Chapter

Current Advances in Immune Checkpoint Therapy

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Abstract

Although immune checkpoint inhibitors (ICIs) have shown survival benefits for patients with metastatic cancers, some challenges have been under intense study in recent years. The most critical challenges include the side effects and the emergence of resistance. Potential opportunities exist to develop personalized immune checkpoint inhibitor therapy based on biomarker discovery. Combinational therapy involving immune checkpoint inhibitors and other forms of anticancer therapies has varied success. This chapter reviews drugs currently undergoing Phase III clinical trials and others that are FDA-approved. We take a critical look at the combinational strategies and address the ever-present challenge of resistance. Moreover, we review and evaluate the discovery of biomarkers and assess prospects for personalized immune checkpoint therapy.

Keywords: immune checkpoint inhibitors, PD-1, PD-L1, CTLA-4, FDA-approved, ICI resistance, combinational therapy, biomarkers

1. Introduction

Immunotherapy including the use of immune checkpoint inhibitors (ICIs) exploits the immune system's components to fight cancer progression. The use of immunotherapy on its own or in combination with conventional cancer treatments such as chemotherapy or radiation has been relatively successful in many cancers [1]. Immune checkpoint proteins are co-inhibitory receptors that are responsible for keeping the immune system in check. Cancer cells exploit these receptor proteins in order to induce tumor tolerance and T cell exhaustion [1, 2]. The FDA has approved treatments for several cancers with immune checkpoint inhibitors that target cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1), and most recently, lymphocyte activation gene-3 (LAG-3). However, there are several additional molecules in clinical trials (Phases I, II, and III) that target immune checkpoint proteins as monotherapy or in combination with other ICIs or different kinds of therapy such as small molecule drugs, chemotherapy, or radiotherapy. Although some adverse reactions occur after treatment with immune checkpoint inhibitors, the main issue encountered is resistance [3]. The most promising strategy to overcome resistance is the use of

combination therapies. However, the identification of reliable biomarkers that can predict resistance and response to ICIs may assist in guiding patient selection and identifying those that will indeed benefit from treatment. Numerous biomarkers have been developed in this regard; however, current biomarkers are challenged with technical limitations. In this review, we present FDA-approved ICIs and novel ICIs in clinical trials. In addition, we address ICI resistance and the use of combinational therapy strategies to overcome it, as well as discuss some of the most extensively studied biomarkers and the limitations associated with each.

2. Approved immune checkpoint inhibitors

T cell activation is critical for normal physiology to suppress carcinogenesis. During carcinogenesis, tumor cells present neoantigens which, in complex with the major histocompatibility complex (MHC), and together with various costimulatory signals, activate naïve T cells through intracellular signals. This process is balanced by signaling through inhibitory molecules called checkpoint inhibitors on the tumor and T cells [4]. However, cancer cells have developed mechanisms to antagonize T cell activation, thereby promoting carcinogenesis. Strategies have been developed to exploit events at the checkpoint synapse to design anticancer therapeutic drugs. In **Figure 1**, the schematic diagram shows the points at which various immunotherapeutic drugs intervene in cancer progression. Currently, approved immune checkpoint drugs target CTLA-4, PD-1, PD-L1, and LAG-3 (**Table 1**). Significantly, all the approved ICIs are indicated for solid tumors, but few are effective against hematological cancers. We discuss their success and current limitations. We consider success to be associated with the overall response rate (ORR), which is generally defined as the proportion of patients who achieve a complete or partial response per RECIST (Response Evaluation Criteria in Solid Tumors) or WHO (World Health Organization) criteria.

2.1 CTLA4 inhibitors

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4 or CD152) is a membrane glycoprotein expressed exclusively on the surface of effector T cells. Despite sharing only 30% sequence similarity with the T cell surface receptor CD28, CTLA-4 has similar structural and functional properties [57]. CTLA-4 and CD28 regulate T cell responses, with CD28 having a stimulatory effect and CTLA-4 having an inhibitory effect. Both receptors bind to B7 ligands (CD80 and CD86) found on antigen-presenting cells (APCs). However, CTLA-4 has been shown to have a higher affinity for these molecules and competes for binding to common ligands [58]. CTLA-4 usually aids in maintaining self-antigen immunity by preventing T cell activation (**Figure 1A**). However, when CTLA-4 binds to B7 ligands present on cancer cells, it exerts an antagonistic effect on T cell activation and results in the evasion of immune responses. Blockade of the CTLA-4/B7 axis invigorates T cell activation and proliferation and therefore presented a unique therapeutic opportunity for cancer patients [59].

Ipilimumab (Yervoy®) is the first immune checkpoint inhibitor that the FDA approved for the treatment of human cancers. Ipilimumab is a humanized IgG1 antibody developed by Bristol-Myers Squibb, and targets CTLA-4, thereby preventing its interaction with B7 ligands (**Figure 1B**). Ipilimumab was initially approved by the FDA for the treatment of late-stage unresectable melanomas in 2011 (**Table 1**). In 2015, it was further approved for cutaneous melanomas [5, 60]. In melanomas,

