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PD-1/PD-L1 AXIS INHIBITORS AS PROMISING STRATEGY FOR MANAGEMENT OF HEPATOCELLULAR CARCINOMA: EXPECTATIONS, BOUNDARIES AND PITFALLS.

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ABSTRACT:
Hepatocellular carcinoma (HCC), the most prevalent primary liver cancer and a leading cause of cancer-related deaths worldwide, has prompted exploration into innovative treatment avenues. Notably, cancer immunotherapy has emerged as a promising strategy, with immune checkpoint inhibitors, specifically targeting the programmed cell death 1 (PD-1) and its ligand (PD-L1), revolutionizing cancer care. PD-1 is a crucial protein in suppressing immune responses and promoting self-tolerance by regulating T-cell function. The PD-1/PD-L1 axis is responsible for immune evasion in cancer cells making it a focal point in cancer therapy. However, despite the potential of PD-1/PD-L1 inhibitors, their clinical utility is hampered by significant immune-related adverse effects. This underscores the urgency to develop novel inhibitors, including small molecules and peptides that target the PD-1/PD-L1 axis to better meet clinical demands. This review focuses on elucidating the biological mechanisms of PD-1/PD-L1 immune checkpoints and their role in both the healthy immune system and the tumor microenvironment. Limitations to this treatment approach include low response rates in certain cancers, immune-related toxicity, and the development of drug resistance. Overcoming these limitations is crucial to expand the use of PD-1/PD-L1 blockade in cancer treatment and improve response rates and survival times for cancer patients.

1. INTRODUCTION

Liver cancer continues to be a global health challenge. Based on estimates of GLOBOCAN 2020, primary liver cancer is the sixth most diagnosed cancer and the third leading cause of cancer death worldwide in 2020 with approximately 906,000 new cases and 830,000 deaths. Rates of both incidence and mortality are 2 to 3 times higher among men than among women in most regions, and liver cancer ranks fifth in terms of global incidence and second in terms of mortality for men. Primary liver cancer includes hepatocellular carcinoma (HCC) (comprising 75%-85% of cases) (Sung et al., 2021). In Egypt, it is considered to be the fourth most commonly occurring cancer (Rashed et al., 2020). Because of the low resectability rate, the high recurrence rate after resection, and poor response to the conservative treatment, the prognosis of HCC is poor with a 5-year survival rate of 6.9% (Yapali & Tozun, 2018). The detection of HCC at an early stage through surveillance and curative therapy has noticeably expanded the 5-year survival. Hence, medical societies encourage systematic screening and surveillance of target populations at high risk for developing HCC to help its detection at early-stage (Tang et al., 2018).

Although, chronic viral hepatitis as chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are considered the most common risk factors for HCC development, here are other many etiologies and risk factors that can contribute in HCC development as; alcohol consumption, tobacco smoke where tobacco was associated with liver disease and an increased risk of mortality was associated with alcohol consumption (Suresh et al., 2020). In addition, diabetic patients show a higher risk (2.5-fold) of developing HCC, independent of alcohol consumption and co-infection with HBV or HCV. Nonalcoholic fatty liver disease (NAFLD) is present in about 70% of the people with diabetes mellitus, and 90% of the people with obesity (Younossi & Henry, 2021). NAFLD can progress to nonalcoholic steatohepatitis (NASH), cirrhosis, and HCC, due to the inflammation caused by fat accumulation in the liver. Another risk factor is the ingestion and exposure to fungal aflatoxins, principally aflatoxin B1 (Chidambaranathan-Reghupaty et al,2021)

Various strategies have been authorized and put into practice for the management of Hepatocellular Carcinoma (HCC), tailored to the specific stage of HCC advancement. Hepatic resection and liver transplantation have been established as the primary curative measures for cases of HCC. Refinements in the selection of suitable patients have led to improved outcomes in surgical resection and noteworthy long-term survival rates, with some cases even extending beyond a decade, particularly for liver transplantation when tumors are down staged beyond the Milan criteria (Tong et al., 2021).

