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Immune Checkpoint Inhibitors in Hepatocellular Carcinoma: A New Era in Treatment of Advanced Disease

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Abstract

Hepatocellular carcinoma (HCC) is the most prevalent primary hepatic cancer with high fatality and recurrence rates. The prognosis of advanced HCC is dismal, and treatment was limited for a decade to sorafenib with limited effectiveness and miserable overall survival. The advent of immune checkpoint inhibitors (ICPIs) provides a considerable step in the treatment of several advanced malignancies including HCC and opens new horizons for this group of patients. Two drugs belonging to ICPIs, namely nivolumab and pembrolizumab, have now been licensed by the US FDA as a second-line treatment of patients who have progressed or have not responded to sorafenib, both are inhibitors of programmed cell death protein 1 (PD-1). Possible synergism of ICPIs, when used in conjunction with drugs active against other checkpoint molecules, targeted drugs, and locoregional modalities, is now investigated in several clinical trials. The current challenge is to evolve predictive biomarkers of tumor response to appropriately select patients who may respond well to ICPIs.

Keywords: Immune checkpoint inhibitors; Hepatocellular carcinoma; Nivolumab; Pembrolizumab

Preamble

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer in the globe and the fourth commonest cause of deaths due to cancer, making up to 75% to 85% of primary liver cancers **(1)**. The prognosis among HCC patients at the early stage gets better due to improvements in diagnostic techniques and treatment options. Many treatment options were available for this patient group, including liver resection, transplantation and percutaneous ablation (PEI, RFA, microwave ablation) **(2)**.

Seventy to eighty percent of patients cannot advantage of these treatment options since they are discovered at a late stage, and the solely available drug was sorafenib with overall survival (OS) of 10.7 months and overall 5-year survival of less than 16% **(3)**. Over the last 10 years, more than 10 drugs have not reached clinical endpoints in phase III studies **(4)**. Favorable outcomes of phase III trials, including regorafenib as a 2nd line treatment in patients progressing on sorafenib; and lenvatinib as a 1st line treatment, have been revealed to have a survival advantage,

but improvement in OS remained unsatisfactory **(5)**.

The role of immunotherapy in HCC therapy

Several immunotherapeutic methods including cytokine-based therapies, cancer vaccines, adoptive cell transfer, and oncolytic viruses have been studied in HCC **(6)**. The aim of cancer immunotherapy was to stimulate immune cell action to eliminate malignant cells; nevertheless, this does not lead to real stimulation of the immune system due to suppression by checkpoint molecules. As a result, its clinical implementation still debatable. Recently, the emergence of immune checkpoint inhibitors (ICPIs) which remove constraints on the immune system, bringing back its function to a normal level, may fundamentally improve HCC immunotherapy **(7)**.

Immune system response to malignant tumors

Once cells turn malignant, MHC molecules of antigen-presenting cells (APCs) identify tumor antigens. APCs then travel to lymph nodes presenting antigens to T cell receptors (TCR) on the surface of immature T cells. Nevertheless, antigen activation solely is inadequate to stimulate immature T cells; a further co-stimulatory signal is needed, which is the attachment of CD28 on T cells to CD80/B7-1 or CD86/B7-2 on APCs, as a result of this second signal, CD8 T cells become stimulated (priming phase). These stimulated T cells then travel via the bloodstream to the tumor location and identify tumor antigens presented by MHC molecules on malignant cells, initiating the destruction of malignant cells through the release of perforin and granzymes (effector phase) **(Figure1) (8)**.

