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Immunotherapy for hepatocellular carcinoma: Current status and future perspectives

Mandlik DS *et al* Immunotherapy for HCC

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Hepatocellular carcinoma (HCC) is one of the world's deadliest and fastest-growing tumours, with a poor prognosis. HCC develops in the context of chronic liver disease. Curative resection, surgery (liver transplantation), trans-arterial chemoembolization, radioembolization, radiofrequency ablation and chemotherapy are common treatment options for HCC, however, they will only assist a limited percentage of patients. Current treatments for advanced HCC are ineffective and aggravate the underlying liver condition. Despite promising preclinical and early-phase clinical trials for some drugs, existing systemic therapeutic methods for advanced tumour stages remain limited, underlining an unmet clinical need. In current years, cancer immunotherapy has made significant progress, opening up new treatment options for HCC. HCC, on the other hand, has a variety of causes and can affects the body's immune system *via* a variety of mechanisms. With the speedy advancement of synthetic biology and genetic engineering, a range of innovative immunotherapies, such as immune checkpoint inhibitors [anti-programmed cell death 1 (PD-1), anti-cytotoxic T lymphocyte antigen-4, and anti-PD ligand 1 cell death antibodies], therapeutic cancer vaccines, engineered cytokines, and adoptive cell therapy have been used for the treatment of advanced HCC. In this review, we summarize the present clinical and preclinical landscape of immunotherapies in HCC, critically discuss recent clinical trial outcomes, and address future perspectives in the field of liver cancer.

Key Words: Hepatocellular carcinoma; Immunotherapy; Immune checkpoint inhibitors; Alpha-fetoprotein; Cancer vaccine; Combination therapies

Hepatocellular carcinoma (HCC) is the world's fifth most frequent malignancy and the third main cause of cancer death[1]. Men are slightly more likely than women to get HCC (3:1 male-to-female ratio). Globally, primary liver cancer caused an estimated 906000 new cases and 830000 fatalities in 2020; the majority of these cases (75%-85%) were HCC[2]. Underlying liver conditions including cirrhosis or chronic hepatitis frequently led to HCC. Hepatitis C virus (HCV), hepatitis B virus (HBV), fatty liver disease like non-alcoholic steatohepatitis, and alcohol consumption are the most prominent risk factors (Figure 1)[3]. The five-year age-standardized rate of relative survival for HCC is only 18.1%, even though surgical intervention is currently the best therapy for HCC. Tumour reappearance is common after tumour removal[4]. In adult males, HCC is the second most common cancer-related killer after lung cancer because of the challenge of earlier detection, which results in the vast bulk of HCC patients receiving a higher stage diagnosis at their first visit and being ineligible for treatments like radiofrequency ablation (RFA) or hepatectomy[5]. Sorafenib and lenvatinib, the two clinically authorised targeted therapy medicines, could barely extend survival time by 2 mo to 3 mo[6,7]. As a result, novel HCC therapy techniques are urgently required.

Immunotherapy is successful and safe in the treatment of solid tumours, resulting in long-term survival and manageable toxicity[8,9]. The liver is a remarkable immunologically tolerant organ that is exceptional in its capacity to accept liver transplants and diminish responsiveness to antigens in bacterial and food products through the portal vein[10]. This tolerogenic trait of the liver and the immunosuppressive tumour microenvironment of HCC may jointly inhibit the formation of anti-tumour immunity against HCC. Cancer immunotherapy may be a compelling therapeutic approach for HCC, which has a metachronous multicentric incidence due to its capacity to trigger systemic and long-lasting anti-tumour actions. For numerous forms of malignancies, along with HCC, the FDA has so far approved seven immune checkpoint inhibitors (ICIs) that target the programmed cell death protein-1 (PD-1), proteins cytotoxic T lymphocyte antigen 4 (CTLA-4), or its ligand programmed cell death-ligand 1 (PD-L1)[11,12]. Other immunotherapeutic approaches,

like immune cells modified with chimeric antigen receptors, adoptive cell treatment, tailored cytokines, and cancer vaccines, are almost ready and provide HCC patients new hope[13-15]. In this review, we first provide an overview of the current state of immunotherapy for HCC (Figure 2), after which we analyse the challenges, opportunities, and potential directions of this field of study.

ANTIBODY-BASED THERAPY

Monotherapy with ICIs

Effector immune cells express inhibitory immunoreceptors called immunological checkpoints that keep the immune system from overreacting. The T cell immunoreceptor with Ig and ITIM domains (TIGIT), lymphocyte-activation gene 3 (LAG3), B and T lymphocyte attenuator, and T cell immunoglobulin and mucin domain containing-3 (TIM3) are only a few of the inhibitory receptors in this group[16]. This physiological process is employed by HCC and other solid tumours to thwart anti-tumour immune reactions[17]. Monoclonal antibodies known as ICIs have the potential to prevent immunological checkpoint proteins from interacting with their ligands. This could improve the immune response against tumours by preventing T cell inactivation and reactivating immune attack and immune recognition. At the moment, ICIs' primary targets are CTLA-4, PD-1, and PD-L1[13]. Most immune cells, myeloid-derived suppressor cells, primarily activated T cells, regulatory T cells (Treg), natural killer (NK) cells, dendritic cells (DC), and monocytes express PD-1, are a member of the CD28 family. When PD-1 binds to its ligands *i.e.*, PD-L1 and PD-L2, that are stimulated in a variety of cancers, including HCC, it can send T cells inhibitory signals and cause tumour cells to bypass the immune system[18].