Local ablation using radiofrequency remains the foundational approach for early-stage HCC treatment, guided by medical imaging, despite progress made in alternative techniques (Balogh et al., 2016). For intermediate-stage HCC, treatments involving loco-regional devices or radiation, such as transarterial chemoembolization (TACE) and transarterial radioembolization (TARE), have demonstrated their effectiveness through Phase II investigations. (Llovet et al., 2021).

Currently, systemic treatments such as tyrosine kinase inhibitors (TKIs), monoclonal antibodies and immune-checkpoint inhibitors (ICIs) have introduced a new dimension to the treatment landscape of HCC, challenging the traditional therapeutic approaches. It is estimated that around 50–60% of HCC patients will have encountered systemic therapies during their lifetime, especially in the more advanced disease stages. Over the last five years, significant strides have been made in the realm of systemic therapies, with research revealing a notable enhancement in both overall survival rates and the quality of life for patients (Ruf et al.,2021). Sorafenib (Nexavar, Bayer) is an oral multitargeted tyrosine kinase inhibitor that exhibits activity against many kinases such as RAS/RAF kinase affecting cellular proliferation and vascular endothelial growth factor receptor (VEGFR) affecting angiogenesis (Morse et al., 2019). Adverse events with this drug are common and sometimes are difficult to tolerate, therefore, dose reduction is often needed (Ayala-Aguilera et al., 2021). Moreover, the development of resistance after 1 or 2 years is almost a rule in most patients who showed partial response or stabilization of the disease while on sorafenib. Currently, there is also a rescue therapy available, regorafenib (Stivarga, Bayer), which has shown improvement in survival among patients who progressed while on sorafenib therapy (Heo & Syed, 2018)

The emerging Cancer immunotherapy of HCC

Physiologically, the liver is exposed to several antigens either food-borne or microbe coming from the gut. T cell-immunity of cancer immunotherapy consists of three steps. First, antigens are presented by antigen-presenting cells (APCs) such as dendritic cells (DCs) as antigenic peptides, which are recognized by the T-cell receptor (TCR; Signal 1) (Jan et al., 2021). The secondary signal is then delivered when B7 proteins (CD80 and CD86) on the APCs engage with CD28 on the T cells, leading to the activation of T cells (Richardson et al., 2020). Subsequently, the activated cancer-specific T cells enter into the tumor sites and recognize tumor-specific antigens thereby destroying the cancer cells as shown in figure 1 (Liu et al., 2021).
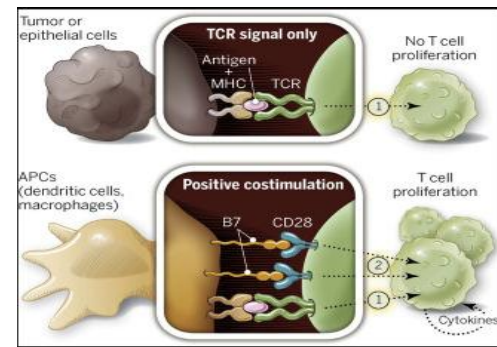
Figure (1): Costimulatory proteins on antigen-presenting cells for T Cells activation pathways (Sharma & Allison, 2015).

The immune mechanism of the body has a critical role in the inhibition or initiation of cancer. However, tumors produce local and systemic immune-suppressive milieu, so they can escape immune surveillance. "Immune escape" has been recognized as one of the emerging hallmarks of cancer in recent years and describes the actions of cancer cells to avoid attack and elimination by the immune system. Immune checkpoint molecules, serving as regulators of immune response to maintain immune tolerance to self-antigens, are often hijacked by cancer cells to achieve immune escape (Schaller et al., 2020).

