Biomarkers to Predict Response to Immune Checkpoint Inhibitor Therapy in Cancer

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Abstract-Traditional treatment modalities for advanced cancer act directly on tumors to inhibit growth or destroy them. Along with surgery, these modalities are predominantly palliative, though associated with toxicity and modest improvements in survival of patients with advanced solid tumors. To address these issues, novel immunotherapies targeting programmed death-1 (PD-1/PD-L1) and cytotoxic T lymphocyte antigen 4 (CTLA4) have been developed and approved by the FDA. These therapies have been proven to provide substantial benefit and success in advanced solid tumors of different types. However, these expensive checkpoint inhibitor therapies extend clinical benefits to only a small subset of patients. Hence, it is crucial to comprehend the determinants and the role of biomarkers that drive response, resistance, and adverse effects. In this review, we have elaborated on the role of various biomarkers both pre-treatment and post-treatment which assist in predicting response to immune checkpoint inhibitors in cancer treatment.

***Keywords*: Immune checkpoint inhibitors, PD1/PD-L1, CTLA4, biomarkers, immunotherapy, solid tumors, predictive biomarkers**

1. INTRODUCTION

1.1 Immune checkpoint inhibitors

Immune checkpoints are important immune system controllers. These pathways are important for self-tolerance because they prevent the immune system from attacking cells indiscriminately. However, some types of cancer possess the ability to protect themselves from attack by stimulation of the immune checkpoint targets (1).

Programmed death-1 (PD-1) is an immune checkpoint that is responsible for limiting excessive immune responses to antigens and thereby preventing autoimmunity (2). It is expressed on various immune cells, such as T lymphocytes. B lymphocytes, Natural killer T cells (NKT), activated monocytes, and dendritic cells (3). There are two ligands for PD-1: PD-L1 and PD-L2. Human activated T lymphocytes, dendritic cells, monocytes, and myeloid cells express PD-L1 (4). PD-L1 expression can be induced by type I and type II interferons. The interaction of PD-1 with its ligands also inhibits CD8+ T cell cytolytic effector functions. In addition to binding to PD-1, PD-L1 can bind to B7-1 on the surface of T cells and induce inhibitory signals in those cells (5). Numerous drugs that target PD-1 or PD-L1 are in various stages of clinical development. Nivolumab [OPDIVOTM, Bristol-Myers Squibb Company] and Pembrolizumab [KeytrudaTM, Merck & Co., Inc.] are humanized PD-1-blocking monoclonal antibodies (mAbs) that have already received approval from the U.S. Food and Drug Administration (FDA) and the European Medical Agency (EMA)(6). Both drugs have been registered for the treatment of patients with unresectable or advanced malignant melanoma (MM), non-small cell lung cancer (NSCLC) with progression on or after platinum-based chemotherapy, and recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN) with disease progression on or after platinum-based therapy.

CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) is also known as CD152 (cluster of differentiation 152). It is a protein receptor that acts as an immune checkpoint and down regulates immune responses. CTLA-4 is expressed on regulatory T cells and unregulated in conventional T cells after activation. It acts as an "off" switch when bound to CD80 or CD86

on the surface of antigen-presenting cells (dendritic cells). CTLA-4 is a member of the immunoglobulin superfamily that is expressed by activated T cells and also transmits an inhibitory signal to T cells. Similar to the T-cell co-stimulatory protein CD28, CTLA-4 binds to antigen-presenting cells' CD80 and CD86, also known as B7-1 and B7-2, respectively. CTLA-4 outcompetes CD28 for its ligands because it binds CD80 and CD86 with higher affinity and avidity. It is to be noted that T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4 [Figure 1].

Specifically, CTLA-4) primarily affects cellular proliferation and PD-1 signaling in T cells predominantly modifies cytokine production such as IFN- ɣ, TNF- α, and IL-2 (7). Nivolumab has also received approval for the treatment of advanced renal cell carcinoma (RCC) progressing after previous therapy and relapsed or progressive classical Hodgkin's lymphoma after autologous hematopoietic stem cell transplantation (HSCT). Pembrolizumab has recently received approval for the first-line treatment of patients with 50 percent or more PD-L1 expression (8).The FDA has also recently granted approval to atezolizumab [Tecentriq, Roche-Genentech], a mAb against PD-L1, for the treatment of patients with locally advanced or metastatic urothelial carcinoma who had disease progression during or following platinum-based chemotherapy, and for the treatment of patients with locally advanced or metastatic NSCLC (9, 10).who had disease progression during or following platinum-containing chemotherapy, and have progressed on an appropriate FDA-approved targeted-therapy if their tumor has EGFR or ALK gene abnormalities. Other drugs in this class, such as durvalumab [Astra-Zeneca] and avelumab [Merck KGaA-Pfizer], were granted breakthrough therapy designation by the US FDA for treatment of patients with PD-L1 positive urothelial bladder cancer and metastatic Merkel Cell Carcinoma, respectively. Effective patient selection tools have not accompanied this impressive development of anti-PD-1/PD-L1 inhibitors and their recent arrival in the clinic. The results of the major clinical studies reveal that despite the remarkable survival benefit obtained with checkpoint inhibition immunotherapy in certain populations, around 40–60% of patients will not benefit from these therapies. These therapies can be expensive and come with potential side effects as well. Thus, it is imperative to identify valid biomarkers of response that help us optimize patient selection. The complicated immune response against cancer involves many different components and is dynamic; making the development of biomarkers for immunotherapeutic more difficult than the development of biomarkers for targeted therapy This study examines both host immune system and tumor-related parameters to present the most recent data on biomarkers of responsiveness to PD-1/PD-L1 and CTLA-4 suppression.