Nivolumab, a PD1 inhibitor, was given expedited approval in the United States in 2017 for this second-line treatment of patients with severe HCC following sorafenib therapy. ICIs have been used to treat HCC in several exploratory trials that have been completed so far. PD-1 and PD-L1 inhibitors pembrolizumab and atezolizumab, which target these receptors respectively, have been steadily proposed as a clinical therapy

strategy for HCC and added to the treatment recommendations of numerous nations. Nivolumab and pembrolizumab cause objective remissions of 15%-20%, which includes complete remissions of 1%-5%, and these remissions are long-lasting and linked with improved survival. Nivolumab had a median response duration of 17 mo in the dose-escalation cohort in 48 patients of the CheckMate 040 investigation, and responders had a survival rate of about 2 years of more than 80%[19]. Statistically longer survival was seen in the 413 patients who took part in phase III clinical trial KEYNOTE-240, which compared pembrolizumab after sorafenib medication to placebo. Pembrolizumab was found to have long-term advantages for some people, as seen by the overall survival curves and progression-free survival. Around 20% of patients who got pembrolizumab stayed in progression-free status for longer than a year, as opposed to merely 7% of the control group[20]. In the phase III CheckMate 459 trial, 743 patients who had never received systemic medications were compared between nivolumab and sorafenib. Patients who received nivolumab had a longer median survival time than those who received sorafenib[21]. The CheckMate 459 study's lengthier follow-up period confirmed nivolumab's better ability to sorafenib to raise the long-term rate of survival[22].

Recent studies presented at the ESMO 2021 Annual Meeting revealed that tislelizumab, a humanised monoclonal antibody (mAb) with substantial adhesion for PD-1, evidenced durable response and was well tolerated in individuals who had previously systemically treated unresectable HCC. In a global, randomised phase 3 trial, the first-line therapies for adult patients having unresectable HCC are tislelizumab and sorafenib (NCT03412773)[23]. The majority of activated T cells and DCs express CTLA-4, a member of the CD28 family. After binding to B7 molecules, it participates in the downregulation of the immune response[24]. The FDA approved ipilimumab, the first ICI, in 2011 for the therapy of patients with severe skin cancer. Tremelimumab is another CTLA-4 inhibitor[25].

Tremelimumab, an IgG2 mAb, has different antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity activities than Ipilimumab, an IgG1 mAb[26]. Tremelimumab demonstrated a potent anti-HCC impact in a clinical trial

conducted in 2013, with a 17.6% of partial response (PR) rate and 76.4% of disease control rate[27]. With the in-depth analysis of CTLA-4 inhibitors' mechanism, some researchers hypothesise that it involves the targeted eradication of Tregs from tumours rather than the immune checkpoint[28]. In human HCC, the presence of TIM3 on tumour-associated macrophages and tumour-infiltrating lymphocytes (TILs) impairs T cells' ability to operate as effector cells, while increasing the activation of Treg cells' suppressor genes[29]. Less differentiated HCC is linked to the highly expressed TIM3[30]. LAG3 appearance is much greater on tumour-specific $CD4^+$ and $CD8^+$ TILs in patients with HCC compared to other immune compartments. Hepatocytes produce fibrinogen-like protein 1, another useful soluble ligand for LAG3[31]. The most recent studies published demonstrate that Siglec-15 inhibits CD44's lysosomal degradation, which encourages the migration of liver cancer cells[31,32]. T-cell immunoreceptor with immunoglobulin and ITIM domains also plays a role in TIGIT[33]. The TIGIT/CD155 pathway increases interleukin (IL)-10 synthesis and decreases IL-12 by DCs to prevent T cell activation[34]. The exploration of Siglec-15, TIM3, TIGIT, and LAG3 inhibitors in HCC in conjunction with PD1 and PDL1 inhibition is supported by the preclinical evidence presented here. According to the findings of current clinical studies, patients medicated with alone ICIs have a lower rate of response; as a result, future therapies will combine ICIs and other therapies.

The results of the worldwide, randomised phase 3 IMbrave150 trial in 2020 showed that atezolizumab plus bevacizumab an anti-angiogenic drug considerably lowered the probability of mortality in patients with severe unresectable HCC and enhanced the rate of patient survival[35]. Lenvatinib, a tyrosine kinase inhibitor (TKI), was combined with pembrolizumab to provide an overall response rate of 46%. Patients with unresectable HCC who were enrolled in the study saw complete responses (CR) and PR in proportions of 11% and 35%, respectively[36]. Similar to this, newer preclinical and clinical investigations have demonstrated that the administration of ICIs in combination with radiation, RFA, and transcatheter arterial chemoembolization (TACE) can enhance the effectiveness of anti-tumour immunotherapy[37,38]. In a phase, Ib/II clinical trial,

camrelizumab and the chemotherapy FOLFOX4 are being used to treat advanced HCC[39].

The HIMALAYA phase 3 trial, which evaluated a single, high dose of tremelimumab, an anti-CTLA-4 antibody, added to durvalumab, an anti-PD-L1 antibody, or durvalumab alone in comparison to sorafenib as first-line treatment in 1171 patients with advanced HCC, has provided additional evidence that single-agent PD1/PD-L1 inhibition has antitumor activity (NCT03298451). Durvalumab monotherapy achieved the goal of overall survival without being inferior to sorafenib while being less toxic[40]. Regarding anti-CTLA-4 monotherapy, the research 22 revealed that tremelimumab monotherapy in patients previously treated with sorafenib after unacceptable toxicity or rejection of sorafenib had a manageable safety profile. However, the benefit-risk ratio was often better when tremelimumab and durvalumab were used together[41].