Immune checkpoints are molecules that act as gatekeepers of immune responses. Under normal circumstances, they allow the immune system to react against infection and malignancy while protecting tissues from any harm that may arise from this action. The processes of maturation, activation, and expansion of T lymphocytes, and inhibition of their apoptosis are elicited by **stimulatory checkpoints** and their ligands while an opposite effect is supported by **inhibitory checkpoints** with their ligands. The most studied checkpoints are the inhibitory pathways consisting of cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), a gene with very high homology to CD28 that starts with T cell activation. CTLA-4 binds B7 molecules, with much higher affinity and stops T cells in lymph nodes at the initial stage of naive T-cell activation (figure 2-a) (Jacquelot et al., 2021).

| (A) | (B) |
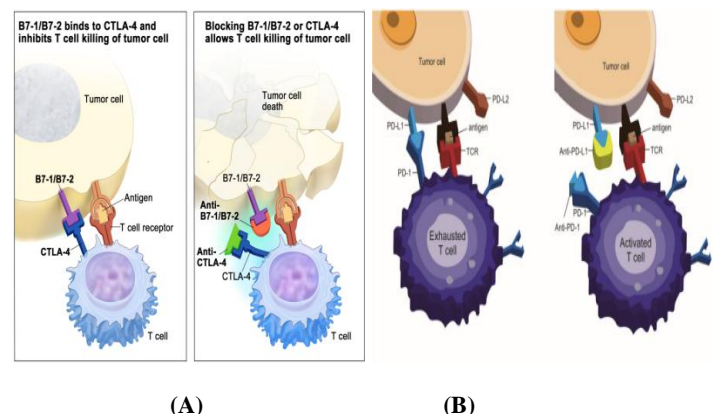
Figure (2): Killing tumor cells by T cells can be achieved by blocking the binding of immune checkpoints with immune checkpoint inhibitors (ICIs) (a) (anti-CTLA4) / (b) (anti-PD-L1 or anti-PD-1) (Sharma & Allison, 2015).

Programmed cell death protein 1 (PD-1, also known as CD279) is an immune checkpoint receptor mostly expressed on the surface of mature, activated T lymphocytes to prevent the T-cell receptor (TCR) signaling at a later stage. Stimulation of PD-1/PD-L1 signaling negatively regulates T cell-mediated immune responses in the peripheral tissues to inhibit effector T cell responses and guard tissues from immune-mediated tissue damage which is also known as peripheral T cell tolerance (Akinleye & Rasool, 2019). Therefore, PD-1 is not expressed on resting T cells but is inducibly expressed after activation by TCR/antigen-loaded MHC and CD28/B7 interactions. PD-1 is also expressed on natural killer cells, and B cells where they may be activated and proliferate in the presence of ligands (Shimizu et al., 2021). The programmed death-ligand 1 (PD-L1, also known as CD274 or B7-H1) and PD-L2 (CD273 also known as B7-DC) are its known ligands. The PD-L1 ligand is expressed on many cell types, including T-cells and APCs, and is up-regulated in response to proinflammatory cytokines such as interferon gamma (IFNγ) and interleukin-4 (IL4) through signal transducer and activator of transcription-1 (STAT1) and IFN regulatory factor-1 (IRF1). While, PD-L2 is expressed on macrophages and dendritic cells (Zhou et al., 2019)