Inhibitory checkpoint molecules are the major targets for cancer immunotherapy because of their impending use in multiple types of solid cancers. Presently, the checkpoint inhibitors that are approved are the ones that block CTLA4 and PD-1 and PD-L1. Immune checkpoints are the molecules that modulate the signals of the immune system by increasing or decreasing them, and they are known to be critical factors in treating infections, cancers, and autoimmune diseases. Immune checkpoint therapy is currently regarded as a cornerstone of cancer treatment. Immune checkpoints play an important role in immune regulation, and the blocking of immune checkpoints on the cell membrane is a potential strategy in the treatment of various types of cancer. Based on this, monoclonal antibodies are developing rapidly, such as those against PD-1 (programmed cell death protein 1). However, the cost involved in the preparation of monoclonal antibodies is too high and their therapeutic effect is still not fully understood. Among the different checkpoint therapies, those involving PD-1 are currently considered the most effective. The PD-1 pathway suppresses activated T cells at the late stage of an immune response, typically in peripheral tissues (11).

Immune checkpoint inhibitor (ICI) therapy is one of the types of cancer immunotherapy. This therapy targets immune checkpoints, which are the key regulators of the immune system and, when they are stimulated, can generate an immune response to an immunologic stimulus. Tumors protect themselves and escape the immune cells by stimulating the immune checkpoint targets. Immune checkpoint therapy can block inhibitory checkpoints, in order to restore immune system function (12). Ipilimumab-a CTLA4 blocker, is the first anti-cancer drug targeting an immune checkpoint, approved in the United States in 2011(13).

ICIs herald a new era in cancer therapy by increasing anti-tumor responses and providing significant survival advantages in multiple tumors(14) describing how anti-tumor responses are increased and significant survival advantages are provided in multiple tumors.PD1/PD-L1 therapies are approved for second-line or first-line treatment in a variety of malignant neoplasm, including melanoma, lung cancer, renal cell carcinoma (RCC), head and neck squamous cell carcinoma (HNSCC) and gastro-esophageal cancer.

However, despite the huge breakthroughs observed in clinical treatment with ICIs, only 30–40% of patients get benefit. Hence, it is crucial to comprehend the determinants that drive response, resistance, and adverse effects. During the past few years, it has been an area of potential research for scientists for the identification and development of predictive biomarkers for assessing response to ICIs. However, in recent years, large amounts of data and comprehensive understanding have been obtained, including new sets of data on tumor genome biomarkers, blood-based biomarkers, gremlin genetics, tumor microenvironment, and host-related factors. Advancements in the improvement of multiplex immune-histochemical technology, next generation sequencing, and a variety of combinational biomarker strategies have emerged during recent years in order to develop multi-factorial synergistic predictive biomarkers for ICIs. Development of a set of these predictive biomarkers will not only provide us a better understanding of the mechanisms of ICIs but also assist in disease management, in achieving decision-making in personalized anti-tumor immunotherapy, monitoring efficacy, tumor prognosis, guiding clinical trial design, as well as for deeper understanding of drug resistance mechanisms. A better knowledge of how these variables interact to affect tumor–host interactions is required to optimize the implementation of checkpoint inhibitor therapy(15).In this review, we summarize the current status of pre-treatment and post-treatment biomarkers and also focus on recently identified molecular and cellular determinants of response that may assist in predicting response to ICIs.

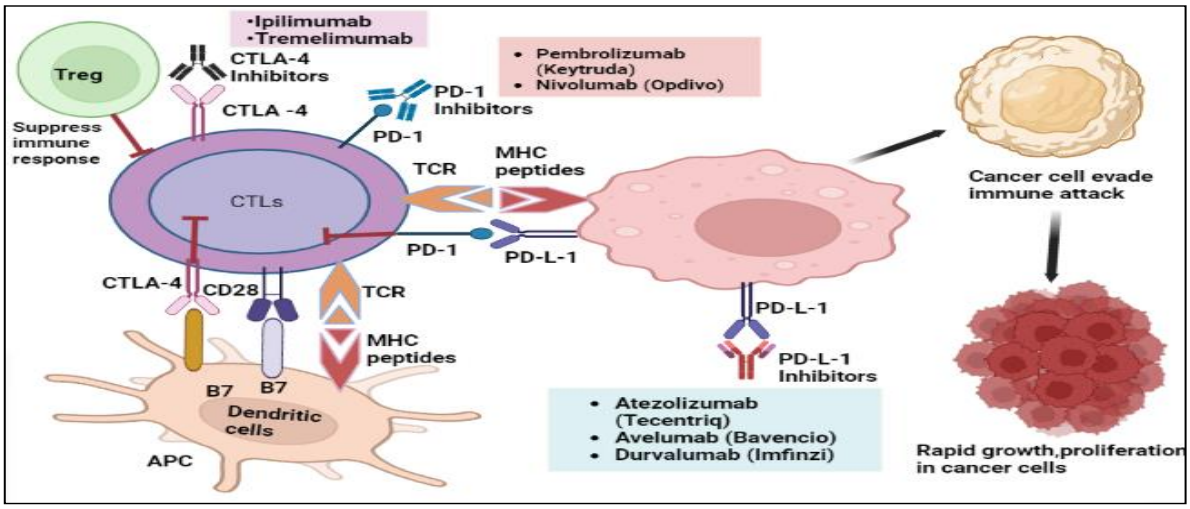

Figure 1. Immune checkpoint Inhibitors. Tregs have a suppressive effect on CD8-T cell activation or because of immunological checkpoints, cancer cells are able to evade immune attack, live, and proliferate. Antigen-presenting cells that produce B7 ligands connect with CD8-T cells' CD28 receptors to activate T cells and the immune system. As an alternative, T cells' activity is suppressed when B7 ligands attach to CTLA-4 expressed on them. Additionally, CTLA-4 boosts Treg activity, which has an immunosuppressive effect. T cells that have been stimulated express PD-1. When PD-1 binds to PD-L1, CTLs become anergic, which further encourages inhibitory signals. Monoclonal antibodies that pharmacologically inhibit immunological checkpoints restore CTL antitumor activity and alleviate immunosuppression.