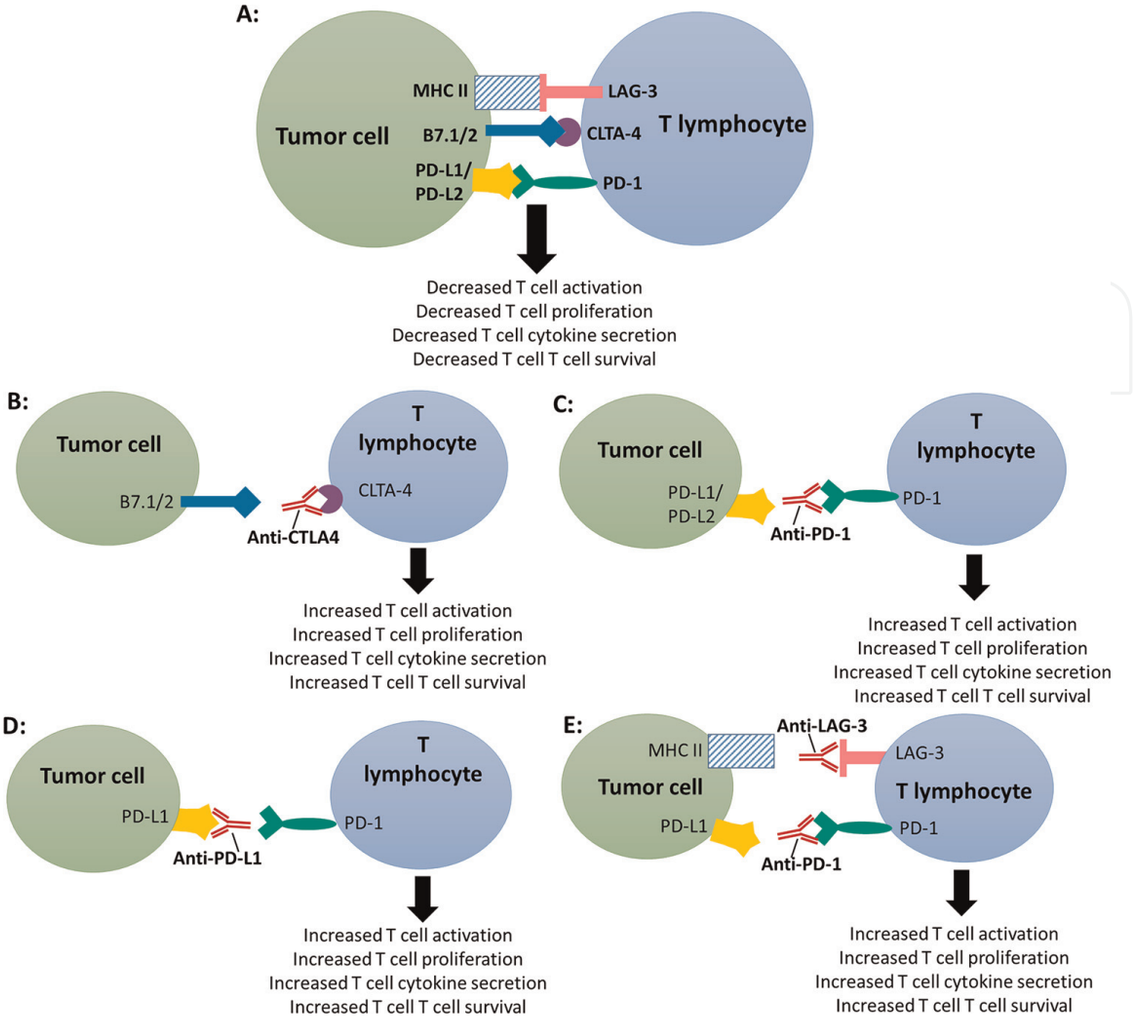

Figure 1.
Mechanism of immune checkpoint inhibitors. (A) Immune checkpoint proteins present on T lymphocytes interact with corresponding ligands on tumor cells, which leads to an alteration in normal T cell phenotypes. The main outcome is the suppression of T cell activation and the resultant decrease in the immune response. (B) Immunotherapy targeting CTLA-4 that disrupts the binding of the B7 family and subsequent signaling. (C) Anti-PD-1 immunotherapy disrupting the interaction with PD-L1/2. (D) The PD-1 and PD-L1 pathway being inhibited by an antibody against PD-L1. (E) The dual blocking of the LAG-3/MHC II pathway and PD-1/PD-L1 pathway, using antibodies that target LAG-3 and PD-1 simultaneously. Blocking these interactions between immune checkpoint proteins and their ligands, using the targeted antibodies, results in a reversal of T cell inhibition.

Ipilimumab exhibited an ORR of 10.9%. In 2018, Ipilimumab received approval for the treatment of renal cell carcinoma (RCC) (ORR 40.4%) and metastatic colorectal cancer (ORR 49%) in conjunction with Nivolumab [7, 61]. More recently, Ipilimumab was approved in conjunction with Nivolumab for non-small cell lung cancer (ORR 36%), malignant pleural mesothelioma (ORR 40%), and hepatocellular carcinoma (ORR 32%) in 2020 (**Table 1**) [40, 62, 63].

Although not yet approved, Tremelimumab is in the final stages of approval. Tremelimumab is a human IgG2 monoclonal antibody that also targets the CTLA-4 receptor. Following the promising results obtained from the HIMALAYA Phase III trial [64] Tremelimumab in combination with Durvalumab (STRIDE (Single T Regular Interval D) regimen) was accepted under Priority Review by the FDA for patients with unresectable hepatocellular carcinoma. The clinical trial demonstrated that patients experienced an improved median overall survival (OS) (16.4 months) and

Drug	Combination	Cancer and reference	Approval year	ORR (95% CI)
CTLA-4 inhibitors				
Ipilimumab	NA	Melanoma [5]	2011	10.9% (6.3–17.4)
	NA	Colorectal cancer [6]	2018	49% (39–58)
	Nivolumab	Renal cell carcinoma [7]	2018	40.4% (26.4–55.7)
	Nivolumab	Hepatocellular carcinoma (HCC) [8]	2020	32%; (9–38)
	Nivolumab	Non-small cell lung cancer [39]	2020	36% (31–41)
	Nivolumab	Pleural mesothelioma [40]	2020	40% (34.1–45.4)
PD-1 inhibitors				
Pembrolizumab	NA	Melanoma [41, 42]	2014	24% (15–34)
	NA	Metastatic non-small [43] cell lung cancer	2015	44.8% (36.8–53.0)
	NA	Head and neck squamous cell carcinoma [44]	2016	16% (11–22)
	NA	Hodgkin's lymphoma [45]	2017	69% (62–75)
	NA	Urothelial carcinoma [46]	2017	24% (20–29)
	Pemetrexed-platinum	Non-squamous NSCLC [47]	2017	47.6% (39.2–56.0)
	NA	Solid tumor with MSI-H or dMMR [9]	2017	39.6% (31.7–47.9)
	NA	Gastric cancer [10]	2017	11.6% (8.0–16.1)
	NA	Cervical cancer [11]	2018	12.2% (6.5–20.4)
	NA	Primary mediastinal large B-cell lymphoma (PMBCL) [12]	2018	45% (32–60)
	Platinum-based chemotherapy	Squamous NSCLC [13]	2018	57.9% (51.9–63.8)
	NA	Hepatocellular carcinoma (HCC) [14]	2018	17% (11–26)
	NA	Merkel cell carcinoma (MCC) [15]	2018	56% (41–70)
	Axitinib	Renal cell carcinoma (RCC) [16]	2019	59% (54–64)
	NA	Esophageal squamous cell cancer [17]	2019	22% (14–33)
	Lenvatinib	Endometrial carcinoma [18]	2019	38.0% (28.8–47.8)
	NA	Non-muscle-invasive bladder cancer (NMIBC) [19]	2020	41% (31–51)
	NA	MSI-R or dMMR colorectal cancer [20]	2020	43.8% (35.8–52.0)
	NA	Cutaneous squamous cell carcinoma (cSCC) [21]	2021	50.0% (36.1–63.9) (localized) 35.2% (26.2–45.2) (metastatic)

Drug	Combination	Cancer and reference	Approval year	ORR (95% CI)
Nivolumab	NA	Melanoma [22]	2014	31·7% (23.5–40.8)
	NA	Squamous NSCLC [23]	2015	20% (14–28)
	NA	Renal cell carcinoma [24]	2015	25% (3.68–9.72)
	NA	Hodgkin's lymphoma [25]	2016	65% (55–75)
	NA	Head and neck squamous cell carcinoma [26]	2016	13.3% (9.3–18.3)
	NA	Urothelial carcinoma [27]	2017	19.6% (15.1–24.9)
	NA	Colorectal cancer (MSI-H) [6]	2017	31·1% (20.8–42.9)
	NA	Hepatocellular carcinoma [28]	2017	20% (15–26)
	NA	Small cell lung cancer [29]	2017	12% (5–23)
	Ipilimumab	Malignant pleural mesothelioma [30, 40]	2020	40% (34.1–45.4)
	Platinum–based chemotherapy or Ipilimumab	Esophageal squamous cell carcinoma [31]	2022	47% (42–53) 28% (23–33)
Cemiplimab	NA	Cutaneous squamous cell carcinoma [32]	2018	49% (31–67) (localized) 47% (35–59) (metastatic)
	NA	Advanced basal cell carcinoma [33]	2021	31% (21–42)
	NA	Non-small cell lung cancer [34]	2021	39% (34–45)
Dostarlimab	NA	Mismatch repair deficient (dMMR) recurrent or advanced solid tumors [35]	2021	41.6% (34.9–48.6),
PD-L1 inhibitors				
Atezolizumab	NA	Urothelial carcinoma [36]	2016	14.8% (11.1–19.3)
	NA	Non-small cell lung cancer [37]	2016	17% (11.0–23.8)
	Nab-paclitaxel	Triple negative breast cancer	2019	56% (51.3–60.6)
	Carboplatin and etoposide	Extensive-stage small cell lung cancer ES-SCLC [38]	2019	60.2% (53.1–67.0)
	Bevacizumab	Hepatocellular carcinoma [20]	2020	33.3% (28.3–38.7)
	Vemurafenib and cobimetinib	Melanoma [48]	2020	66.·3% (60.1–72.1)
Avelumab	NA	Merkel cell carcinoma [49]	2017	31·8% (21.9–43.1)
	NA	Urothelial cancer [50]	2017	18.2% (8.2–32.7)
	Axitinib	Renal cell carcinoma [51]	2019	55.2% (49.0–61.2)
Durvalumab	NA	Urothelial carcinoma [52]	2017	17.0% (11.9–23.3)
	NA	Non-small cell lung cancer [53]	2018	28.4% (24.3–32.9)