The PD-L1/PD-1 interaction in the tumor microenvironment leads to T cell dysfunction, exhaustion, apoptosis (figure 2-b), and IL-10 amplification in a tumor mass generating a state of resistance from cytotoxic T cells (CD8+) mediated tumor cell killing (Raskov et al., 2021). It promotes the development and progression of cancer by enhancing tumor cell proliferation and survival. Recent studies suggest that tumors are highly infiltrated with regulatory T (Treg) cells which further suppress effector immune responses (Scott et al., 2021). PD-L1 is broadly overexpressed in many types of cancer cells, including melanoma, breast cancer, lung cancer, lymphoma, bladder cancer, glioblastoma, kidney cancer, and ovarian cancer, and is supposed to play a crucial role for cancer cells to escape from immune surveillance (Chen et al., 2020). Since the binding affinity of PD-1 with PD-L1 is 3 times greater than the affinity between PD-1 for PD-L2, and PD-L1 expressions in tumor cells are controlled by the stimulation of pro-inflammatory cytokines such as IFN-γ and TNF-α. Therefore, PD-L1 is responsible for tumor immune modulation. The structure of PD-1 comprises four parts: an immunoglobulin variable region (IgV), a transmembrane region, immunoreceptor tyrosine-based inhibitory motifs (ITIMs), an immunoreceptor tyrosine-based switch motifs (ITSMs) (Figure 3) (Jimbu et al., 2021). The interaction of PD-1 and PD-L1 stimulates phosphorylation of ITIMs and ITSMs to be in the intracellular domain of PD-1, thus leading to recruitment of tyrosine acid phosphatase Src homology phosphatase 1 (SHP-1) and Src homology phosphatase 2 (SHP-2) (Patsoukis et al., 2020). These phosphatases cause dephosphorylation of several key proteins in the T cell antigen receptor (TCR) signaling pathway and inhibit signaling pathways downstream of the TCR, such as the mammalian target of rapamycin (mTOR), (Ras/MAPK/ERK): rat sarcoma (RAS), (MAPK/MEK/(ERK) (Jiang et al., 2019), PI3K, protein kinase B (PKB/AKT)

(Patsoukis et al., 2020). In turn, it causes inhibition of related genes transcription, arresting the T cell cycle progression and the expression of related proteins, and eventually, it reduces the production of cytokines, the proliferation and differentiation of T cells, causing loss of their immune function (Wu et al., 2021).
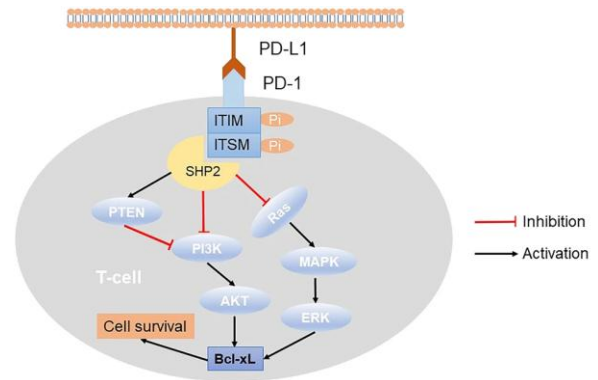

Figure (3): Immune checkpoint PD-1/PD-L1 signaling pathway (Wu et al., 2021)

The liver is distinguished by a strong intrinsic immune suppressive microenvironment performing a crucial role in host defense yet more in the maintenance of self-tolerance that is considered a major barrier to an effective antitumor activity prompted by immunotherapeutic interventions. Additionally, to the presence of cells with immune suppressive functions, the tumor microenvironment of HCC is characterized by a great expression of immune checkpoint molecules. High expression of PD-L1 in HCC has been largely observed on Kupffer cells likewise on tumor cells as well as on tumor-infiltrating lymphocytes. This is very often associated with high PD-1 expression on CD8+ T cells and is correlated with a higher risk of cancer recurrence or metastasis and cancer-related death (Oura et al., 2021)

The PD-L1/PD-1 axis blockade can reactivate the anergy T cells and hence has been considered the key strategy to counteract the immune resistance of cancer (Figure 2) (Chen et al., 2020). It has been studied that the visibility of tumor cells for the host immune system is regained by targeting either PD-1 or its receptors (PD-L1/PD-L2) by being blocked by monoclonal antibodies which leads to the suppression of the cancer cells (Jelinek et al., 2018). To this point, six monoclonal antibodies targeting the PD-L1/PD-1 axis were approved by the US Food and Drug Administration (FDA) and over 15 antibodies are in clinical development for treatments of patients with several cancers and have achieved great success in treating different types of cancers, particularly the advanced and refractory ones. Pembrolizumab (Keytruda®), nivolumab (Opdivo®), and cemiplimab (Libtayo) are three agents targeting PD-1, while atezolizumab (Tecentriq®), avelumab (Bavencio®), and durvalumab (Imfinzi®) block PD-L1 instead (Chen et al., 2020). Collectively, these agents are approved for the treatment of a wide variety of malignancies, comprising metastatic melanoma, head and neck squamous cell carcinoma, non-small-cell lung cancer (NSCLC), renal cell