1.2 Approved checkpoint inhibitors

CTLA4, PD-1, and PD-L1are currently approved checkpoint inhibitors across the world. PD-1 is known as the transmembrane programmed cell death 1 protein, which interacts with PD-L1 (PD-1 ligand 1, or CD274). Food and drug administration (FDA) has approved immune checkpoint inhibitors in various malignancies [Table 1]. PD-L1 on the cell surface binds to PD-1 on an immune cell surface, which inhibits the immune system from generating response (16). It is a known phenomenon that up regulation of PD-L1 on the cell surface inhibits T cells to attack the tumor and as a result tumor escapes immune response. Therefore, antibodies that bind to either PD-1 or PD-L1 block this interaction and therefore allow the T-cells to restore its function to attack the tumor. James P. Allison and Tasuku Honjo won Nobel Prize in the discoveries in basic science of checkpoint inhibitor therapies in 2018 (17).

S.No	Name	Brand Name	Marketing rights	Target	Approved	Indications (April 2021)
1	Ipilimumab	Yervoy	Bristol-Myers Squibb	CTLA-4	2011	metastatic melanoma, renal cell carcinoma, colorectal cancer, hepatocellular carcinoma, non-small cell lung cancer, malignant pleural mesothelioma
2	Nivolumab	Opdivo	Bristol-Myers Squibb (North America) + Ono Pharmaceutical (other countries)	PD-1	2014	metastatic melanoma, non-small cell lung cancer, renal cell carcinoma, Hodgkin's lymphoma, head and neck cancer, urothelial carcinoma, colorectal cancer, hepatocellular carcinoma, small cell lung cancer, esophageal carcinoma, malignant pleural mesothelioma
3	Pembrolizumab	Keytruda	Merck Sharp & Dohme	PD-1	2014	metastatic melanoma, non-small cell lung cancer, head and neck cancer, Hodgkin's lymphoma, urothelial carcinoma, gastric cancer, cervical cancer, hepatocellular carcinoma, Merkel cell carcinoma, renal cell carcinoma, small cell lung cancer, esophageal carcinoma, endometrial cancer, Squamous cell carcinoma
4	Atezolizumab	Tecentriq	Genentech/Roche	PD-L1	2016	bladder cancer, non-small cell lung cancer, breast cancer, small cell lung cancer, hepatocellular carcinoma, metastatic melanoma
5	Avelumab	Bavencio	Merck KGaA and Pfizer	PD-L1	2017	Merkel cell carcinoma, urothelial carcinoma, renal cell carcinoma
6	Durvalumab	Imfinzi	Medimmune/Astra Zeneeca	PD-L1	2017	non-small cell lung cancer, small cell lung cancer
7	Cemiplimab	Libtayo	Regeneron	PD-1	2018	squamous cell carcinoma, basal cell carcinoma, non-small cell lung cancer

Table1:- List of approved checkpoint inhibitors till 2021(FDA Approval history, 2021)

2. Predictive biomarkers to assess response to checkpoint inhibitor therapy

Tumor cells express antigens that are recognized by the immune system and hence trigger an immune response, which is known as immune surveillance. These antigens can be tumor specific or host specific, or antigens associated with the tumor that are also expressed on normal cells. There are multiple mechanisms by which the tumor escapes immune surveillance, such as loss of antigen presentation, loss of antigen expression, and inhibition of immune response through expression of molecules such as the immune checkpoint control modulators PD-1/PD-L1, which have immune suppressive effects (18). It has been proven that blocking the checkpoint pathways restores CD8 T cell function, promotes T cell responses and promotes tumor regression. Blockade of checkpoint pathways (like PD-1/PD-L1) enhances antitumor immune responses by decreasing the number and/or suppressive activity of regulatory T cells and by rescuing the activity of effector T cells in tissues and the tumor microenvironment, therefore, generating an immune response against tumors. Herein, we elaborate on the established research progress of predictive biomarkers that can be utilized for enhancing the efficacy of checkpoint inhibitor therapies in cancer [Figure 2].