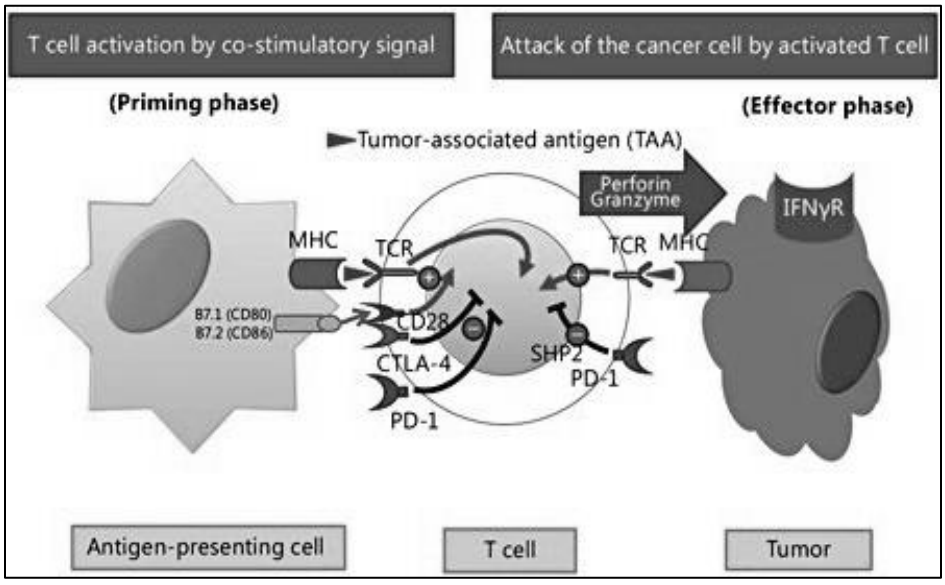

Figure (1): Attack of malignant cells by activated T cells **(7)**.

Immune checkpoints

Immune checkpoints are immune system regulators, different types of cells engaged in the immune reaction express these molecules, which includes T and B cells, NK cells, monocytes, tumor-associated macrophages, dendritic cells (DC), and myeloid-derived suppressor cells (MDSC) **(9)**.

The main role of these molecules is to inhibit persistent T cell activity after preliminary activation and involvement of antigen-specific T cells. Therefore, the majority of these molecules exhibit immunosuppressive action which inhibits excessive T cell activity toward infection, reduces tissue damage, hence preventing autoimmunity **(10)**. Nevertheless, in the tumor microenvironment (TME), reduced immune activity can contribute to tumor progression **(11)**.

The extensively investigated immune checkpoints in human malignancy are programmed cell death protein 1 (PD-1) and its ligands (PD-L1/PD-L2), and cytotoxic T lymphocyte protein 4 (CTLA-4) **(10)**.

CTLA-4 mechanism

CTLA-4 system controls the development of stimulated lymphocytes. CTLA-4 is expressed on regulatory T cells (Tregs) and temporarily on a wide spectrum of T cells during the early activation phase **(7)**. It competes with the CD28 stimulating molecule to attach to CD80 and CD86 molecules on APCs inhibiting T cell stimulation. It activates Tregs that lead to self-tolerance **(12)**.

PD-1 mechanism

PD-1 is expressed on B and T lymphocytes, NK cells, and myeloid cells. Several cytokines induce its expression, especially IFN-γ **(13)**. PD-1 suppresses T cell stimulation by disrupting TCR signaling through interactions with PD-L1 and PD-L2 leading to T cell exhaustion. PD-1 is implicated in the development of Tregs **(14)**.

Immune escape mechanisms in HCC and the role of immune checkpoints

Under normal circumstances, checkpoint mechanisms have an important role in the process of hepatic immunotolerance. In HCC, several immune disturbances lead to tumor persistence and growth. These derangements include defective antigen processing, rising levels of T-regs and other immunosuppressive cells, decreased numbers of $CD4^+$ T cells, elevated expression of checkpoints and impaired formation of cytokines **(6)**.

In TME, PD-1 triggers apoptosis of T-cells and facilitates immune escape, a mechanism used by malignant cells through expression of PD-L1 or PD-L2 **(12)**. In HCC, PD-1 upregulation was reported on T cells, and PD-L1 is substantially expressed on both malignant cells and stromal cells. Elevated local PD-L1 levels have been linked with an elevated risk of postoperative recurrence **(15)**. Elevated levels of $PD\text{-}1^+$ T cells is linked with disease advancement after resection **(16)**. As regards CTLA-4 fewer data exist about its role in HCC. However, the elevation of CTLA-4 levels on hepatic dendritic cells is associated with T-cell suppression and apoptosis **(17)**. Elevated levels of CTLA-4 expression by Tregs are associated with the diminished formation of T cell cytotoxic enzymes **(18)**. Activation of Tregs via CTLA-4 pathway has been linked with both reduced T cell levels in the TME and, more clinically significant, reduced OS in HCC patients **(19)**.

ICPIs in HCC treatment

Currently, ICPIs reveal favorable outcomes in many malignancies and are

approved in melanoma, non-small cell lung cancer, Hodgkin's lymphoma, renal cell carcinoma, colorectal cancer (high microsatellite instability type), and Merkel cell carcinoma **(20)**.

Many ICPI-related medications are evaluated for the treatment of patients with advanced HCC in different clinical studies either alone or in conjunction with other drugs (e.g. other ICPIs, tyrosine kinase inhibitors, anti-VEGFs) or with locoregional modalities (e.g. RFA, TACE, ^{90}Yttrium radioembolization), these drugs and the current clinical trials evaluating them are summarized in **Tables (1,2) (21,22).**

Table (1): ICPIs under assessment in the major clinical trials for HCC **(21)**.