Figure 3 depicts several immune checkpoint inhibitors in HCC. Table 1 presents a summary of the clinical trials including ICIs treatment for HCC conducted during the previous 3 years.

Dual therapies with ICIs

Combining PD-1 and CTLA-4 inhibitors: The concept of combining inhibitors of several immune checkpoints, including as PD-1 and CTLA-4, is now being investigated in advanced HCC. Dual treatments combining PD-1 and CTLA-4 inhibitors have been studied in other cancer types, such as melanoma. The CheckMate 040 trial, which evaluated nivolumab and ipilimumab, an anti-CTLA-4 antibody, in 148 patients with advanced HCC who had progressed on sorafenib, provided the first clinical data[42]. Nivolumab and ipilimumab were administered to patients in three separate arms (Arm A, Arm B, and Arm C) at various doses and frequencies. The trial reported a 49% disease control rate, a 31% overall response rate, and 17-mo median duration of response. The longest overall survival was 23 mo for patients in arm A. The patient side-effect profile was deemed acceptable. To treat side effects, more than 50% of patients required systemic steroids. Overall, the trial's findings were positive, and the

FDA eventually approved the use of nivolumab and ipilimumab together. Additionally, the combination is presently being evaluated in the CheckMate 9DW phase 3 study as a first-line treatment for advanced HCC in comparison to sorafenib or lenvatinib (NCT04039607). Similar to this, durvalumab and tremelimumab together exhibited strong action in unresectable HCC, with a response rate overall of 17.5%[43].

ICIs with anti-vascular endothelial growth factor antibodies: The combination of a PD1/PD-L1 inhibitor with a vascular endothelial growth factor (VEGF) inhibitor has been established as a novel paradigm for the treatment of advanced HCC in light of the encouraging results of the IMbrave150 phase 3 trial (Finn *et al*, 2020). Atezolizumab plus bevacizumab had initially shown good safety and promising anticancer activity in patients with untreated advanced HCC in a phase 1b study[44]. The next phase of the IMbrave150 trial compared sorafenib and atezolizumab in patients with advanced HCC who had never received systemic therapy and were randomly assigned (2:1) to either study arm (Finn *et al*, 2020). Compared to sorafenib, the combination of atezolizumab plus bevacizumab therapy lowered the death risk by 42%. The IMbrave150 trial, which is significant, excluded patients with problems related to portal hypertension, such as severe ascites and esophageal or gastric varices at high risk of bleeding. Atezolizumab and bevacizumab combination, the current standard therapy that has replaced the TKIs sorafenib and lenvatinib, were approved as a first-line treatment for unresectable HCC in the United States and Europe as a result of the IMbrave150 trial. Inhibiting PD-L1, which activates the immune system (especially T-effector cells), and inhibiting VEGF, which lessens VEGF-mediated immunosuppression and encourages T-cell infiltration in the tumor microenvironment, are probable synergistic anticancer agents that account for its effectiveness[45].

Dual therapies combining immune checkpoint with multikinase inhibitors: An alternative to antibody-mediated VEGF suppression may be achieved by combining ICIs with TKIs instead of anti-VEGF antibodies. The multicohort COSMIC-021 phase 1b

trial is evaluating the combination of cabozantinib and atezolizumab in advanced solid tumours, including HCC (NCT03170960). Additionally, it is being evaluated in the COSMIC-312 phase 3 trial (NCT03755791) as a first-line treatment for patients with advanced HCC in comparison to sorafenib[46]. As a secondary outcome metric, cabozantinib/monotherapy is also contrasted with sorafenib in this study. A predetermined interim analysis for overall survival, however, failed to achieve statistical significance. Early 2022 is when its final analysis should produce results[47]. In 104 patients with unresectable HCC who had not previously undergone systemic treatment, the combination of lenvatinib and pembrolizumab showed reasonable results in a phase 1b trial (Finn *et al*, 2020). This combination is currently being compared to lenvatinib monotherapy in the LEAP002 phase 3 trial (NCT03713593)[48]. Finally, the combination apatinib and camrelizumab, an anti-PD-1 antibody is under clinical development. An overall response rate of 50% was found in a phase 1 study of individuals with advanced HCC[49]. Additionally, a phase 3 trial is currently comparing the combination to sorafenib in the first-line setting for patients with advanced HCC (NCT03764293).

Bispecific antibody therapy

Bispecific antibody (BsAbs), in contrast to monoclonal antibodies, are made primarily using recombinant DNA technology and may concurrently and precisely bind two antigens or epitopes[50]. BsAb can target immunological checkpoints and tumour-associated antigens (TAAs) to alter immunosuppression in the tumour environment. It can also directly increase the action of immune cells against tumours. As a result, they outperform monoclonal antibodies in aspects of synergistic effects and can mediate a wide range of particular biological effects. Most of the time, BsAbs bridge the gap between immune cells and tumour cells to attract and activate them to attack tumour cells[51]. An EpCAM/CD3 bispecific antibody called Solitomab (AMG110, MT110) is humanised. Bispecific T-cell Engager (BITE), which can lyse HCC cell lines almost completely *in vitro* when it binds to gd T cells, is created by joining the anti-EpCAM

single-chain variable fragment (scFv) to the anti-CD3 scFv via a Gly4Ser linker[52]. To remove glypican-3 (GPC3) + HCC cells, a different BsAb called GPC3/CD3 BITE is expected to entice CTL. In one work, an IgG-shaped TriFab, that can be employed to activate two antigens sequentially or for targeted delivery of tiny and large payloads, was created by joining two anti-GPC3 Fab fragments to one asymmetric third Fab-sized binding module using flexible linker peptides[53].