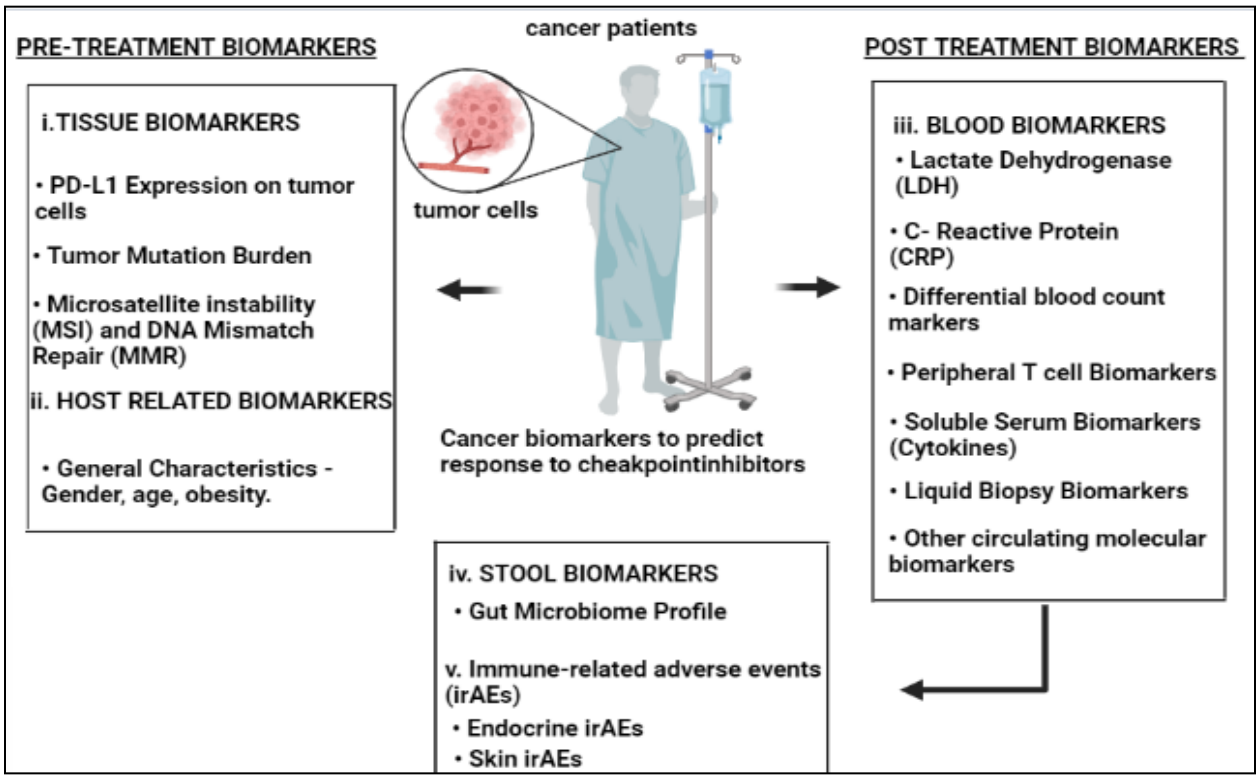

Figure 2. A comprehensive overview of predictive biomarkers of response to immune checkpoint inhibitors.

2.PRE-TREATMENT BIOMARKERS

2.1. TISSUE BIOMARKERS

2.1.1 PD-L1 Expression on tumor cells

Immunohistochemistry (IHC) detection of PD-L1 (B7-H1) is the most common and well-established clinical predictive biomarker for predicting response to checkpoint inhibitor therapy (12). Over expression of PD-L1 on tumor cells facilitates immune evasion by inhibiting cytotoxic T cell functions. Therefore, it is considered that over expression of PD-L1 on tumor correlates with a poor prognosis (19). Multiple studies in various cancer types have demonstrated a positive correlation between PD-L1 expression and response to ICIs, and now some studies have also used it in first-line combination therapy (20, 21). Pembrolizumab is presently approved by the FDA for NSCLC, in which PD-L1 expression $\geq 50\%$ of tumor cells in first-line treatment and $\geq 1\%$ in second-line treatment (22, 23). However, because PD-L1 expression on tumor cells may be the result of IFN- production by tumor infiltrating T cells, which are associated with responders, PD-L1 alone cannot always be a positive predictive marker in all cancers (24, 25). Some studies have reported that PD-L1 negative patients also benefit clinically with treatment with ICI or combination treatment with ICI (26). Therefore, PD-L1 is not yet a comprehensive and independent biomarker in clinical practice to assess the responses to ICIs, with the following challenges presently. Firstly, no standard definition of the cut-off range of PD-L1 expression across cancer types has been established. Several different cut-offs are considered. Secondly, PD-L1 expression on tumor cells and immune cells is a dynamic process due to which the evaluation at a particular time point (from formalin-fixed, paraffin-embedded tissue samples) is usually insufficient to predict response to ICI therapy (27). The predictive value of PD-L1 at different biopsy sites varied, which also created discrepancies in results (28). In addition to these, there are multiple assays for performing the PD-L1 test and antibodies are not standardized because of which the results are not always directly comparable (29). Currently, the tumor

proportion score (TPS) is used to calculate the PD-L1 positive score, which is based primarily on PD-L1 expression on tumor cells. But PD-L1 is also expressed on immune cells such as lymphocytes, macrophages, and stromal cells, and thus comes the concept of "combined positive score" (CPS), which is the proportion score of the sum of PD-L1 expressed by tumor cells and tumor-associated immune cells (30). In addition, PD-L1 expression on immune cells is also considered separately as one of the biomarkers to distinguish the population that is going to benefit, called the immune positive score (IPS). Finally, prior cancer therapies such as radiation, chemotherapy, and others may alter PD-L1 expression via tumor-infiltrating lymphocytes that secrete IFN- (31).

These limitations explain the diversity of results obtained from PD-L1 expression and also refer to a huge clinical need to develop a set of more sensitive and specific biomarkers that can predict response to ICI therapy. Nevertheless, over-expression of PD-L1 definitely assists in patient selection and approved biomarker prediction for a better response to checkpoint inhibition.

2.1.2 Tumor Mutation Burden

Tumor Mutation Burden is defined as the number of non-inherited mutations per million bases (Mb) of an investigated genomic sequence, and its measurement is performed by next-generation sequencing (32). Tumour mutational burden (TMB) is known as a genetic characteristic of tumor tissue and is relevant in cancer research and treatment. TMB has shown immense potential as a predictive biomarker with numerous applications, including patient response to immune checkpoint inhibitor (ICI) therapy in a variety of solid cancers. Tumor mutational burden (TMB) is a potential biomarker which is associated with response to immune checkpoint inhibitor therapies. It has been shown to differ distinctly among tumor types and also among patients within tumor types. Higher TMB is commonly observed in cancers associated with mutagens such as ultraviolet light exposure in melanoma and smoking in non–small-cell lung cancer (NSCLC) (33).