Drug	Combination	Cancer and reference	Approval year	ORR (95% CI)
	Platinum-etoposide chemotherapy	Extensive-stage small cell lung cancer [54, 55]	2020	68% (62–73)
LAG-3 inhibitor				
Relatlimab	Nivolumab	Melanoma [56]	2022	43.1% (37.9–48.4)

Table 1.
FDA-approved immune checkpoint inhibitors.

overall response rate (20.1%) when compared to Sorafenib treatment (13.8 months and 5.1%, respectively). An approval decision in the fourth quarter of 2022 is expected. These data suggest that a combination of an ant-CTLA-4 and an anti-PD-L1 as a strategy may be a feasible approach.

2.2 PD-1 inhibitors

Programmed cell Death 1 (PD-1) (also known as CD279) is a co-inhibitory transmembrane protein that is expressed on antigen-stimulated T and B lymphocytes, natural killer (NK) cells, and myeloid suppressor dendritic cells (MDSCs). PD-1 is activated via antigen recognition or cytokine stimulation and results in the modulation of immune response intensity [65]. PD-1 ligands, namely, programmed death ligands 1 and 2 (PD-L1 (B7-H1) and PD-L2 (B7-DC)), are widely expressed on antigen-presenting cells. The interaction between PD-1 and its ligands results in the inhibition of lymphocyte proliferation or activation, culminating in T cell exhaustion (**Figure 1A**) [65–68]. To date, the FDA approved four PD-1 immune checkpoint inhibitors for the treatment of human cancers, namely Pembrolizumab (Keytruda®), Nivolumab (Opdivo®), Cemiplimab (Libtayo®), and Dostarlimab-gxly.

Pembrolizumab (MK-3475 or Lambrolizumab, Keytruda) is a humanized IgG4 antibody against PD-1, developed by Merck. The FDA initially approved it for the treatment of patients with unresectable or metastatic melanoma in September 2014 after the KEYNOTE-001 clinical trial (NCT01295827) [69]. These patients had to have had prior unsuccessful treatment with Ipilimumab. Pembrolizumab binds to the PD-1 receptor, thereby disrupting the PD-1 pathway and resulting in the restoration of the antitumor immune response of T lymphocyte cells (**Figure 1C**) [70–72]. Pembrolizumab has subsequently been approved for treatment predominantly as monotherapy and occasionally as part of a combinational therapy for an additional 16 cancer types (**Table 1**). The overall/objective response rates to Pembrolizumab ranges from 12 to 69% in these various cancers. The adverse reactions to Pembrolizumab include both immune-related adverse effects (irAEs) and infusion-related reactions. irAEs include encephalopathy, pneumonia, nephritis, hepatitis, myocarditis, and colitis. However, the most common adverse effects (reported in ≥20% of patients) are fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhea, nausea, rash, pyrexia, cough, dyspnea, constipation, pain, and abdominal pain [65, 69].

Nivolumab (Opdivo, ONO4538, MDX-1106, or BMS-936,558) is a genetically engineered, fully humanized IgG4 mAb against PD-1 developed by Bristol-Myers Squibb. Like Pembrolizumab, the FDA-approved Nivolumab for the treatment of unresectable or metastatic melanoma, which had progressed after prior treatment with

Ipilimumab. Nivolumab was approved in December 2014 after the CheckMate-037 trial, which tested its efficacy when combined with chemotherapy. Nivolumab selectively inhibits the interaction between the PD-1 and its ligands, PD-L1, and PD-L2. It achieves this by binding to the PD-1 receptor and interfering with the negative regulation of T lymphocyte activation and proliferation caused by the PD-1 pathway, including the antitumor immune response [73, 74]. Since Nivolumab was first approved for the treatment of melanoma in 2014 [22], it has subsequently been approved by the FDA for the treatment of an additional seven cancer types, either in monotherapy or as part of combination therapy (**Table 1**). The overall/objective response rates to Nivolumab ranges from 12 to 65% in the various cancers. Nivolumab is also the most used ICI for combination with CTLA-4 inhibitors and most recently a LAG-3 inhibitor. Serious adverse effects to Nivolumab include increased risk of severe immune-mediated inflammation in the lungs, the colon, the liver, and the kidneys, immune-mediated hypothyroidism and hyperthyroidism and autoimmune diabetes [75–77].

Cemiplimab (REGN2810, SAR439684, Libtayo) is a human IgG4 anti-PD-1 mAb developed by Sanofi/Regeneron. It was approved in September 2018 for the treatment of metastatic cutaneous squamous cell carcinoma (cSCC) or locally advanced cSCC in patients who did not qualify for surgery or radiation [32, 78]. cSCC has a high mutational burden and is therefore hard to treat. Cemiplimab binds to the PD-1 receptor and blocks its interaction with PD-L1, resulting in the upregulation of cytotoxic T cells and an increase in the antitumor activity of the immune system (**Figure 1C**) [78–80]. After its first approval in 2018, it was further approved by the FDA in 2021 for the treatment of two additional cancers, namely basal cell carcinoma and non-small cell lung cancer (**Table 1**). The overall/objective response rate to Cemiplimab ranges from 31 to 49% in the three cancer types. Reported adverse effects of Cemiplimab include severe and fatal immune-mediated adverse reactions in any organ, system, or tissue, including pneumonia, colitis, hepatitis, endocrine disorders, adverse skin reactions, nephritis, and renal dysfunction. In addition, severe infusion-related reactions (Grade 3) can also occur. However, the most common adverse reactions are fatigue, rash, and diarrhea [65, 78, 79].

Recently, in August 2021, the FDA accelerated the approval of the novel PD-1-humanized IgG4 monoclonal antibody known as Dostarlimab-gxly (Jemperli, GlaxoSmithKline LLC) for patients with mismatch repair deficient (dMMR) recurrent or advanced solid tumors after clinical trial NCT02715284 [81]. The overall response rate was 41.6% (95% CI: 34.9, 48.6), with a 9.1% complete response rate and a 32.5% partial response rate. The most reported adverse reactions in patients with dMMR solid tumors were fatigue, anemia, diarrhea, and nausea. Most common Grade 3 or 4 adverse reactions included anemia, fatigue, increased transaminases, sepsis, and acute kidney injury. In a few patients, immune-mediated adverse reactions are associated with Dostarlimab. These include pneumonitis, colitis, hepatitis, endocrinopathies, nephritis, and dermatologic toxicity. In 2022, Dostarlimab was preferred for treating colon cancer, as a small group of 12 patients in clinical Phase II responded positively to the drug, with a 100% complete response rate. This is a rare phenomenon in clinical trials (NCT04165772) [82]. In addition, no adverse events of Grade 3 or higher or relapse were reported. However, the FDA has not yet approved Dostarlimab for the treatment of colon cancer.