ADOPTIVE CELL TRANSFER

Adoptive cell transfer (ACT), an immunotherapy that harnesses the patient's immune cells or those of a healthier donor to combat cancer, has currently emerged as a crucial component of cancer treatment[54]. ACT could be stimulated and replicated *in vivo* and has a long-lasting anti-tumour impact, in contrast to antibodies or other targeted medications. As a result, the ACT is also known as a "living" therapeutic strategy[55]. Since the majority of ACT's effector cells come from the patient, it is regarded as a highly personalised cancer therapy. ACT is more targeted than chemotherapy because enlarged or genetically altered effector cells can identify and target tumour antigens[56]. Adoptive cell transfer, as opposed to checkpoint inhibition therapy, which passively administers autologous lymphocytes after ex vivo cultivation, aims to improve the outcomes for patients with HCC[57]. Table 2 lists the ACT clinical studies for the HCC treatment that were published at clinicaltrials.gov in the previous 3 years.

Cytokine-induced killer cell: IL-2, interferon-alpha (IFN)-, and anti-CD3 monoclonal antibodies are used to create cytokine-induced killer cell (CIK) cells, a heterogeneous population of immune cells, from human peripheral blood mononuclear cells (PBMC) in a laboratory setting[58]. NK cells, NKT cells, and CTLs make up the majority of CIK cells (CTLs). Through adhesion molecules, CIK can lyse tumour cells and recognise them without the assistance of the major histocompatibility complex (MHC). Shi *et al*[59] showed that autologous CIK cells can effectively enhance the immunological condition of HCC patients by using them to treat primary HCC in a phase I clinical study and

finding that the signs and features of HCC patients were diminished without notable adverse effects. Clinical studies also have demonstrated that CIK cell treatment could be utilised to cure individuals having primary HCC that is inoperable as well as HCC patients who have had their tumours removed. Takayama *et al*[60] published the results of a 150-patient clinical trial of CIK therapy for postoperative HCC. They discovered no significant treatment adverse effects and an 18% lesser recurrence rate in the treatment group than in the control group, indicating that CIK cells treatment can lower reappearance rates and lengthen recurrence-free survival in patients with postoperative HCC[60]. Additionally, conventional therapies and CIK cell therapy have frequently been combined by researchers. As opposed to TACE alone, TACE combined with CIK cells may increase progression-free survival in HCC patients. T and NKT cells dramatically increased with the addition of CIK cells to local radio frequency hyperthermia, whereas alpha-fetoprotein (AFP) levels decreased, according to research by Wang *et al*[51]. Although 17% of patients reported experiencing pyrexia, chills, myalgia, and fatigue, these symptoms were not severe enough to need therapy to be stopped[62]. These results suggest that the combination of CIK cells having TACE or RF hyperthermia is a harmless and efficient therapy for HCC patients.

Tumor-infiltrating lymphocytes: TIL is a part of the host immune response against tumours, which also consists of regulatory T cells (Treg), T cells, NK cells, and B cells[63]. TIL is 50-100 times highly effective as compared to lymphokine-activated killer cells at curing mice with severe metastatic cancer, according to experiments[64]. A phase I clinical trial in individuals with primary HCC established the viability of TIL treatment[65]. Because TILs are derived from surgical tumour tissues and can detect several antigens, they have a stronger tumour-inhibitory effect than medications that target certain antigens or mutations. TILs in HCC are uncommon, however, prior research has shown that despite this, they can significantly affect tumour recurrence and patient prognosis[66]. Clinical research with 150 HCC patients demonstrated that adoptive TIL treatment improved recurrence-free survival following liver resection[60].

Patients who received surgical resection of their HCC and had considerable lymphocyte infiltration had a lower recurrence rate and a greater survival rate than those who did not have significant lymphocyte infiltration[67]. However, isolating TILs from HCC tumour tissues and expanding them *in vitro* is difficult. Additionally, only a small number of HCC patients could survive lymphocyte deletion, which is necessary before TIL infusion[68].

Chimeric antigen receptor T Cell: ACT research is now focused on Chimeric antigen receptor T Cell (CAR-T) treatment, novel immunotherapy for cancer wherein T cells are genetically altered to recognise particular TAA[69]. Hematological cancers can be successfully treated with CAR-T cell therapy. The FDA has approved CAR-T cells that target CD19 and B-cell maturation antigen (BCMA) for the management of acute B-cell lymphocytic leukaemia, several lymphomas, and multiple myeloma[70]. CAR-T therapy for liver malignancy is currently being developed because of the variety of solid tumours, the absence of specific targets, and the liability to the tumour microenvironment[71]. Conventional T cells have a T cell receptor (TCR) structure that is reliant on MHC antigen presentation. The CAR structure is independent of MHC antigen presentation, evades MHC molecule restriction, and resolves the issue of tumour immune escape caused by MHC downregulation[72] (Figure 4). To date, an increasing series of clinical trials have been carried out to show the effectiveness of CAR-T cell treatment in solid tumours. GPC3, a 580 amino acid long heparan sulphate proteoglycan, is overexpressed in HCC yet not at all in healthy tissues[73,74]. Gao *et al*[75] created CAR-T cells targeting GPC3 for the first time and showed that GPC3 CAR-T cells can successfully eradicate the proliferation of HCC cells both *in vitro* and *in vivo*. In a recent study, researchers found that by dividing the CAR design into two parts, HCC tumours may be removed with a lower level of proinflammatory cytokines (split GPC-3 CAR-T cells)[76]. A different study used patient-derived xenograft (PDX) HCC models and showed that GPC3 CAR-T cells reduced tumour development, however to differing degrees because of variations in PDL1 expression on cancer cells[77]. This demonstrates