carcinoma (RCC), Hodgkin's lymphoma, urothelial carcinoma, gastric carcinoma, and hepatocellular carcinomas. Yet, the durability of clinical benefits and long-term remissions by therapeutic antibodies have been limited to a small percentage of patients with specific types of cancer (Kooshkaki et al., 2020).

Some studies have revealed that it is impossible that macromolecular antibody drugs can efficiently penetrate tumor tissues reaching all the tumor regions and accumulating at a sufficient concentration. Additionally, the immunogenicity of antibody drugs will stimulate the body to generate anti-antibodies that cause the efficacy to be lost (Wu et al., 2021). Meanwhile, the immune system function is destroyed causing the immune tolerance to be imbalanced which then results in unsuppressed immune responses, which may be clinically considered to be autoimmune side effects that cause collateral damage to normal organ systems and tissues, comprising the liver, gastrointestinal tract, skin, lung, and endocrine system (Postow, 2015). In the dermatological safety analysis of melanoma patients, skin toxicity was noted in 34% of patients treated with nivolumab and 39% of patients treated with pembrolizumab (Weber et al., 2017). Additionally, it was shown in clinical follow-up data that not all patients are sensitive to monoclonal antibody therapy. In the treatment of melanoma, PD-1 antibodies gave a response rate of 50%. However, for other solid tumors the overall response rate was generally low, ~15%–20% (Ai et al., 2020). Monoclonal antibodies are also expensive, difficult to produce, store, and transport, which may restrict the clinical application of PD-1/PD-L1 antibody drugs (Wu et al., 2021)

Thus, blocking the interaction of PD-1/PD-L1 by small molecules may be a promising replacement to monoclonal antibodies in cancer treatment. As small-molecule inhibitors targeting the PD-1/PD-L1 signaling pathway are considered to have significant advantages compared to monoclonal antibodies. They can deal with these problems as they are more suitable for oral administration, have lower manufacturing costs, can lower the target residence time by controlling the half-life of the drug, thus can avoid serious immune-related adverse events to occur, can be easily transported and stored, have better stability, and superior membrane permeability (Wang et al., 2021). Moreover, small molecules are more capable to repress tumor growth and migration than antibodies and have favorable biosafety. They have regulated and controllable pharmacokinetic characteristics that allow them to overcome the existing problems of antibody drugs in a way that allows them to replace monoclonal antibodies or serve as complementary therapies. In addition, they show a better therapeutic index and allow for more flexible clinical dosing, as well as they favor acute and reversible action due to their shorter half-lives and they have fewer systemic side-effects. Additionally, they have a better tissue and tumor penetration that they can also stimulate intracellular pathways downstream of checkpoint proteins to overcome the drawbacks of mAbs (Chen et al., 2020; Wu et al., 2021)
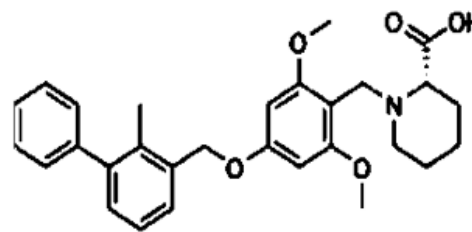
Figure 4: Chemical structure of PD-L1 small molecule inhibitor (BMS-1) (Wu et al., 2021).