2.3 PD-L1 inhibitors

The Programmed Death receptor Ligand 1 (PD-L1) plays a vital role in the downregulation of T cell activation in the tumor microenvironment (TME). PD-L1

(B7-H1) and PD-L2 (B7-DC) are the two ligands known to bind to the PD-1 receptor described earlier [66, 83, 84]. Under normal physiological conditions, the PD-1/PD-L1 interaction moderates excessive immune cell activity, thereby preventing the development of autoimmunity and tissue destruction due to hyperactivation of the immune system. Cancer cells in the TME exploit this regulatory mechanism by overexpressing PD-L1 on their surface. The interaction between PD-L1 on tumor cells and PD-1 on cells (T cells) negatively regulates T-cell-mediated immune responses in the TME, resulting in T cell exhaustion and limitation of effector T cell responses [66, 84, 85]. Consequently, cancer development and progression are enhanced by maintaining tumor cell proliferation and survival. Therefore, the PD-L1 signaling represents an attractive target for novel anticancer therapy.

The development and clinical application of immune checkpoint inhibitors targeting the PD-1/PD-L1 axis have significantly enhanced antitumor immunity, produced durable responses, and prolonged survival in cancer patients. Currently, there are three FDA-approved PD-L1 inhibitors, namely, Atezolizumab, Durvalumab, and Avelumab, for treating several solid cancers such as non-small cell lung cancer and metastatic melanoma [85] (**Table 1**). Atezolizumab was the first PD-L1 immune checkpoint inhibitor to be approved by the FDA in 2016 for the treatment of advanced or metastatic urothelial carcinoma (UC) [46]. Studies from clinical trial results revealed that treatment with Atezolizumab increased the ORR and was linked to the PD-L1 status of patients. Patients with less than 5% PD-L1 expression detected saw 9.5% ORR compared to 26% in patients with PD-L1 expression greater than 5% after the 14.4 month follow-up.

Atezolizumab (MPDL3280 or Tecentriq®, Genentech) is a fully humanized IgG1 monoclonal antibody. Its mechanism of action involves binding to PD-L1, thereby blocking PD-L1 interaction with the PD-1 receptor. The disruption of this interaction between immune (PD-1) and PD-L1-expressing tumor cells in the TME results in the reactivation of T-cell-mediated antitumor cytotoxicity. Clinical data have demonstrated that Atezolizumab is safe and efficacious in a wide range of solid tumors and hematologic malignancies [20, 46, 86]. Following its approval for the treatment of UC, the drug has been further approved for the treatment of non-small-cell and extensive stage lung cancer [87, 88]. The treatment of NSCLC and ES-SCLC with Atezolizumab improved the ORR by 17% compared to conventional chemotherapy.

Durvalumab (MEDI4736 or ImfinziTM, AstraZeneca) is another fully humanized IgG1 monoclonal antibody like Atezolizumab that binds with high affinity and specificity to PD-L1, blocking the interaction with PD-1. The US FDA first approved the immune checkpoint inhibitor in 2017 to treat locally advanced or metastatic urothelial carcinoma (UC) [89]. Following its approval, Durvalumab received further accelerated approval for treating unresectable stage III NSCLC following platinum-based chemotherapy and radiotherapy [90]. The introduction of Durvalumab in the treatment of UC and NSCLC improved the ORRs by 17% and 28.4%, respectively. In 2020, the drug was approved to treat extensive stage small cell lung cancer [54]. Currently, Durvalumab is being tested in combination with targeted therapies, chemotherapy, and immunotherapy to maximize its activity and improve patient survival rates.

Avelumab (MSB0010718C or Banvecio®, Merck and Pfizer) is another fully humanized IgG1 monoclonal antibody that binds to PD-L1. Banvecio® binds and blocks PD-L1 expressed in tumor cells resulting in T-cell-mediated antitumor immune response, particularly T cell reactivation and cytokine production [91]. The FDA accelerated the approval of Avelumab for treating 12-year-old and older patients with

metastatic Merkel cell carcinoma (MCC) in March 2017 [49]. The approval was based on the observed improved ORRs by 31.8% compared to chemotherapy. Avelumab was further approved in May 2017 for the treatment of locally advanced or metastatic UC with disease progression during or following platinum-based chemotherapy [50]. The treatment improved ORR by 18.2%. Avelumab's most recent approval is for the treatment of renal cell carcinoma [51]. Avelumab is currently being tested in combination with traditional cancer therapies in emerging new small molecules (that have synergistic or complementary functions) in clinical trials. Several other PD-L1 immune checkpoint inhibitors are currently in preclinical and early-phase clinical trials [83].

2.4 LAG-3 inhibitors

The lymphocyte activation gene-3 (LAG-3) (CD223) is a membrane receptor protein that is predominately expressed by activated CD4+ and CD8+ T cells, regulatory T cells (Tregs), and natural killer (NK) cells. LAG-3 can also be expressed to a lower extent by B cells and plasmacytoid dendritic cells (DCs) [92]. It interacts with its primary ligand, the major histocompatibility complex class II (MHC-II) (**Figure 1A**), as well as other ligands, including galectin-3, liver sinusoidal endothelial cell lectin (LSECtin), α-synuclein, and fibrinogen-like protein 1 (FGL1). These interactions result in immune cell exhaustion and decreased cytokine secretion [92–95]. Blocking LAG-3 alone cannot reverse T cell exhaustion; however, combining it with a PD-1 inhibitor has been shown to decrease tumor size [96]. Therefore, in March 2022, the combination of Relatlimab (anti-LAG-3) and Nivolumab (anti-PD-1) was approved by the FDA for the treatment of advanced or metastatic melanoma (**Figure 1E**) [56]. The most common adverse reactions (≥20%) were musculoskeletal pain, fatigue, rash, pruritus, and diarrhea. The most common laboratory abnormalities (≥20%) were decreased hemoglobin, decreased lymphocytes, increased aspartate aminotransferase (AST), increased alanine aminotransferase (ALT), and decreased sodium. Currently, there are 17 small molecule drugs targeting LAG-3 in clinical trials comprising of mono and combination treatments (**Table 2**). Furthermore, Tebotelimab (MGD013) is a bispecific DART molecule designed to independently or coordinately block PD-1 and LAG-3 and is being investigated in patients with HER2-positive gastric cancer or gastroesophageal junction cancer (GEJ) (NCT04082364).

3. Immune checkpoint inhibitors in phase III clinical trials

Clinical trials are underway on novel immune checkpoint inhibitors and new combinations of already FDA-approved ICIs. Novel emerging immune checkpoint inhibitors include drugs that target lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and ITIM domain (TIGIT), T cell immunoglobulin and mucin domain containing-3 (TIM-3), V-domain immunoglobulin suppressor of T cell activation (VISTA), B7 homolog 3 protein (B7-H3), inducible T cell costimulatory (ICOS), and B and T lymphocyte attenuator (BTLA). Currently, at least nine novel ICIs have reached Phase III clinical trials (**Table 2**). We note that in addition to the drugs listed in **Table 2**, there are more than 50 other agents (antibodies and small molecules) targeting immune checkpoint proteins that are in Phase I and II [106].