that combining CAR-T treatment and ICIs is a workable way to increase the efficiency of curing PD-L1- + ve HCC. The secreted glycoprotein known as AFP is extensively expressed in foetuses but only modestly so in adults. However, AFP is once again expressed when HCC manifests in adults[78]. Traditional CAR-T cells are only able to identify tumour surface antigens; they are unable to identify intracellular antigens. Liu *et al*[79] developed some distinctive CAR-T cells that could bind to the AFP158-166 peptide-MHC complex and then damage HLA-A*02:01+/AFP+ tumour cells because all intracellular antigens are delivered by MHC class I molecules. A phase I clinical trial (NCT03349255) assessing the security and performance of CAR-T cells in patients with AFP-expressing HCC was successfully finished in the interim. As a result, using CAR-T cells to target intracellular antigens is an effective way to treat HCC. The c-Met tyrosine kinase receptor could promote hepatocyte survival, regeneration, and proliferation[80].

HCC development and progression can be aided by c-Met overexpression. As a result, c-Met is being evaluated as a possible target for the therapy of HCC. Jiang *et al*[81] developed CAR-T cells that simultaneously targeted c-Met and PD-L1, and they discovered that these cells significantly cytotoxic attacked c-Met+ PD-L1+ HCC cells. NKG2DL ligands (natural-killer group 2 member D ligands) are present in a variety of primary malignancies, along with HCC, yet not found in healthy tissues[82]. Therefore, NKG2DL might be a good candidate for HCC immunotherapy. Innovative NKG2D-CAR-T cells that specifically target NKG2DL expressed on HCC cells were developed by Sun *et al*[83]. They also discovered that NKG2D-CART cells only targeted ruptured HCC cells with elevated NKG2DL expression and had no effect on the NKG2DL negative cell line. The xenograft model further demonstrated that NKG2D-CAR-T cells may prominently limit *in vivo* tumour growth. Type I transmembrane glycoprotein CD147 was discovered in high levels in HCC and various solid tumours[84]. When Tet-On inducible CD147-CART cells were administered Dox, Zhang *et al*[85] found that Tet-On inducible CD147-CART cells can successfully rupture numerous HCC cell lines *in vitro* and reduce the development of cancer cells in the HCC xenograft model. The safety of hepatic artery infusions (HAI) of CD147-CART cells for severe hepatocellular

carcinoma was recently evaluated in a phase I trial (NCT03993743). Mucin 1[86], EpCAM[87], and CD133 are further potential targets antigens for HCC CAR-T treatment[88,89]. The majority of the above-stated targets, though, are TAAs, which are expressed at low levels in both cancerous and healthy cells, leading to both on- and off-target toxicities in normal tissue. The most significant objective for future researchers will be to identify new targeted antigens and improve the effectiveness and safety of CAR-T treatment for HCC.

Chimeric antigen receptor NK cells: The distribution of NK cells in the liver is substantially greater as compared to the peripheral blood and spleen. As a result, NK cells are thought to be important in the prevention of HCC and are assumed to be a promising source for cell therapy in the treatment of HCC[90]. The technique utilised to produce CAR-T cells could likewise be employed to generate CAR-NK cells from NK cells. Furthermore, because CAR-NK cells have a shorter lifespan as compared to CAR-T cells, they can lower the danger of autoimmune response and tumour transformation[91]. CAR-NK cells could also be generated from various resources, such as the peripheral blood mononuclear cells (PBMC), NK92 cell line, umbilical cord blood (UCB), and induced pluripotent stem cells (IPSC). Since CAR-NK cells could be delivered "off-the-shelf", abolishing the requirement for customised and patient-specific products, so is the case with CAR-T therapies currently, the danger of syngeneic xenograft responses and graft-versus-host (GVHD) sickness is reduced[92]. The therapeutic potential of GPC3-specific CAR-NK cells for HCC was investigated by Yu *et al*[93] in 2018. *In vitro* co-culture experiments with GPC3$^+$ HCC cells revealed considerable cytotoxicity and cytokine generation by GPC3-specific CAR-NK cells. Additionally, cytotoxicity was unaffected by soluble GPC3 and TGF-b, and hypoxic (1%) conditions did not significantly alter anti-tumour efficacy. In a different study, Tseng *et al*[94] developed CD147-specific CAR-T and CAR-NK cells to treat HCC using CD147 as the target antigen. The results indicated that numerous malignant HCC cell lines *in vitro*, as well as HCC tumours in xenograft and PDX animal models, could be

successfully eliminated by CD147-specific CAR-NK cells. In a human CD147 transgenic mouse model, GPC3-synNotch-inducible CD147-specific CAR-NK cells preferentially destroy $GPC3^{+}CD147^{+}$ however not $GPC3^{-}CD147^{+}$ HCC cells and don't induce substantial on-target/off-tumour damage. The lack of effective gene transfer techniques in primary NK cells is one of the fundamental obstacles to CAR-NK immunotherapy. Numerous current investigations have shown that retroviral vectors may successfully transduce larger NK cells with efficiency ranging around 27% to 52% after just one round of transduction[95]. The insertional mutations linked to retroviral transduction and the detrimental actions on primary NK cell survival, however, are the most significant drawbacks of this strategy in a clinical setting.