Target	ICPI	Trade name
PD-1	Nivolumab	OPDIVO
	Pembrolizumab	KEYTRUDA
	Tislelizumab	
	Camrelizumab	
	Spartalizumab	
PD-L1	Durvalumab	IMFINZI
	Atezolizumab	TECENTRIQ
	Avelumab	BAVENCIO
CTLA-4	Tremelimumab	
	Ipilimumab	YERVOY

The first clinical trial on ICPIs in advanced HCC patients that demonstrated favorable outcome was a phase II study of tremelimumab (anti-CTLA-4) in advanced HCC patients and cirrhosis due to HCV who experienced disease advancement while on sorafenib. 17.6% of patients show a partial response with a good safety profile **(23)**.

Successful anti–CTLA-4 treatment encourages to evaluate other ICPIs. Nivolumab (anti–PD-1) was evaluated in advanced HCC patients (CheckMate-040 trial). Of the 212 evaluated patients, the overall response rate (ORR) was noticed in 20% of patients, in addition, the response was comparable in patients with or without previous sorafenib treatment. Based on these results, nivolumab was approved as a second-line treatment for advanced HCC patients, awaiting the results of a phase III study of the first-line nivolumab versus sorafenib **(24)**.

Pembrolizumab (anti–PD-1) was assessed in advanced HCC patients who developed disease advancement following sorafenib treatment (KEYNOTE-224 trial). The study revealed ORR in 1 7% of patients **(25)**. Based on these findings, an accelerated FDA license was offered to pembrolizumab for HCC patients with prior sorafenib therapy **(26)**.

Side effects of ICPIs

Regarding the safety of ICPIs, reports from undergoing clinical trials reveals that these drugs are reasonably tolerated in HCC patients, and the toxicity is milder than that of cytotoxic drugs and targeted therapies. However, ICPIs can generate autoimmunity-related side effects including type 1 DM, hypothyroidism, hyperthyroidism, or myasthenia gravis. The majority of these side effects can be managed by the stoppage of ICPIs and starting steroids **(7)**. Immune-related side effects are the least common in patients receiving anti-PD-L1 antibodies and most common in patients receiving anti-CTLA-4 antibodies. Other side effects include xerostomia, hepatitis, enteritis, dermopathy, arthritis, adrenal hypofunction and uveitis **(27)**.

Table (2): Undergoing major trials of ICPIs in HCC treatment **(22)**.