Target	Drug, clinical trial number, and year	Cancer	Protocol
CTLA-4 [97]	Tremelimumab NCT03298451 2017	Advanced hepatocellular carcinoma (HCC)	Durvalumab + Tremelimumab vs. Durvalumab monotherapy vs. Sorafenib
LAG-3 [98]	Relatlimab NCT03470922 2018	Advanced melanoma	Relatlimab + Nivolumab vs. Nivolumab monotherapy
LAG-3 [99]	MGD013 NCT04082364 2019	Gastric cancer (GC) or gastroesophageal junction cancer (GEJ)	Margetuximab, Retifanlimab, Tebotelimab, and Chemotherapy
TIGHT [100]	Tiragolumab NCT04294810 2020	Non-small cell lung cancer (NSCLC)	Tiragolumab + Atezolizumab Versus Placebo + Atezolizumab
TIGHT [101]	Tiragolumab NCT04256421 2020	Extensive-stage small cell lung cancer (ES-SCLC)	Atezolizumab + Carboplatin and Etoposide (with or without Tiragolumab)
TIM-3 [102]	Sabatolimab NCT04266301 2020	Myelodysplastic syndrome (MDS) or chronic myelomonocytic leukemia-2 (CMML-2)	MBG453+ Azacitidine
B7-H3 [103]	Enoblituzumab NCT04129320 2019	Head and neck squamous cell carcinoma (HNSCC)	Enoblituzumab + MGA012 or MGD013
B7-H3 [104]	131I-Omburtamab NCT03275402 2017	Neuroblastoma, central nervous system, or leptomeningeal metastases	131I-omburtamab + Radioimmunotherapy
ICOS [105]	GSK3359609 NCT04128696 2019	Head and neck squamous cell carcinoma (HNSCC)	GSK3359609 + Pembrolizumab

Table 2.
Immune checkpoint inhibitors in phase III clinical trials.

4. Resistance to immune checkpoint inhibitors

One of the most significant challenges in immune checkpoint therapy is the development of resistance, whether it is primary (the patient never responds to treatment) or acquired (the patient initially responds to treatment but stops responding after the commencement of therapy). Resistance can also be intrinsic or extrinsic to tumor cells [107]. Intrinsic resistance occurs when cancer cells alter processes related to immune recognition, cell signaling, gene expression, and DNA damage response. Resistance to immune checkpoint inhibitors is associated with loss of immunogenic neoantigens, an increase of immunosuppressive cells, and the upregulation of alternate immune checkpoint receptors [27, 108]. Response to ICIs can also vary by tumor type, with the highest response rates found in tumors with a high mutational burden, such as melanoma, lung, and bladder cancers.

In contrast, tumors with lower tumor mutational burden (TMB), such as prostate and pancreas, show a lower response [109]. However, ICI response can vary among tumors with a similar TMB, thus suggesting that response to ICI is influenced by several other factors [110]. These factors may include PD-L1 expression or induction, deficiencies in DNA mismatch repair (MMR), levels of tissue-specific neoantigens and tumor-

infiltrating lymphocytes (TILs), endogenous retroviruses (RVs) epigenetic alterations, and oncogenic alterations [27, 108, 111, 112]. Extrinsic resistance occurs external to tumor cells throughout the T cell activation process. Tumors can have different immunophenotypes, such as variation in type, density, and location of immune infiltrates, and these differences can affect the response to ICI therapy. In general, inflamed tumors generally respond better to ICI therapy [113, 114]. In addition, the tumor microenvironment (TME) also plays a big role in treatment response, contributing to both primary and acquired resistance. The TME is complex and comprises various immune and stromal cells, the extracellular matrix, surrounding vasculature, and cytokines [114, 115]. This scenario further complicates the development of drug resistance.

Resistance can also be attributed to contextual factors, which include the gut microbiome, expression of human endogenous retroviruses, and gender. The response to ICI therapy influenced by gut microbiomes is thought to involve the activation of dendritic cells, upregulation of MHC-II, and the increased levels of effector T cells [107, 116–118]. High expression of human endogenous retroviruses (RVs) in tumors resulted in a phenotype consistent with immune checkpoint activation in various cancer types. Furthermore, the abnormal expression of ERVs appears to activate epigenetic changes such as histone methylation [111, 119]. Overall, the abnormal expression of ERVs indicates a positive response to ICI treatment.

With overall response rates for most cancers to FDA-approved drugs generally being between 10 and 50%, this indicates that in at least 50% of patients, either primary or acquired resistance is occurring. Two of the most promising strategies by which we can overcome resistance are combinational therapy and identifying predictive biomarkers of ICI therapy.

5. Combinational therapy as a strategy to overcome resistance

In the past decades, patients diagnosed with various cancers that did not respond well to traditional methods such as chemotherapy and radiotherapy received very poor prognoses. Moreover, these conventional cancer therapy methods are also known to cause damage to healthy normal cells. Since then, various cancer therapies targeting disordered proteins, immune cells, and components of the tumor microenvironment (TME) have been developed to improve prognosis. Small molecules and immunotherapy have drastically improved the prognosis for some patients. Despite that, a limited number of patients obtain benefits from the treatment. This is attributed mainly to low response and acquired resistance during the treatment, and severe side effects also lead to unfavorable outcomes. To overcome this, researchers are investigating the potential of combining ICIs with various other treatments, including chemo/radiotherapy and targeted therapies. Immunotherapy based on single targets often results in serious side effects, unresponsiveness, or overreaction. In contrast, combinational immunotherapies show synergistic outcomes with higher efficacy and safety. Strategies combining immunotherapy and conventional therapies like radiotherapy and/or chemotherapy have demonstrated promising clinical and basic research results. However, the underlying mechanisms are still unclear.

5.1 Combination of two or more immune checkpoint inhibitors

Checkpoint inhibitors that target CTLA-4 and the PD-1/PD-L1 axis are promising candidates for combination immunotherapy. The rationale behind the dual

checkpoint inhibitor treatment is the synergy of inhibiting both CTLA-4 and PD-1 with Nivolumab plus Ipilimumab which was the first combination immunotherapy to be licensed in the US and Europe and has been used in the treatment of melanoma for several years [120]. Clinical studies have shown that the combination of Ipilimumab with Nivolumab significantly improved overall survival rates to 57% compared to Nivolumab (43%) and Ipilimumab (25%) alone in melanoma patients after a 6.5-year follow-up to assess efficacy and safety [120]. Following its first approval for the treatment of advanced melanoma in 2017, the combination is now used for the treatment of advanced RCC, HCC microsatellite instability-high (MSI-H), or mismatch repair deficient (dMMR) metastatic colorectal carcinoma (CRC), NSCLC, and malignant pleural mesothelioma (MPM) as shown in **Table 1** [61, 63, 121, 122]. Combination therapy has significantly improved the clinical outcomes for most patients. Long-term follow-up (42 months) in RCC patients revealed an improved overall response rate of 42% (Nivolumab + Ipilimumab) versus 26% in patients treated with Sunitinib, a small molecule monotherapy [123]. Furthermore, durable long-term efficacy was observed, especially among patients with more than 1% PD-L1 expression [62].