TCR-engineered T Cell: TCR-Engineered T Cell (TCR-T) cells are created by altering T cells with the exogenous TCRs gene to precisely identify tumour antigen peptides-MHC complexes[96]. All tumour-derived proteins could indeed be broken down via proteasomes and displayed by MHC, allowing TCR-T cells to target both tumour surface and intracellular antigens. TCR-T therapy should therefore be more widely applicable compared to CAR-T therapy. HCV infection affects 130-150 million people worldwide and could result in conditions like HCC[97]. Spear *et al*[98] engineered T cells with a high affinity, HLA-A2-restricted, HCV NS3:1406-1415-reactive TCR to create HCV-specific TCR-T cells. The findings demonstrated that HCV-specific TCR-T cells may cause a relapse of established HCV+ HCC *in vivo*, indicating that HCV-specific TCR-T therapy might be an efficient technique for curing HCV-associated HCC. A lesser proportion of HCC tissues produced from HBV infection detain HBV gene expression, which may become TCR-T targets. In 2011, Gehring *et al*[99] identified TCR-T cells specific for the HBV surface antigen in PBMC of patients with chronic HBV and HBV-related HCC. These HBV-specific TCR-T cells may recognise HCC tumour cells and have a variety of uses.

Additionally, a phase I clinical experiment was carried out to assess the safety and efficiency of HBV-specific TCR-T in stopping HCC reappearance following

transplantation of the liver[100] (NCT02686372). As previously stated, AFP is another TAA linked to HCC. Docta *et al*[101] recently mentioned the discovery of a human HLAA2/AFP158-specific TCR, and AFP-specific TCR-engineered autologous T cells from HCC patients are now being used in a clinical investigation (NCT03132792). In 2018, we found a large number of HLA-A2/AFP158-specific TCRs from HLA-A2 transgenic mice using an immunisation technique that comprised recombinant lentiviral priming and peptide boosting[102]. These TCRs were expressed on human T cells, and both *in vitro* and *in vivo* tests revealed strong anti-tumour efficacy. Additionally, the outcomes of comprehensive X-scans showed that there is little to no cross-reactivity between these TCR T cells and human cells. A clinical trial including these TCRTs for the treatment of HCC patients has begun (NCT03971747). GPC3[103], New York oesophagal squamous cell cancer 1 (NY-ESO-1)[104], and human telomerase reverse transcriptase (hTERT) are further options for HCC TCR-T treatment[105]. However, because TCRs are promiscuous, TCR-T cells might cross-react with healthy tissue MHC-peptide complexes, resulting in off-target damage. TCRs produced in mice and humans can both cause off-target harm.

Despite being produced in humans, the melanoma-associated antigen (MAGE)-A3/HLA-A1TCR induced severe cardiac damage via targeting the cardiac muscle protein titin[106]. However, while NY-ESO-1 TCRT has demonstrated clinical anti-tumour efficacy, the majority of similar TCRTs haven't been confirmed to be useful for patients. Various aspects could be addressed to increase the anti-tumour efficacy of TCR-T therapy, such as extending TCR-T *in vivo* survival, enhancing tumour infiltration, and preventing T cell depletion.

CYTOKINES RELATED TO ADVANCED HCC

Key elements of the immune system, cytokines are crucial in the immunological response to cancer. Recently, there has been a lot of interest in using cytokines to treat cancer as the immune system is competent in identifying and eliminating cancer cells[107]. In 1986, the United States FDA approved the use of IFN-α as the first cytokine

for the treatment of hairy cell leukaemia (HCL)[108]. In 1992, the use of high-dose IL-2 as a therapy for metastatic renal cell carcinoma (mRCC) and metastatic melanoma was approved (MM). Besides its original approval, IFN-α has been used to treat a variety of cancers, including mRCC, melanoma, follicular lymphoma when combined with bevacizumab, and Kaposi's sarcoma linked to the AIDS virus. Numerous factors can be used to categorise advanced diseases in HCC. The major liver disease societies approve the BCLC staging, which has been well verified[109]. In the BCLC staging system, level C is the advanced stage, and stage D is the final stage[110]. Multiple cytokines and stimulatory substances have been linked to the likelihood of progressive disease in HCC patients.

Interleukin-10: IL-10 is a highly effective anti-inflammatory cytokine[111]. The majority of activated immune cells, along with macrophages and monocytes, secrete IL-10, which reduces the secretion of inflammatory mediators, prevents the formation of antigens, and controls several other immunological properties[112]. While its significance in viral infections is widely known, its function in HCC is less clear. Interpreting IL-10 data for HCC is complicated by the finding that HCC patients have greater levels of IL-10 than cirrhotic patients and healthy controls, although not in viral hepatitis patients[113]. In one investigation, it was discovered that patients with IL-10 levels of more than 12 pg/mL had inferior post-operative results. It has also been investigated how IL-10 affects HCC which is incurable. IL-10 levels in serum were revealed to be a poor prognostic predictor in a prospective analysis of 74 patients with unresectable HCC, with a considerably poorer median survival (3 mo *vs* 12 mo; $P = 0.02$)[114]. Patients with elevated IL-10 levels in serum performed markedly worse even than patients with decreased IL-10 levels in a larger trial of 222 patients with unresectable HCC (mainly related to HBV) [hazard ratio (HR) 2.2][114]. Elevated IL-10 levels dramatically decreased overall survival in patients with advanced disease (BCLC stage C) from 10.2 mo to 3.5 mo, compared to those with low IL-10 levels, who exhibited average mortality of 10.2 mo[115].