High TMB is associated with increased expression of tumor-specific neoantigens, a subset of which can be recognized by the immune system. Higher numbers of somatic mutations in tumor DNA have been hypothesized to increase the probability of the immune system recognizing and eliminating tumor cells during treatment with checkpoint inhibitor therapy. One of the main survival and escape mechanisms in tumors, among others, is to increase the expression of immune checkpoint molecules that can bind to tumor-specific T-cells and inactivate them, preventing tumor cells from being detected and killed (34).ICIs have the potential to improve patients' responses and survival rates by helping the immune system target tumor cells. Various studies on TMB have shown an association between the survival of patients and TMB values (32). Apart from being a predictive biomarker for response to therapy, TMB also assists in identifying individuals that can benefit from ICI therapy with cancers that generally have low TMB values. Furthermore, it has been shown that tumors with higher TMB values usually result in a higher number of neoantigens, the antigens that are presented on the tumor cell surface and are usually a result of missense mutations. So, TMB is considered a good predictor of neoantigen load and also helps in finding patients who may benefit from ICI therapy by enhancing the possibility of detecting the neoantigens. Before TMB may be utilised as a trustworthy biomarker, it is crucial to note that multiple sequencing platforms and bioinformatics pipelines have been used to estimate it. As a result, it is crucial to standardise TMB quantification methodologies and procedures. (35). There have been some efforts to standardize these methods. Significant correlations between TMB and patients' response to therapy have been proven in several cancer types, including small cell lung cancer (SCLC), NSCLC, melanoma, urothelial carcinoma, and human papilloma virus (HPV)-negative HNSCC. However, there are studies which suggest that TMB alone cannot clearly distinguish responders and predict non-responders to ICI therapy in cancer types, but as per approval given in April 2020 by the Food and Drug Administration (FDA), TMB can be used as a companion diagnostic biomarker for ICI therapy. TMB cut-off values differ depending on cancer type and assay platform (36).However, according to the NCCN and FDA guidelines Version 2.2021, a TMB score of 10 is considered TMB-high, and checkpoint inhibitor therapies are an option, such as ultraviolet light exposure in melanoma and smoking in non–small-cell lung cancer (NSCLC). High TMB is associated with increased expression of tumor-specific

neoantigens, a subset of which can be recognized by the immune system. Higher numbers of somatic mutations in tumor DNA have been hypothesized to increase the probability of the immune system recognizing and eliminating tumor cells during treatment with checkpoint inhibitor therapy. One of the main survival and escape mechanisms in tumors, among others, is to increase the expression of immune checkpoint molecules that can bind to tumor-specific T-cells and inactivate them, preventing tumor cells from being detected and killed (37).ICIs have the potential to improve patients' responses and survival rates by helping the immune system target tumor cells. Various studies on TMB have shown an association between the survival of patients and TMB values (32). Apart from being a predictive biomarker for response to therapy, TMB also assists in identifying individuals that can benefit from ICI therapy with cancers that generally have low TMB values. Furthermore, it has been shown that tumors with higher TMB values usually result in a higher number of neoantigens, the antigens that are presented on the tumor cell surface and are usually a result of missense mutations. So, TMB is considered a good predictor of neoantigen load and also helps in finding patients who may benefit from ICI therapy by enhancing the possibility of detecting the neoantigens. However, it is important to note that different sequencing platforms and bioinformatics pipelines have been used to estimate TMB and it is important to harmonize TMB quantification protocols and procedures before it can be used as a reliable biomarker (35). There have been some efforts to standardize these methods. Significant correlations between TMB and patients' response to therapy have been proven in several cancer types, including small cell lung cancer (SCLC), NSCLC, melanoma, urothelial carcinoma, and human papilloma virus (HPV)-negative HNSCC. However, there are studies which suggest that TMB alone cannot clearly distinguish responders and predict non-responders to ICI therapy in cancer types, but as per approval given in April 2020 by the Food and Drug Administration (FDA), TMB can be used as a companion diagnostic biomarker for ICI therapy. TMB cut-off values differ depending on cancer type and assay platform (36).However, according to the NCCN and FDA guidelines Version 2.2021, a TMB score of 10 is considered TMB-high, and checkpoint inhibitor therapies are an option.

TMB cannot predict therapy response alone due to the complexity of tumor-immune interactions and tumour heterogeneity, and its clinical applicability is limited due to the difficulty in obtaining tissue samples and the high cost of the test involved when compared to other tests.

2.1.3 Microsatellite instability (MSI) and DNA Mismatch Repair (MMR)

Microsatellites are repeated sequences of DNA that are made up of repeating units of one to six base pairs in length. The length of these microsatellites is extremely variable from person to person and contributes to the individual's DNA "fingerprint," so each individual has microsatellites of a particular length. The condition of genetic hypermutability resulting from impaired DNA mismatch repair (MMR) is called microsatellite instability (MSI) (38). The presence of MSI shows phenotypic evidence that MMR is not functioning normally. The role of MMR is to correct the errors that spontaneously occur during the process of DNA replication, such as single-base mismatches or short insertions and deletions. DNA polymerase errors are corrected by the proteins involved in MMR by inserting the appropriate sequence in their place. Cells with abnormal MMR function are unable to correct errors that occur during DNA replication and, as a result, accumulate errors. This results in the formation of novel microsatellite fragments. Polymerase chain reaction-based assays can disclose these novel microsatellites and provide evidence for the presence of MSI (39).

Microsatellite instability is connected with colon cancer, gastric cancer, endometrium cancer, ovarian cancer, hepatobiliary tract cancer, urinary tract cancer, brain cancer, and skin cancers. MSI is most prevalent in colon cancers. There are over 500,000 colon cancer cases worldwide each year. Based on findings from over 7,000 patients stratified for MSI-High (MSI-H), MSI-Low (MSI-L), or Microsatellite Stable (MSS) colon cancers, those with MSI-H tumors had a more positive prognosis by 15% compared to MSI-L or MSS tumors. Lynch syndrome is associated with MSI-H tumors, but MSI-H can also occur in patients without Lynch syndrome, and confirmation of Lynch syndrome requires testing of germline DNA.