More recently, the combination of Relatlimab and Nivolumab, known as Opdualag, was approved by the FDA for treating advanced or metastatic melanoma in patients aged 12 years and older. The approval was based on results from the RELATIVITY-047 clinical trials [98]. The combination treatment with Relatlimab +Nivolumab was at 47.7% compared to 36% in the Nivolumab monotherapy group after 12 months of follow-up. As described in the introduction, Relatlimab inhibits LAG-3 while Nivolumab inhibits PD-1, which are both often expressed by immune cells in the TME (**Figure 1E**). The expression of PD-1 and LAG-3 negatively regulates T cell tumor infiltration and proliferation, respectively. Combination immunotherapy has become an attractive avenue for the treatment of resistant cancers following the Ipilimumab + Nivolumab treatment of various cancers. Currently, several Phase III/IV clinical trials are ongoing to test the safety and efficacy of dual checkpoint inhibitor therapy combining two or more ICIs as listed in **Table 3**.

5.2 Combination of immune checkpoint inhibitors with conventional therapies (chemotherapy/radiotherapy and small molecules)

In some instances, chemotherapeutic agents have appeared to impact the immune system positively. The positive effects of standard chemotherapy on tumor immunity are mainly reflected in inducing immunogenic cell death and disrupting tumor escape strategies. Experimental data have shown that some anticancer chemotherapeutic agents can stimulate naïve immune cells to induce immunogenic cancer cell death [133]. For this reason, chemotherapy in combination with immune checkpoint inhibitors is an attractive strategy for synergistic combination treatment in cancer. Several studies using murine models have shown that chemotherapeutic agents such as cyclophosphamide, fluorouracil (5-FU), and Gemcitabine can reduce Tregs, improve circulating NK cells, and augment tumor-infiltrating T cells, respectively [134, 135]. Indeed, a combination of PD-L1 inhibitor (Nivolumab) plus Gemcitabine and Cisplatin significantly improved the ORR over monotherapy in a Japanese Phase I clinical trial [136]. Since then, several ICI and chemotherapy combination treatments have been investigated to improve patient response rate and survival.

To date, there are several ICI and chemotherapy/radiation combination therapies that have been approved by the FDA. Others are currently in Phase III/IV clinical trials as listed in **Table 3**. Pembrolizumab combined with standard chemotherapy has

Protocol	Disease (refs)	ORR (95% CI)
Ipilimumab + Nivolumab	Unresectable stage III or IV melanoma [124]	No data available
	EDSCLC + after completion of platinum-based chemotherapy (CheckMate 451) [125, 126]	9.1% (5.9–13.2)
	NSCLC combined with two cycles of chemotherapy [62]	45.4% (38.4–52.4)
	Esophageal squamous cell carcinoma [31]	42% (34–50)
Pembrolizumab + Ipilimumab	Metastatic NSCLC [127]	45.4% (39.5–51.4)
Chemoradiotherapy + Temozolomide (chemo) + Nivolumab	Glioblastoma [128]	ORR not measured
Ipilimumab + Paclitaxel and Carboplatin	Squamous NSLC [129]	44% (39–49)
Nivolumab + chemotherapy (capecitabine and oxaliplatin every 3 weeks or leucovorin, fluorouracil, and oxaliplatin every 2 weeks)	Advanced gastric, gastroesophageal junction, and esophageal adenocarcinoma [130]	57.1% (34.0–78.2)
Ipilimumab + Etoposide and Platinum chemotherapy	Extensive-stage small cell lung cancer [131]	62% (58–67)
Spartalizumab + Dabrafenib and Trametinib	BRAF V600-mutant unresectable or metastatic melanoma [132]	69% (62.6–74.1)

Table 3.
Current combination therapies.

become the first such combination therapy to be licensed for first-line use in patients with metastatic non-squamous NSCLC in the US and Europe after a trial showed that the combination enhanced overall survival at 12 months by 69.2% compared to 49.4% in the monotherapy group [137]. Since then, Pembrolizumab in combination with Axitinib, a vascular endothelial growth factor (VEGF) inhibitor has been further approved for the treatment of RCC. The ORR favored the Pembrolizumab/Axitinib group (59.3%) over the sunitinib group (35.7%). Atezolizumab and Durvalumab, both targeting the PD-L1, have been FDA-approved in combination with chemotherapy as a first-line treatment for advanced SCLC [138]. The approval was based on the IMpower133 and CASPIAN clinical trials which both evaluated Atezolizumab and Durvalumab, respectively, in combination with etoposide and carboplatin-based chemotherapy. Both studies revealed improved overall survival (OS) by Atezolizumab + chemotherapy (12.3 months); Durvalumab + chemotherapy (13 months) compared to chemotherapy alone (10 months) [54, 139].

6. Predictive biomarkers of therapy dynamics

6.1 Genomic biomarkers

6.1.1 Tumor mutational burden

Tumor mutational burden (TMB) refers to the frequency of non-synonymous mutations and is directly related to the neoantigen load. A high frequency of

mutations generally results in a high rate of neoantigen production, thereby increasing the probability of an immune response [140]. Therefore, TMB has been investigated and validated as a predictive biomarker for ICI response by numerous studies.

The association between TMB and a response to ICI has been extensively studied in NSCLC patients, however, with variable outcomes. After whole-exome sequencing (WES), a high mutational burden (>178 mutations per sample) observed in NSCLC patients treated with Pembrolizumab correlated to better ORR (68%) compared to patients with a low mutational burden (0%). Therefore, low TMB was correlated with poor efficacy in patients and is considered a marker of primary resistance to ICI treatment [140]. Similarly, a study with 4064 NSCLC patients showed that a high TMB had a significantly higher OS compared to a low TMB [141]. Numerous other studies have also shown a similar association between TMB and ICI response [142–144]. In contrast to these observations, a study whereby NSCLC patients were treated with Pembrolizumab and chemotherapy showed that TMB with >175 mutations per exome was not able to predict a response [145]. It is important to note that some tumors with a low TMB are still capable of responding to ICI. This highlights that, although TMB is a good indicator of ICI response, it is not the only determinant factor. On a broader scale, the correlation between TMB and response to ICI has been demonstrated across 27 tumor types [146]. The KEYNOTE-158 study with 750 participants showed that TMB-high tumors were associated with better overall response rates (28%) and progression-free survival (24%) compared to TMB-low tumors (7% and 14%, respectively). Interestingly, 12.5% of the TMB-high cohort were also mismatch repair deficient and were even more likely to respond to ICIs [147]. These studies provided compelling evidence for the use of TMB as a biomarker to determine benefit from ICIs.

Despite the association between TMB and ICI response, there are challenges that complicate the use of TMB as a biomarker in the clinic. TMB is typically measured using whole-genome sequencing (WGS), whole-exome sequencing (WES), or targeted next-generation sequencing (NGS). WES has been the standard method of choice but is resource-intensive and time-consuming and is most often used in a research setting. Therefore, in a drive for a more feasible detection method, multiple NGS panel assays were developed which targets specific sites of the genome [148]. The current challenge is the standardization of the method in terms of the regions that are targeted and sequencing depth [149]. The definition of TMB and sampling methods also limit its use. Variations in cancer types means there is no standard cut point in the definition for a high TMB or low TMB, and each tumor type may have its own optimal threshold to predict a response [150]. In addition, the sampling methods are invasive, and single biopsies can often lead to misclassification of the TMB due to tumor and intratumor heterogeneity. A study showed that 20% of NSCLC and 52% of urothelial cancers were misrepresented as a high TMB. Further multi-sample analysis revealed a low TMB [151]. Lastly, it would be useful to test the effect of TMB on a protein level for neoantigens, since only a subset of mutated genes result in potent neoantigens that are able to elicit an immune response [152]. Although numerous studies have provided supportive evidence for TMB as a predictive biomarker for ICI response, assessment of combination or multiple biomarkers in conjunction with TMB may have a stronger predictive value.