Interleukin-37β: The wideset of the 5 distinct isoforms of IL-37β (named IL-37a-e) is IL-37β[116]. This cytokine, which is generated by epithelial cells, macrophages, and monocytes inhibits the production of pro-inflammatory cytokines as well as EMT by activating the STAT3/IL-6 pathway[117]. Additionally, recombinant IL-37β studies *in vivo* on mice showed that the tumour volume was lowered compared to the untreated controls[118]. IL-37 β serum levels were found to be negatively correlated with the survival of advanced HCC in a study of HBV-related HCC patients. Higher levels of IL-37β in the subjects' bodies led to prolonged overall survival and disease-free survival[119]. Similarly, in a cohort with a high percentage of HBV-HCC, higher IL-37β expression in HCC tissues was linked with better DFS and survival[118]. These HCC findings, along with decreased IL-37β expression and production in metastatic tumours, suggest that IL-37β is implicated in signalling pathways that control metastasis and may have an impact on histopathologic prognosis[119].

CC Chemokine Ligand 20: CC Chemokine Ligand 20 (CCL20) (also known as macrophage inflammatory protein-3 alpha) links with CC chemokine receptor 6 (CCR6), causing immune cells to be chemoattracted to the zone of inflammation. CCL20 have been demonstrated to play several functions in rheumatoid arthritis, general inflammation, and various cancers[120]. *In vitro* and *in vivo* studies have revealed a function for the CCL20-CCR6 axis in HCC generation, development, and invasion[121]. Furthermore, in an investigation of 33 specimens from 22 patients, CCL20 overexpression was detected in tumours, indicating a function in hepatocarcinogenesis[122]. A role in tumour invasion, angiogenesis, and the development of hepatic malignancies has been suggested by several studies that have detected significant amounts of CCL20 and its receptor CCR6 in HCC and colorectal cancer liver metastases. The relationship between CCL20 expression and tumour grading, however, was only identified in one small study including 11 HCC patients (TNM stage 3 *vs* 2)[123]. Tumour-infiltrating regulatory T cells may be preferentially

attracted to the tumour via the CCR6-CCL20 axis, according to an investigation of 293 HCC patients. This study found a favourable correlation between the number of regulatory T cells infiltrating the tumour and CCL20 expression in the tumour. Significantly, patients with HCC who had larger levels of tumour-infiltrating regulatory T cells had a worse prognosis[124].

IL-6: The predictive importance of pre-treatment blood IL-6 levels in the condition of advanced HCC was examined in research on 128 sorafenib-treated HCC patients divided into a finding and validating cohort. In both groups, a high level of serum IL-6 before therapy was a reliable indicator of poor overall survival. The time to progression and progression-free survival were comparable independent of pretreatment IL-6 levels, hence there was no association with sorafenib efficacy. Furthermore, pretreatment IL-6 levels were not associated with macrovascular invasion or extrahepatic dissemination[125]. Although promising, further research, which is presently being undertaken, is required to establish the function of IL-6 in HCC therapeutic response. Interestingly, recent research in cellular models has shown that blocking IL-6-related pathways reduces sorafenib resistance[126].

Angiopoietin-2: A major regulator of vascular development, angiopoietin-2 (ANG-2) is nearly entirely generated by epithelial cells and supports the actions of other endothelial-acting cytokines[127]. Superior pretreatment In the SHARP research, the first randomised placebo-controlled experiment to assess the effect of sorafenib in advanced HCC as well as the prognostic significance of multiple cytokines, ANG-2 levels were associated with shorter overall survival in the overall cohort as well as in the sorafenib arm. However, no link was discovered between sorafenib-associated survival and therapy interaction analyses. Nonetheless, those with higher plasma ANG-2 levels at week 12 had a smaller total survival and time to progression than those who did not have an increase in plasma levels[128]. A year after, the Okayama Liver Group (Japan) then performed a longitudinal analysis and retrospective investigation of serum

cytokines in 2 independent sorafenib-treated advanced HCC cohorts. Increased pretreatment ANG-2 levels, as in the SHARP research, were linked to a worse overall survival rate[129]. Furthermore, when the researchers assessed ANG-2 in a prospective cohort, the difference was not statistically significant, most likely because there were fewer patients, even though patients with progressive disease had higher ANG-2 levels at the beginning of treatment than those having the non-progressive disease. ANG-2 levels, on the other hand, increased only in patients with worsening disease over time[129].

Hepatocyte growth factor: *In vitro* and animal models have revealed that hepatocyte growth factor (HGF) could either promote or prevent the development of HCC[130]. In both the sorafenib arm and the overall cohort of the SHARP trial, increased pretreatment plasma HGF levels were a significant independent predictor of worse overall survival. Surprisingly, sorafenib was linked with a greater benefit in terms of total survival and time to progression in patients with reduced HGF amount at the start of therapy. Additionally, in the therapy arm, a reduction in median HGF plasma levels at 12 wk was not associated with overall survival but was associated with a lengthier duration of progression[128]. In a prospective cohort, the Okayama Liver Group discovered pretreatment serum HGF levels to be a potential independent marker of overall survival, albeit significance was lost following multivariate analysis. Additionally, only in the retrospective cohort, HGF pretreatment levels were higher in patients with the progressive disease compared to non-progressive disease[131].