Target		Design	Clinical trial number	Phase	Endpoint
ICPIs as monotherapy					
PD-1	**Nivolumab**	**Nivolumab vs. Sorafenib**	**NCT02576509**	**3**	**OS**
	Nivolumab	**Nivolumab vs. placebo**	**NCT03383458**	**3**	**PFS**
	Pembrolizumab	**Pembrolizumab vs. placebo**	**NCT03062358**	**3**	**OS**
	Pembrolizumab	**Pembrolizumab**	**NCT03337841**	**2**	**RFS**
	Tislelizumab	**Tislelizumab**	**NCT03419897**	**2**	**ORR**
	Tislelizumab	**Tislelizumab vs. Sorafenib**	**NCT03412773**	**3**	**OS**
	Camrelizumab	**Camrelizumab**	**NCT02989922**	**2/ 3**	**ORR/OS**
PD-L1	**Avelumab**	**Avelumab**	**NCT03389126**	**2**	**ORR**
Combination with other immune-based therapies					
PD-1 and CTLA-4		**Nivolumab + Ipilimumab**	**NCT03682276**	**1/2**	**ORR**
		Nivolumab + Ipilimumab	**NCT03510871**	**2**	
		Nivolumab +/- Ipilimumab	**NCT03222076**	**2**	**Safety**
		Nivolumab +/- Ipilimumab	**NCT03203304**	**1**	**Safety**
		Tremelimumab vs. Tremelimumab + Durvalumab vs. Sorafenib	**NCT03298451**	**3**	**OS**
		Tremelimumab vs. Durvalumab vs. Tremelimumab + Durvalumab	**NCT02519348**	**2**	**Safety**
PD-L1 and TIM-3		**LY3300054 +/- LY3321367**	**NCT03099109**	**1**	**Safety**
PD-1 and LAG-3		**REGN2810 +/- REGN3767**	**NCT03005782**	**1**	**Safety/ORR**
Combination with molecular targeted agents					
PD-L1 and anti-VEGF		**Atezolizumab + Bevacizumab**	**NCT02715531**	**1**	**Safety/ORR**
PD-L1 and anti-VEGF		**Atezolizumab + Bevacizumab vs. Sorafenib**	**NCT03434379**	**3**	**OS/ORR**
PD-1 and TKI		**Pembrolizumab + Lenvatinib vs. Lenvatinib**	**NCT03713593**	**3**	**PFS/OS**
PD-1 and TKI		**Pembrolizumab + Lenvatinib**	**NCT03006926**	**1**	**Safety/OR/DOR**
PD-1 and TKI		**Camrelizumab +Apatinib**	**NCT02942329**	**1/2**	**OS**
PD-1 and TKI		**Spartalizumab + Sorafenib**	**NCT02988440**	**1**	**Safety**
PD-1 and c-MET inhibitor		**Spartalizumab +/- Capmatinib (INC280)**	**NCT02795429**	**1/2**	**Safety/ORR**
PD-1 and anti-TGF-β		**Spartalizumab +/- NIS793**	**NCT02947165**	**1**	**Safety**
PD-1 and FGFR4 inhibitor		**Spartalizumab +/- FGF401**	**NCT02325739**	**1/2**	**Safety/TTP/ORR**
PD-1 and TKI		**Nivolumab +/- Lenvatinib**	**NCT03418922**	**1**	**Safety**
PD-1 and TKI		**Nivolumab + Cabozatinib**	**NCT03299946**	**1**	**Safety/Completion**
PD-1 and anti-VEGF		**Nivolumab + Bevacizumab**	**NCT03382886**	**1**	**Safety**
PD-1 and TKI		**Pembrolizumab + Regorafenib**	**NCT03347292**	**1**	**Safety**
PD-1 and TKI		**Pembrolizumab + Sorafenib**	**NCT03211416**	**1/2**	**ORR**
PD-L1 and TKI		**Avelumab + Axitinib**	**NCT03289533**	**1**	**Safety**
PD-L1 and DNMT inhibitor		**Durvalumab + Guadecitabine**	**NCT03257761**	**1**	**Safety/ORR**
CTLA-4, PD-1 and anti-OX40		**Nivolumab + INCAGN01949 vs. Ipilimumab + INCAGN01949 vs. Nivolumab + Ipilimumab + INCAGN01949**	**NCT03241173**	**1/2**	**Safety/ORR**
PD-1 and antiphosphatidyl-serine		**Pembrolizumab + Bavituximab**	**NCT03519997**	**2**	**ORR**
Combination with local therapies					
PD-1 and ischemia		**Nivolumab + TACE**	**NCT03143270**	**1**	**Safety**
PD-1 and radiation		**Pembrolizumab + TACE**	**NCT03397654**	**1/2**	**Safety**
PD-1 and radiation		**Nivolumab + Y90**	**NCT03033446**	**2**	**ORR**
CTLA-4, PD-L1 and ischemia		**Tremelimumab + Durvalumab + Radiation**	**NCT03482102**	**2**	**ORR**
PD-1 and HSV oncolytic virus		**Pembrolizumab +/- Talimogene Laherparepvec (T-VEC)**	**NCT2509507**	**1**	**Safety/ORR**

TIM-3: T-cell immunoglobulin domain and mucin domain 3; LAG-3: lymphocyte activation gene-3; c-MET: tyrosine-protein kinase MET; DNMT: DNA methyltransferase; PFS: progression-free survival; RFS: recurrence-free survival; TTP: time to progression

Predictors of therapeutic response to ICPIs

Most HCC patients do not respond to ICPIs (70-90%). Recognizing the mechanisms which cause resistance may help to guide future therapy and contribute to the development of efficient combination therapies. For instance, upregulation of alternative immune checkpoints such as

indoleamine 2,3-dioxygenase and TIM-3 was found to make tumors insensitive to ICPIs. The suppression of these additional upregulated checkpoints can reverse immunosuppression and promote the probability of combination therapy **(28)**.

There are a number of promising possible predictors of therapeutic response to ICPIs which could allow better selection of patients including PD-L1 expression in malignant tissue, elevated levels of lymphocytes infiltrating the tumor, intact IFN-γ signaling, the existence of $CD8^+$ T lymphocytes in the TME, or a high risk of tumor mutation. It is noticed that the stimulation of the Wnt/β-catenin pathway in HCC patients is linked with resistance to ICPI and can be used as a biomarker of resistance **(29)**.

Conclusion

Treatment of advanced HCC is challenging and has been confined to sorafenib for the past decade with a modest impact on OS and considerable toxicity. ICPIs show encouraging results in the setting of treatment of advanced HCC with manageable side effects and may provide new hope for improved OS in this highly lethal tumor. Many studies are currently performed to assess the effectiveness of ICPIs either alone or in combination with other modalities. The development of predictive biomarkers is greatly required to identify patients for whom the therapeutic response is more likely to occur.

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