Hence, small-molecule inhibitors offer an alternative treatment strategy either alone or complementary with therapeutic antibodies to reduce the chance of drug resistance and low clinical response. As of today, although a few patents and publications have revealed a series of small-molecule inhibitors targeting the PD-1/PD-L1 pathway (Qin et al., 2019), there are no FDA-approved small-molecule modulators for the PD-1/PD-L1.Three main hot spots on the interface of PD-L1/PD-1 were identified: the hydrophobic pocket, the six-membered aromatic ring, and the aliphatic hydrocarbon branch, respectively (Figure 4), which are considered to be the binding sites of small molecule drugs and to disrupt the PD-L1/PD-1 interactions, a large number of small molecules with different structures have been synthesized (Wang et al., 2021; Wu et al., 2021). CA-170, developed by Curis/Aurigene in 2015, has entered Phase I/II clinical trials for treatment of advanced solid tumors and lymphomas with promising results. (Li & Tian, 2019; Chen et al., 2020). Nonpeptide-based small molecule inhibitors have been developed by The BMS company. The structures of two groups of small molecule inhibitors that include a biphenyl core structure have been revealed: one structure is based on the 2-methyl phenyl methanol scaffold (such as found in BMS-200), and the other is based on the 2-methyl-3-biphenyl methanol scaffold (such as found in BMS-202) (Bailly & Vergoten, 2020).

The suggested mechanism of action of such small molecule compounds is that they work on the surface of the PD-L1 protein to stimulate the dimerization of PD-L1, while BMS-202 is bound to two dimerized PD-L1 cylinders in the shape of a hydrophobic cavity. The surface of the PD-L1/PD-L1 interaction after dimerization is highly similar to that of the PD-1/ PD-L1 interaction, resulting in the inability of PD-1 and PD-L1 to undergo normal interactions, which eventually blocks the signaling pathway (Wu et al., 2021). Another suggested mechanism of action: It has been reported that most of the immune-related receptors and ligands, including PD-1 and PD-L1, are extensively glycosylated. Four N-glycosylation sites have been recognized at the extracellular domain of PD-L. The glycosylation is necessary for its stability and its engagement with PD-1, the small-molecule PD-L1 inhibitor BMS1166 effectively blocked PD-L1 exporting from endoplasmic reticulum (ER) and further glycosylation, leading to its failure to interact with PD-1 to activate its signaling (Chen et al., 2020).

Although PD-1/PD-L1 checkpoint blockade can result in impressive therapeutic responses, many patients can only be partial responders to therapy. Patients not responding to initial therapy with PD-1/PD-L1 blockade are referred to as having "primary resistance" to therapy (Zhao et al., 2020).

Moreover, there is a large group of patients who, despite showing a strong initial response to therapy, can further develop progressive disease. This phenomenon, in which disease is refractory to the continuation of therapy, is known as "acquired resistance" to PD-1/PD-L1 blockade immunotherapy. The mechanisms for both types of resistance can be overlapping and/or multifactorial. Additionally, each patient's individual environmental and genetic factors can create a unique therapeutic landscape for a given patient (Nowicki et al., 2018).

Tumor-intrinsic factors that contribute to primary and acquired resistance to PD-L1/PD-1 immunotherapy correspond to a genetic and signaling landscape that prevents immune cell infiltration in the tumor microenvironment (TME) (figure 5). Resistance to PD-L1 blockade immunotherapy is often associated with insufficient tumor antigenicity, PD-L1 overexpression and defects in IFN signal transduction within cancer cells and modifications in the regulation of oncogenic pathways (Lei et al., 2020).