6.1.2 Mismatch repair deficiency and microsatellite instability

Mismatch repair genes such MLH-1, MSH-2, MSH-6, and PMS-2 are responsible for DNA repair. Loss of function in these genes is referred to as mismatch repair

deficiency (MMR-D). It leads to the accumulation of mutations during replication at a significantly higher rate than normal as well as the development of microsatellite instability (MSI) [153]. MMR-D/MSI is especially common in pancreatic, endometrial, cervical, prostate, and gastrointestinal cancers, including colorectal, gastric, and small intestinal cancer [154]. These tumors are particularly rich in frameshift mutations resulting in a high neoantigen load. Additionally, these tumors have also been found to contain a high level of infiltrating immune cells. These factors frequently enhance the immune response. Therefore, MMR-D can be used as a predictive biomarker for determining ICI response.

Clinical trials have shown that Pembrolizumab has durable outcomes in patients with MMR-D/MSI tumors. A study evaluating the efficacy of Pembrolizumab in colorectal cancer patients with and without MMR-D as well as MMR-D non-colorectal cancer patients showed promising results. For colorectal cancer with MMR-D, an overall response rate of 40% was observed whereas, for non-colorectal cancers with MMR-D, an overall response rate of 71% was observed. In contrast, patients without MMR-D exhibited an ORR of 0%. These results demonstrated that MMR-D patients produce a more favorable response to ICI treatment and are ideal candidates. This study led to the recommendation for MMR-D testing in metastatic colorectal cancer. In 2017, the FDA approved Pembrolizumab for patients with solid MMR-D/MSI tumors. This represents the first FDA approval for cancer treatment based on a genetic biomarker alone [155].

6.1.3 IFN pathway profiles

Activated CD8+ T cells secrete IFN-γ following binding to the MHC–peptide complex. IFN-γ is a cytokine that activates immune cells and stimulates an immune response. In the tumor cell, JAK/STAT signaling is activated by IFN-γ which results in the release of chemokines to promote an anticancer response. Moreover, IFN-γ triggers the upregulation of MHC-1 and PD-L1 expression promoting antigen presentation in APCs. IFN-γ expression was found to predict a positive response to PD-1 immune checkpoint inhibitors in melanomas and NSCLC. Conversely, mutations in IFN pathway genes such as IFNGR1/IFNGR2, JAK1/JAK2, STAT, and IRF1 have been associated with poor outcomes and resistance in patients receiving ICI therapy [156, 157]. In melanomas and MMR-D colorectal cancers, the loss of function in JAK1 and JAK2 have also been identified as mechanisms of both primary and secondary resistance to ICIs [158, 159].

A study including NSCLC and melanoma patients treated with Nivolumab and Pembrolizumab, respectively, indicated that increased expression of IFN-γ correlated with improved OS and PFS [160]. Similarly, another study investigating a four-gene IFN-γ signature (IFN-γ, CD274, LAG3, and CXCL9) in NSCLC patients treated with Durvalumab revealed that a positive signature for the gene set was associated with higher ORRs, PFS, and OS in comparison with signature-low patients [161]. It has also become increasingly common to assess IFN-γ in combination with other biomarkers such as TMB. A study in melanoma patients assessed both inflammatory gene profiles and the TMB. Patients treated with Pembrolizumab exhibiting high levels of both biomarkers had an ORR of 54% compared to an ORR of 14% in patients with low expression levels [162]. Furthermore, in melanoma patients treated with neoadjuvant Ipilimumab and Nivolumab, tumors with high IFN gene signatures and TMB displayed a 100% response rate, while tumors with low expression profiles of both had a 37% response rate [163, 164]. Similar results have been observed for NSCLC and

renal cell carcinoma [165]. These studies demonstrate the emerging role of inflammatory gene expression profiles as a predictive biomarker for ICI response. Challenges associated with the use of such gene panels arise from the replication of results due to intratumor heterogeneity and sampling methods, once again highlighting the limitations of single region sampling.

6.2 Tumor-immune microenvironment biomarkers

6.2.1 PD-L1

ICIs that target PD-1 or PD-L1 aim to disrupt the PD-1/PD-L1 axis, allowing cells to mount an antitumor response by preventing T cell downregulation [166]. Consequently, PD-L1 expression is one of the most extensively studied predictive biomarkers for response to ICI therapy. In the KEYNOTE-001 study, patients with PD-L1 expression of more than 50% had an ORR of 45% and improved PFS and OS. In comparison, patients who displayed 1–49% PD-L1 expression had an ORR of only 17% [167]. This study ultimately led to the approval of Pembrolizumab in NSCLC patients who display more than 50% PD-L1 and established the expression of PD-L1 as a companion predictive biomarker for patient selection. Positive correlations have also been seen for gastric cancer, colorectal cancer, and hepatocellular carcinoma [17, 168, 169]. Subsequent trials for PD-L1 as a predictive biomarker led to approvals by the FDA for urothelial, triple-negative breast cancer (TNBC), head and neck, gastric, esophageal cancers, and cervical cancer at various cut points.

PD-L1 expression has significant spatial and temporal heterogeneity. Expression varies between sites of the same tumor and between metastatic sites. Given this, the use of PD-L1 as a predictive biomarker has limitations. Detection is usually carried out using immunohistochemistry, but it is not adequately standardized. Even in the same cancer type, there are variations in thresholds. There are five main PD-L1 diagnostic antibodies that are available for detection. These antibodies have only been validated in the context of its companion drug trial: Pembrolizumab (Dako 22c3), Nivolumab (Dako 28–8), Durvalumab (Ventana SP263), Avelumab (Dako 73–10), and Atezolizumab (Ventana SP142). Variations in detection between assays have been noted. Dako 73–10 scores more cells as positive and Ventana SP142 scores more as negative leading to misinterpretations [170]. Detection of PD-L1 is frequently observed in patients who respond to anti-PD-1/ PD-L1 immunotherapies. However, [43] reported that even when NSCLC tumors displayed more than 50% PD-L1 staining, approximately half of the subset of patients still had primary resistance to Pembrolizumab. This study suggested that PD-L1 expression alone may be insufficient at predicting resistance. As with TMB, it is critical to note that PD-L1 does not preclude response to treatment. In the study mentioned earlier, although PD-L1-positive patients had a higher response rate, 15% of PD-L1-negative patients still responded [171].