MMR is a key DNA repair mechanism for identifying and repairing erroneous deletions and insertions of bases that might occur during DNA replication and

recombination (40). MMR deficiency is a positive predictive biomarker for response to ICI in colorectal cancer (41). These findings are indicative of the greater number of mutations that are unresolved by MMR, which would make the tumor more immunogenic. In a recent publication, it has been shown that MMR/MSI markers will guide treatment decisions for ICI in multiple tumor types (42). However, cases with MSS and intact MMR tumors have also shown favorable responses to ICI, making it an indefinite biomarker and further studies are required for its real predictive value. In May 2017, two immune checkpoint inhibitors for PD1 and PD-L1, which are pembrolizumab (Keytruda) and nivolumab (Opdivo), got approval by the Food and Drug Administration (FDA) for patients with metastatic CRC with MMR-D or MSI-H, denoting significant survival benefit. This finding is considered independent of PD-L1 expression assessment, tissue type, and tumor location. According to NCCN guidelines, MSI-H must be 40% unstable, MSI-L 20% unstable, and MSS 5% stable. All other markers are stable. Studies have shown a sustained clinical response to immune checkpoint inhibitors with remarkable clinical improvement in patients with MSI-H or MMR in solid cancers. Additionally, disease progression after an initial positive response to ICIs indicates acquired resistance mechanisms. A paradigm shift in cancer diagnosis and treatment strategies based on next-generation sequencing is generation sequencing is currently under way.

The approval of anti-PD-1 therapy for the treatment of MSI-H/dMMR tumors has marked the first step towards revolutionizing cancer treatment strategies including checkpoint inhibitor therapy. MSI status is currently considered as a sensible surrogate marker for predicting immunotherapeutic response; however, further studies are needed to investigate more precise biomarkers which will significantly advance precision cancer medicine.

2.1.4 Tumor Infiltrating Lymphocytes (TIL)

Tumor immune infiltration is classified as immune-inflamed, immune-excluded, and immune-desert (43). Inflammation is described by the presence of CD8+ and CD4+ T cells in the tumor microenvironment and also by the expression of immune checkpoint molecules (44) , which indicates a potential anti-tumor immune response to ICIs treatment (45,46) found that immune-excluded tumours have different immune cell types in the aggressive margin but cannot infiltrate into the tumour parenchyma, whereas immune-desert tumours have an absence of abundant T cells in the tumour parenchyma or stroma and a poor response to ICI-treatment (45). The presence of TILs in different tumor types and stages has been shown to have remarkable prognostic potential. A high abundance of CD8+ T cells at the invasive margin as well as tumor environment have been observed in responders. Recently, the immunoscore concept (47) was established on the basis of the tumor microenvironment (TME), which differentiates between responders and non-responders based on the density of two lymphocyte populations (CD8+ and CD45RO+ memory T cells). In studies done on colorectal cancer, multivariate analysis and immunoscore significantly showed relevance in predicting ICI efficacy and survival (48). The value of immunoscore for predicting ICI efficacy is also being validated internationally in clinical trials of melanoma and NSCLC (47). A deeper assessment of active immune responses within the TME by immune geneexpression profiling is essential to predict the clinical benefit of ICIs therapies. Expression of cytotoxic T cell markers such as CD8A, perforin 1, granzyme B; Th1 cytokines, chemokines, and other immune-related genes (NGK7, IDO1) in tumor microenvironment of tumor biopsies was remarkably different in subsets of responders and non-responders, making them a potential candidate to be established as a biomarker. (49). In a study by more than 299 immune-related genes were compared in patients with recurrent breast cancer 1–5 years post treatment and those without recurrence post 7 years and later, and they found that five genes (IGK, GBP1, STAT1, IGLL5, and OCLN) were highly overexpressed in patients with recurrence-free survival. In addition to this, IFN-γ-induced immune gene signatures may be effective biomarkers for predicting the clinical benefit of treatment with ICIs (50). Based on the receiver operating characteristic curve (ROC curve), optimised cut-off values for IFN-scores can achieve a positive predictive value of 59% for responders and a negative predictive value of 90% for non-responders (51).

2.1.5 Host Germline Genetics

Pathogens are the strongest selective forces in human evolution, and the continuous interaction between

humans and microorganisms leads to a huge amount of immunologically associated gene variation found in humans. One of the major mechanisms of immune escape is dysfunction in the antigen presentation pathway, which in-turn promotes tumor progression. For example, tumors down regulate HLA-I expression by acquiring damaging mutations in HLA-I genes or harboring loss of heterozygosity (LOH) of HLA-I genes, wherein the HLA-I halotype is somatically lost (52). Some patients with germline heterozygous HLA-I loci can harbor somatic LOH in their tumors which is associated with a reduced response to ICI therapy. Immune gene variation also impacts the efficacy of ICI therapy. The HLA genes in the human genome encode the key components of immune genicity and are known as the most polymorphic genes. HLA class I (HLA-I) diversity is characterized by a remarkable sequence variation in the peptide binding region (53). Studies have found that the more diverse array of HLA-I molecules was associated with good response and survival to ICI (54). It is possibly due to the broader presentation of tumor antigens to the T cells. Additionally, the association of HLA-I heterozygosity with extended survival was increased when correlated with the TMB (55). Patients treated with ICI therapy who expressed hetereozygosity at HLA-I loci were able to undergo better clonal expansion of their TCR repertoires. Additionally, specific HLA-I super types (HLAB44) are associated with survival after ICI therapy (56).

The present findings indicate that small differences in the number of available HLA-I molecules influence the strength of anti-tumor T cell responses after ICI.

2.1.6 HOST RELATED BIOMARKERS

General Characteristics

Several studies have shown that immune responses can also be gender-specific. A meta-analysis of a large number of melanoma and NSCLC patients has reported that gender differences in efficacy of ICIs were significantly higher in males as compared to females. (57). A significant correlation has also been observed between age and immune response. Aging is associated with a decline in immune response and has significant effects on both innate and adaptive immune responses (58). However, several studies done in melanoma patients have reported a significantly higher tumor response in patients over 60 as compared to lower-aged patients treated with ICI therapy (59). Obesity and inadequate fat distribution in the body have also been shown to affect tumor prognosis and response to ICI. It has been shown that the phenomenon of T cell exhaustion is promoted by obesity, which leads to immune ageing and also promotes tumor growth (60).

Presently, there is no substantial evidence to support the mechanisms by which general characteristics at baseline level influence the efficacy of ICI therapy. However, this could be used for patient selection and stratification in the future by further studies.