1- The loss of tumor antigenicity

It is a major escape mechanism for many tumor types. This is mainly caused by cancer immunoediting, a process by which the immune system exerts selective pressure over the most immunogenic cancer cell variants. Effector T cells will eliminate the most immunogenic cancer cells and regulate tumor progression for some time (Workenhe et al., 2021). However, the less immunogenic cancer cell variants will overgrow and progress. Consequently, tumor immunoediting represents a strong mechanism of acquired resistance to immunotherapies. The surviving cancer cells will show a decreased level of tumor antigen expression, or a downregulation in antigen presentation molecules such as lack of major histocompatibility complex (MHC I) (Chocarro de Erauso et al., 2020). In this context, no endogenous T cell responses can be raised against these tumors thus causing ICI therapies to fail. For instance, genomic instability or epigenetic alterations in pre-existing tumor cell variants can allow these cancer cells to evade ICI therapies. And this will facilitate tumor growth, immune evasion, and tumor escape (Polania et al., 2021).
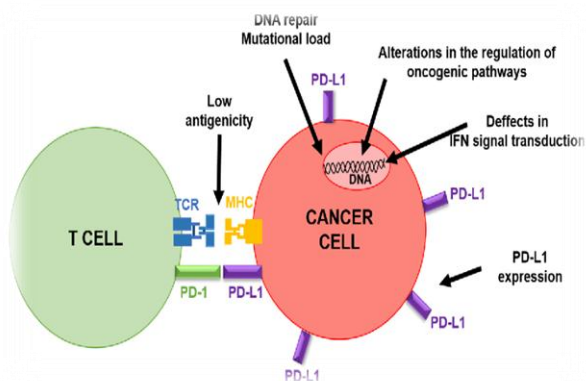
Figure (5): Schematic summary of cancer-intrinsic characteristics affecting clinical response to PD-L1/PD-1 blockade therapies. The figure illustrates the interaction of a T cell with a cancer cell, highlighting cancer cell-intrinsic factors that can inactivate T cell activities (Chocarro de Erauso et al., 2020).

2- PD-L1 overexpression:

The PD-L1 expression is regulated by two general types of immune resistance, such as (I) innate immune resistance, and (II) adaptive immune resistance. In innate immune resistance, the PD-L1 expression is caused by Phosphatase and tensin homolog (PTEN) downregulation leading to activation of PI3K-Akt tumorigenic signaling in glioblastomas (Han et al., 2020). PD-L1 expression also occurs through the signal transducer and activator of transcription 3 (STAT3) upregulation. The STAT3 activation is increased by pro-inflammatory cytokines, such as IL-6 and the IL-6-STAT3 axis is considered as one of the critical pathways in immune suppression (Xu et al., 2021). In adaptive immune resistance, the PD-L1 expression is induced in some tumors by the secretion of pro-inflammatory IFN-γ from tumor cells that causes neutralization of CD8+ cytotoxic T cell-induced antitumor immune responses. Furthermore, PD-L1 can also stimulate cancer cell growth by altering the activity of AKT/mTOR, autophagy, and glycolysis (Ju et al., 2020).

Besides, tumor-extrinsic factors and resistance to PD-L1/PD-1 blockade therapies involve: Irreversible T cell exhaustion in TME, expression of further immune checkpoint molecules and their ligands for example (CTLA-4), differentiation and extension of immunosuppressive cell populations and release of immunosuppressive cytokines and metabolites both systemically and within the TME (IL-10, IL-6, IL-17, IFN-γ) (Figure 6) (Fares et al., 2019).

Other than tumor cells, elements associated with primary or acquired resistance comprise exhausted T cells, Tregs, myeloid-derived suppressor cells (MDSCs) that are major cellular components of the TME that stimulate immune evasion and tumor growth by preventing T-cell infiltration and activation (Diskin et al., 2020; Theivanthiran et al., 2020), and other inhibitory immune checkpoints and cytokines. T cell exhaustion is demonstrated by dysfunction, sustained expression of inhibitory receptors, and different transcriptional status with functional effector or memory T cells. Tregs are known to suppress effector T cell (Teff) responses by secretion of certain inhibitory cytokines, such as IL-10, IL-35, and transforming growth factor-beta (TGF-β) (Jiang et al., 2021). It has been shown that depletion of Treg cells from the TME can strengthen the antitumor immune response. Moreover, response to PD-1/PD-L1 blockade therapy was shown to be associated with an increased ratio of Teff to Treg (Huang et al., 2021).