6.2.2 Tumor infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TILs) encompass lymphatic cell populations that invade the tumor tissue. TILs may promote an antitumor response (CD4+), exert cytotoxic antitumor activity (CD8+), or even limit a response (FOXP3+ Treg). These cells have therefore been associated with prognosis and response to ICI in many

cancer types, including NSCLC, TNBC, colorectal cancer, and melanoma. The density, location as well as phenotype of TILs give an indication of the response. In melanoma patients treated with Pembrolizumab, the spatiotemporal dynamics of TILs showed that the presence of CD4+ and CD8+ T cells at the infiltrative margin of the tumor was associated with patients who respond to treatment. The high density of cells allowed for increased infiltration into the tumor parenchyma of responders [172]. Another study revealed that responders had high levels of stromal TILs (50%) in comparison with non-responders (15%) for TNBC patients treated with Pembrolizumab [173]. An investigation into the temporal dynamics of TILs showed that an increase in TILs at 3 weeks, compared to the baseline reading, was correlated with response in melanoma patients treated with Ipilimumab [174]. Furthermore, the phenotype of TILs may also be used as a prognostic biomarker. A study showed that CD69+ CD103+ tumor resident CD8+ T cells were associated with improved survival in melanoma [175]. In contrast, FOXP3 tregs have been associated with poor survival in numerous cancer types [176]. The prognostic value of TILs has also been demonstrated by combining detection with PD-L1 expression to allow for better accuracy in determining response. Patients who exhibited high CD8+ TILs and low PD-L1 had an OS of approximately 93% in comparison with patients with low CD8+ TILs and high PD-L1 (61%). The authors suggested that CD8+ TIL combined with PD-L1 expression was better at predicting response than each biomarker alone [177].

6.3 Blood-based biomarkers

6.3.1 Circulating tumor DNA and tumor cells

The noninvasive nature of blood biopsies reduces patient suffering and provides certain advantages such as overcoming the heterogeneity issues of single sample tissue biopsies. It also allows multiple sampling throughout the disease progression and acquisition of real-time data. Therefore, there it is imperative to develop reliable blood-based biomarkers [178]. Emerging studies have linked circulating DNA (ctDNA) and circulating tumor cells (CTCs) found in the peripheral blood with response to ICI. In a study with melanoma patients, detectable baseline ctDNA that persist during treatment correlated with a poor response of only 6%. However, when ctDNA was initially detectable and became undetectable at 12 weeks, the response rate was 77% and when ctDNA was undetectable at both the baseline and 12 weeks, the response rate was 72% [179]. Thus, ctDNA may serve as a biomarker of response. Studies went further to assess TMB from the ctDNA. In NSCLC, it was shown that blood TMB correlated with tissue TMB and was associated with ICI response [180]. CTCs have also been suggested as prognostic biomarkers. In NSCLC patients, blood sampled before and after treatment with Nivolumab showed that high levels of CTCs before treatment was associated with an increased risk of disease progression and death [181].

6.3.2 Soluble biomarkers

Some indicators such as neutrophil-to-lymphocyte ratio (NLR), lactate dehydrogenase (LDH), and various cytokines (IL-6 and IL-8) have been studied as biomarkers of response to ICI in a variety of tumors [182]. Neutrophils that express PD-L1

attenuate the antitumor response by binding to PD-1 T cells. Therefore, NLR has been suggested to have a predictive role for response to ICIs in melanoma and NSCLC. A study of melanoma patients treated with Ipilimumab demonstrated that patients with an NLR > 3 had a poor OS and PFS [183]. Similar results were shown in another study where an NLR >5 was also associated with a lower OS and PFS [184]. In advanced solid tumors, the OS of high NLR patients was 8.5 months, while the OS of patients with a low NLR was 19.4 months [185]. Changes in LDH during ICI treatment correlates with patient response. A study showed that patients who displayed an elevated baseline serum LDH value had a shorter OS at 12 months (44%) compared to patients with normal LDH values (71%). Moreover, a 10% increase from the baseline level during ICI treatment also indicated poor ICI efficacy [186]. Lower levels of the cytokine IL-6 at the baseline and on treatment have been correlated with improved response, while higher levels of IL-6 correlate with a shorter OS [187]. Additionally, in NSCLC and melanoma, it was reported that lower levels of IL-8 were associated with improved treatment responses, while higher baseline IL-8 levels were associated with poorer OS [188].

6.4 Biomarkers associated with the gut

Studies have suggested the association of the bacterial species in the gut with ICI responses. Bacterial species such as *Akkermansia muciniphila* have been observed and correlated with ICI response, whereas species such as *Ruminococcus obeum* have been correlated with resistance [189, 190]. The use of antibiotics prior to ICI treatment was also associated with a shorter overall survival and progression-free survival. As such, it has been suggested that careful consideration should be given when prescribing antibiotics in patients starting ICI treatment [190]. This is still an emerging field of study and further evidence is needed (**Table 4**).

Biomarker (Ref)	Method of detection	Indication
Biomarkers associated with the tumor genome		
TMB [146, 147]	WES and NGS gene panels on tissue and blood samples	High mutational burden correlates with high response rates and improved OS and PFS. Low TMB associated with primary resistance.
MMR-D and MSI [155]	WES on tissue samples	Somatic MMR-D and MSI correlates with high response rates. FDA approved genetic biomarker for patient selection.
IFN pathway profiles [160, 161]	Gene panels and transcriptome on tumor sample	Increased expression of IFN-γ correlated with improved OS and PFS. Mutations in the IFN pathway associated with poor outcomes and resistance.
Biomarkers associated with the tumor immune microenvironment		
PD-L1 [167]	IHC staining of tumor cells and immune cells	High PD-L1 density (> 50% expression) predicts improved response rates, OS, and PFS. FDA approved biomarker for patient selection.
TILs [172]	Anti-CD4 and anti-CD8 IHC staining on tissue samples	High CD4 and CD8 density or increase in density correlates with higher response rates. FOXP3 Tregs associated with poor survival.

Biomarker (Ref)	Method of detection	Indication
Biomarkers associated with the peripheral blood		
ctDNA and CTCs [179, 181]	FACS on blood sample	Detectable and persistent ctDNA correlates with poor response. High CTCs prior to treatment associated with disease progression and death.
Soluble biomarkers [183, 186]	IHC, FACS, and enzymatic assays	High NLR associated with poor response, OS, and PFS. Increase in LDH correlates with poor response.
Biomarkers associated with the gut		
Microbiota [189, 190]	Shotgun metagenomic analysis of feces	Distinct species profiles correlate with responses. *Ruminococcus* correlated with resistance.

Table 4.
Biomarkers associated with the tumor genome, tumor-immune microenvironment, peripheral blood, and gut that predict response to ICI.

7. Conclusions

The heterogeneity of tumors has introduced a profound complexity in our understanding of carcinogenesis and the numerous challenges in developing strategies for the treatment of cancer. The recent developments in immunotherapy enable us to devise interventions that promise to improve cancer therapy. Immune checkpoint inhibitors (ICIs) are recently developed drugs that promise to increase overall response. Our evaluation of ICIs shows that the PD-1-PD-L1/L2 pathway is the most targeted pathway. With PD-1 inhibitors in particular having been FDA-approved for the largest variety of cancers. PD-1 inhibitors have been found to have a good response in monotherapy but have recently been frequently tested as part of combinational therapy with other ICIs, such as CTLA-4 and LAG-3. This dual targeting of immune checkpoint proteins has resulted in some of the most promising outcomes. Despite these successes, there are challenges of serious adverse events and the development of resistance. The serious adverse events must be addressed because they are of Grade 3–4. Attempts to overcome them are in progress. Resistance occurs in a significant percentage of patients and therefore urgently needs to be addressed. The two main strategies targeting resistance are the use of combinational therapies and biomarker identification.

Acknowledgements

BR is funded by Buboo (Pty) Ltd., The Innovation Hub, Hatfield, Gauteng, Pretoria, 0200, South Africa.

STM is funded by the National Research Foundation of South Africa (GUN 121878) and the University of South Africa.

Conflict of interest

The authors declare no conflict of interest.

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