POST TREATMENT BIOMARKERS

2. 2 BLOOD BIOMARKERS

Peripheral blood is a non-invasive method to explore potential biomarkers to predict response to ICI. In several studies, a substantial association with clinical benefit and response has been observed and validated.

2.2.1 Lactate Dehydrogenase (LDH)

LDH is a housekeeping enzyme that is released by metastatic tumors. Therefore, serum LDH correlated positively with tumor mutation burden. Most studies have observed no correlation between baseline values of LDH and response. Nevertheless, dynamic changes in LDH from baseline to week 12 were observed to be correlated with response in several studies (61). Hence, elevated serum LDH may be one of the prognostic biomarkers for exclusion of patients from ICI treatment.

2.2.2 C- Reactive Protein (CRP)

Acute-phase protein C reactive protein (CRP) correlates favourably with TMB. It is a prognostic biomarker in cases of melanoma and its elevated serum concentrations are associated with no response to ICI (62). In malignancies such as gastrointestinal, renal, pancreas, bladder, and hepatocellular cancer, CRP has been shown to affect the prognosis. In some studies, elevated CRP levels are shown to be associated with poor response in NSCLC (63).

2.2.3 Differential blood count markers

Since ICI therapy works by activating the T lymphocytes of the host, the number of lymphocytes and other circulating immune cells has been shown to affect its efficacy. Increased counts of neutrophils are found in the peripheral blood of cancer patients and

have been correlated with worse overall survival and no response to ICI therapy in melanoma patients (64). An increase in the count of lymphocytes is correlated with a response to ICI therapy (65). It has been reported in several studies that neutrophil-to-lymphocyte ratio (NLR) is also used as a prognostic biomarker for predicting response to therapy (66). High baseline NLR is associated with poor response to ICI therapy in the case of melanoma, NSCLC, and RCC (67). NLR is considered to be a good prognostic biomarker, but it is not treatment specific and, alone, cannot be used as a predictive biomarker. Some studies have found that a high eosinophil count correlates with response to ICI therapy. Myeloid-derived suppressor cells (MDSCs) also play an important role in melanoma and other malignant tumors. Immunosuppressive, particularly for T cells, MDSCs and granulocytic and monocytic (mo-MDSCs) are immunosuppressive. A higher number of mo-MDSCs was negatively correlated with response to ICI in melanoma patients (68). All these differential blood count biomarkers have the potential to be used as predictive markers for ICI, but further studies are required to investigate their predictive cut-off values in different malignancies.

2.2.4 Peripheral T cell Biomarkers

Peripheral blood analysis provides us with deep knowledge regarding the immune responses that are induced by blocking the PD-1 pathway. T cells are known as the effector cells of ICI treatment. Therefore, a detailed analysis of the T cells and their subsets in the peripheral blood can be beneficial and serve as a potential biomarker for ICI. Several studies have shown that the increase in the cytotoxic CD8 T cells post ICI therapy, as compared to the baseline, has been shown to be responders to therapy (69). Proliferation of the PD-1+ CD8 T cells in the peripheral blood of lung cancer patients has also been shown to correlate with response to ICI therapy. CyTOF analysis performed on melanoma patients in several studies has demonstrated the increase in natural killer cells and their subsets post-therapy as compared to the baseline level (before the initiation of therapy) and can therefore serve as potential biomarkers for ICI treatment after validation in a larger cohort of patients with various malignant conditions (70). Additionally, high levels of circulating T regulatory cells at baseline level were also associated with responders to therapy. T cell co-stimulatory markers such as Inducible T cell Co-stimulator (ICOS), which is expressed by activated T cells and Tregs, have also been shown to be enhanced post-ICI in responders, clearly showing the activation of T cells post-therapy, which is required (71). Apart from these, studies have reported and correlated the presence of circulating tumor cells (CTCs) in the peripheral blood with the metastatic process in tumors, and PD-L1 is a highly expressed ion in CTCs from patients with advanced head and neck cancer, which shows that PD-L1^{+} CTCs may be considered as a predictive biomarker of response to ICI (72).

To summarize, T cells, being the effector cells of ICI, are the current focus of biomarker research in the field of advanced malignant conditions eligible to receive ICI therapy. The approaches towards it appear to be promising, but no biomarkers have yet been established to be used in clinical practice, and hence larger studies are required for it to be validated.

2.2.5 Soluble Serum Biomarkers (Cytokines)

The relationship between inflammatory cells, cancer and pro-inflammatory proteins such as chemokines (which regulate tumor growth and angiogenesis) are well known in the field of cancer. These inflammatory chemokines are also involved in metastasis (73). Soluble serum biomarkers, which include immune regulatory molecules such as cytokines and soluble checkpoint receptors with their binding partners, also correlate with the clinical benefit of ICI treatment. Interferons (IFNs) are cytokines that activate the immune cells and increase MHC-I expression on cancer cells, thereby improving cytotoxic CD8+ T cell recognition and tumor cell destruction. This interaction assists in the use of IFN gene signatures for selecting the appropriate ICI therapy in various malignant conditions (74). These cytokines are produced and act on both cancer cells and immune cells. Some studies have shown serum IFN-, IL-6, and IL-10 levels were significantly higher in non-responders as compared to responders (75). Recently, in a study, it was shown that mutations in the IFN receptor signalling pathway are responsible for acquired resistance to anti-PD1 therapy in melanoma (76). In another study, resistance to CTLA-4 inhibition was demonstrated by genomic alterations in the IFN-ɣ pathway genes. These findings support the relevance of tumour genomic data about IFN-related genes as a

predictor of response as well as patient selection for ICI therapy (77).Hence, tumor genetic signatures of IFN-ɣ, may be used in the predictive model of response to ICI therapy in the near future.