The irregular cellular signaling transduction that promotes the expression of the PD-1/PD-L1 axis is also a crucial factor associated with the resistance to immunotherapy. Such as: The mitogen-activated protein kinase (MAPK) signaling pathway is an important signal transduction system that regulates cell proliferation, differentiation, invasion, metastasis (Peng et al., 2018). Recent research showed that the inhibition of the MAPK pathway prevented epidermal growth factor and IFN-γ-induced PD-L1 protein expression in lung adenocarcinoma cells (Stutvoet et al., 2019). Likewise, inhibition of mitogen-activated protein kinase kinases 1 and 2 (MEK1/2) can significantly prevent PD-L1 expression in renal cell carcinoma (Jalali et al., 2019; Han et al., 2020). Oncogenic

PI3K/AKT pathway has been proved to be associated with primary resistance to PD-1/PD-L1 checkpoint inhibition as it controls a variety of cellular processes including apoptosis, proliferation, motility, angiogenesis, and glucose metabolism, and contributes to tumor development and progression. Activation of PI3K/AKT may lead to PD-L1 expression by increasing extrinsic signaling or decreasing expression of negative regulators, such as phosphatase and tensin homolog (PTEN) (He et al., 2021).

The WNT/β-Catenin signaling pathway: T cell elimination from cancers has also been shown by the stabilization of β-catenin resulting in constitutive WNT signaling pathway (Li et al., 2019b). Murine tumors lacking β-catenin responded effectively to immune checkpoint therapy whereas β-catenin-positive tumors did not. NFκB: Which is a downstream target of AKT has been shown to regulate PD-L1 expression. AKT activates NFκB, which up-regulates PD-L1 transcriptionally. JAK/STAT3/INF-α: STAT3 has been proven to bind to the PD-L1 promoter to regulate its expression transcriptionally (Deldar Abad Paskeh et al., 2021). It has been found that a JAK2 inhibitor suppressed the upregulation of PD-L1 at both the mRNA and protein levels. Hence, these results proved that the JAK/STAT pathway regulates the expression of PD-L1 (Li et al., 2019a; Han et al., 2020).

The IFN-γ pathway is emerging as a key player in primary, adaptive, and acquired resistance to checkpoint blockade therapy. It has both favorable and damaging effects on antitumor immune responses. Interferon-γ is produced by tumor-specific T cells that have identified their related antigen on cancer cells or APCs induces an effective antitumor immune response. However, continuous exposure to IFN-γ can lead to immunoediting of cancer cells, resulting in immune escape (Jorgovanovic et al., 2020) . One mechanism by which cancer cells could escape the effects of IFN-γ is by decreasing the expression or mutations in molecules in the IFN-γ signaling pathway, which occurs through the IFN-γ receptors JAK1 and/or JAK2 and the STATs (Dhatchinamoorthy et al., 2021).

Taken all together, cancer immune-checkpoint inhibition could be considered as a promising era that could deeply reshape the strategy of cancer therapy and HCC. This raised fact is based on growing clinical evidence that had shown its efficacy in a variety of solid tumors and hematologic malignancies. While the negative results of some of these clinical trials shed the light to the potential boundaries and pitfalls of using cancer-immune check point inhibition including either de-novo, due to insufficient tumor antigenicity and/or acquired resistance, due to PD-L1 overexpression. Extensive preclinical and clinical investigations are urgently required to verify several notions including the advantage of small molecule inhibitors of PD-L1 over the traditional inhibition by monoclonal antibodies. Moreover, evaluation of emerging combinations with PD-L1 inhibitors that could manipulate signaling pathways and regulate PD-L1 overexpression with subsequent overcoming potential resistance and maximize cancer management

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