2.2.6 Liquid Biopsy Biomarkers

Biomarkers of circulating tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) is found in the blood of the human body and is defined as the DNA that arises from cancerous cells and tumors. Most DNA is inside a cell's nucleus. Cell death occurs and they are replaced by new cells when the tumor progresses. The DNA of broken cells with their contents is thereafter released into the bloodstream. ctDNA basically comprises small fragments of DNA which usually comprise fewer than 200 nucleotides in length. The genomic information related to the response to ICIs can be obtained from ctDNA. Numerous studies have found that a high number of ctDNA mutations are associated with improved overall survival and a poor response in cancer patients treated with ICI (78). ctDNA can also be a useful marker for the identification of pseudoprogression during ICI therapy. The association of TMB based on ctDNA levels and clinical benefit was also validated in tumor patients, confirming it to be a promising predictive biomarker. An association was also observed between high hypermutated ctDNA levels and non-responders to therapy for diverse malignancies treated by ICI (79). To summarize, dynamic monitoring of ctDNA can predict response to ICI therapy during the course of the treatment process in a non-invasive manner, thereby improving the sensitivity and specificity of predicting response.

2.2.7 Other circulating molecular biomarkers

Exosomes are single-membrane organelles that are secreted by many types of cells, including cancer cells and immune cells. The main molecular components of exosomes are cell-derived proteins; lipids, glycoconjugates, and nucleic acids (80). Exosomes show a variety of activities, such as remodelling the extracellular matrix (ECM) as well as mediating the intercellular transmission of signals and molecules. There have been multiple studies demonstrating the variety of roles of exosomes in cancer progression as well as suppression. As cell-derived nanovesicles, exosomes have potential uses in ICI because of their immunogenicity and molecular transfer functions (81). Moreover, some new studies suggest that tumor cell-derived exosome DNA (ExoDNA) activates the immune cells and can act as a key regulator of checkpoint immunotherapy as well as regulate tumor immunity (82). In ongoing clinical trials, exosomes are considered immunotherapeutic vaccines, markers of cancer diagnosis, prognosis, recurrence, and metastasis, or drug delivery carriers for cancer treatment. Plasma exosomes can also provide relevant information about the tumor and ICI therapy.

2.3 STOOL BIOMARKERS

2.3.1 Gut Microbiome Profile

As shown in several studies, the microbiota profile also plays a significant role in stimulating and inhibiting the immune response (83). The gut microbiome is significantly associated with improved responses to ICI therapy in several cancers such as melanoma, NSCLC, RCC, and urothelial carcinoma (84, 85, 86). It has been reported that commensal bifidobacteria enhanced PD-1 anti-PD1 antibody response by enhancing the function of dendritic cells. Also, baseline microbiota enriched with Faecalibacterium species and other fermicutes generated a better response in patients treated with ICI as compared to microbiota enriched with bacteroides. A significant correlation was also observed between the response to ICI and microbiota enriched with Akkermansia muciniphilia. Another analysis found that the enrichment of bacteroides caccae in all ICI responders, and specifically Faecalibacterium prausnitzii, Bacteroides thetaiotaomicron and Holdemania filiformis when treated with anti-PD1 therapy (87). Collinsella aerofaciens, Enterococcus faecium, and Bifidobacterium longum relative abundance has also been linked to melanoma responders. All the studies reported that an imbalance in gut microbiota is associated with immune dysfunction in non-responders. It is to be noted that the efficacy of ICI as per microbiota profiling is associated with geographical location, antibiotic treatment, different cancer types, dietary habits, and microbial sequencing technique. The present studies indicate a relevant association between response and microbiome profile. However, further prospective studies are required to establish gut microbiota as a predictive biomarker to be used across different cancer types.

2.4 Immune-related adverse events (irAEs)

The various spectrums of side effects caused by the ICI therapy are known as irAEs. irAEs affect almost every organ of the body, including the skin, gastrointestinal tract, lung, endocrine, musculoskeletal, and various other systems. Different types of irAEs are associated with different tumor types. ICIs cause tumor regression and irAEs through enhanced immune response. Several studies have shown a relevant association between these two. In a multivariate analysis, it was shown that low grade irAEs were associated with better response to ICI therapy in non-melanoma patients and that early development of overall irAEs was associated with better survival in NSCLC patients receiving ICI therapy (88). In addition to this, an association was also observed between endocrine irAEs and vitiligo and a better prognosis in melanoma patients; thyroid dysfunction was associated with a better response in NSCLC patients receiving ICI therapy (2). Additional studies are required to confirm certain irAEs' ability to be used as a prognostic biomarker to predict response to ICI.

3. SUMMARY

To conclude, we are yet to establish a predictive model for ICIs efficacy. The current mechanisms and understanding of how to assess the clinical response to ICI therapy are unambiguously indicative of the fact that there cannot be a single biomarker to predict the response to this therapy. Since these therapies are highly expensive and effective only for 30–40% of total patients, it is imperative to develop biomarkers that can predict and assess response to therapy. Therefore, the development of a comprehensive predictive biomarker model that takes different components into consideration is primarily essential for utilizing ICI therapy to its full strength. Importantly, this type of predictive model will provide a one-of-a-kind opportunity to assess confounding factors and the individual contributions of each of these factors to the response to ICIs. Comprehensive predictive models will need a permutation of different types of data sets for training and constant evaluation. These variables include DNA sequencing data for calculation of TMB, genetic alterations, RNA sequencing data to evaluate whether the immune phenotype will favor sensitivity to ICIs, germline DNA sequencing data for HLA diversity, IHC for PDL1 expression, TME, commensal microbiota, and expression of other checkpoint molecules. Furthermore, as more knowledge about the molecular determinants of response to ICIs becomes available, these predictive models will require a continuous process of model update and re-evaluation. For precision immuno-oncology, such biomarker models for response to ICIs will have profound implications in the area of checkpoint inhibitors. Ultimately, clinical use will be governed not just by the science but also by feasibility and reproducibility in the "real world" clinical setting, as well as cost and investment to establish prospective validation. The ongoing, intensive work to establish and understand biomarkers for ICI response prediction holds great promise for maximizing patient benefit from these transformative therapies.

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