VEGF: Multityrosine kinase inhibitors, such as Sorafenib, target VEGF signalling as a major cytokine causing angiogenesis. The SHARP study looked at VEGF as a predictive marker in addition to ANG-2 and HGF. Similar to ANG-2, higher pretreatment VEGF amounts were linked to reducing survival. Nevertheless, the Sorafenib arm did not convert its predictive value. Interesting, the Sorafenib group's average plasma VEGF levels were significantly greater than those of the placebo group[128]. Furthermore,

VEGF levels were shown to be higher in individuals those later experienced disease advancement compared to non-disease progression in a retrospective study on HCC patients administered with sorafenib by the Okayama Liver group. Furthermore, higher baseline VEGF levels were linked to shorter overall survival and progression-free survival, which is consistent with the results of the SHARP trial. Nevertheless, the multivariate analysis failed to identify VEGF as a predictor of total survival[131]. Later, the same study team verified this finding in a prospective cohort of HCC patients receiving sorafenib therapy[129]. A decrease in plasma VEGF levels at 8 wk from baseline was shown by Tsuchiya *et al*[132] to be an independent predictive factor linked with one-year survival following Sorafenib therapy in a small cohort of HCC patients.

THERAPEUTIC VACCINE

Tumour vaccines are substances that boost particular immune reactions to tumour antigens. For patients whose tumours don't have a lymphocytic infiltration that can be treated with ICI, immune-enhancing techniques may be helpful. One of the earliest immunotherapeutic methods applied in HCC among these methods is vaccination. In comparison to studies looking at adoptive cellular therapies and checkpoint inhibitors, mentioned clinical trials for such tumour vaccines in HCC are presently being conducted far less frequently. This is partly due to earlier disappointing trial results as well as the relative ineffectiveness of other tumour vaccines. This could be because it was formerly challenging to identify the proper tumour antigens, but that is now possible because of recent technology advancements permitting huge parallel DNA sequencing. Therefore, inducing an immune response remains a promising treatment strategy for HCC, either by itself or more likely in combination including an immune modulator. To date, several agents have been looked at in this area.

The high recurrence rate cannot be avoided by current treatments for advanced-stage HCC due to their insufficient efficacy. Vaccines have already been suggested as potential solutions to this problem, able to enhance clinical results when used in conjunction with existing recognised systemic treatments. Though they are harmless

and have immunologic effects, the few trials that have been undertaken so far have only shown disappointing/poor clinical results/efficacy[133]. The majority of them are quite old, and they are all phase I or II trials (Table 1). Peptide-based or DC-based vaccines are two categories of HCC vaccination methods now in use (Table 1). Additionally, this could be further divided into peptide-loaded and tumour lysate-pulsed DCs. Epitopes from the oncofetal antigens AFP[134,135], glypican 3 (GPC-3)[136], and the hTERT peptide GV1001 are the principal antigens used for peptide-based vaccinations in HCC[137]. Additionally, peptides from AFP and AFP coupled with MAGE-1 and GPC3 have been tested in clinical trials utilising DCs loaded with peptides[138]. HepG2 (a hepatoma cell line) and autologous tumour lysates have both been employed in clinical trials[138,139]. All of these vaccination methods are risk-free, and even though clinical outcomes were subpar, the majority of them produced antigen-specific immune reactions without inducing toxic or autoimmune reactions. The various characteristics of HCC tumours and the vaccine's design, or even the combination of the two, may be to blame for this poor performance.

TAA-based vaccines are prone to tolerance mechanisms because they are not entirely tumour specific, which is reflected in the dearth of highly reactive clones against them[140]. As a result, responses are typically insufficient to stop tumour development. Additionally, as previously mentioned, the immunosuppressive HCC environment is not favourable for immune responses since it is a clear, prominent reflection of the intrinsic hepatic environment[141]. In addition, the restricted amount of TAAs expressed in HCC tumours makes it possible for immune evasion through Ag loss. Regarding their manufacturing, antigenic repertoire, and the variety of patients to be cured, the various vaccination modalities employed in HCC patients each have their benefits and drawbacks, which are listed in Table 2. Clinical trials for HCC have not yet fully utilised all vaccine strategies. Vaccination clinical trials in HCC with reported results are shown in Table 3. By changing the vaccination platform or adding new tumour antigens, vaccines may be improved. The first entails techniques like tumour cell fusion and *in vivo* DC-targeted immunization[140,141]. The assumption behind targeted vaccines is that

by linking the antigen to antibodies, ligands, or viruses, non-target cell Ag delivery is prevented, potentially reducing any negative effects. In a recent preclinical model, we showed that this tactic increases the therapeutic effectiveness of ICI if it is paired with other treatments[142]. WT-1, ROBO1, FOXM1, and NY-ESO-1 are additional TAAs that may be utilised in upcoming clinical studies about a larger antigenic repertoire[143,144]. Finally, future HCC anticancer vaccination strategies should take into account neoAgs because of their tumour selectivity and possible increased immunogenicity[145].

Dendritic cells

DCs are expert antigen-presenting cells with a wide range of responsibilities, including the processing, presentation, and absorption of TAAs. Allogeneic DCs make up a significant portion of vaccines because they provide the antigen as well as the secondary co-stimulation required to effectively elicit a T cell activation. DCs from peripheral blood are isolated, grown ex vivo, and activated with cytokines like granulocyte-macrophage colony-stimulating factor (GM-CSF) to produce primed DCs for reinfusion. Another appealing, targeted strategy involves injecting such cells to cause the activation of effector cells, trigger a cascade of tumour rupture, and cause more TAA secretion[146]. To boost the effectiveness of the vaccine and optimise this TAA priming, a variety of approaches may be used. DCs might be cultured with tumour lysate or the fusion of DCs and tumour cells, or they may be transduced with DNA or RNA encoding recognised TAAs[141]. Ilixadencel (pro-inflammatory allogeneic DCs activated by GM-CSF and IL-4) was injected intratumorally in a newly published phase I trial, where 17 patients received it either alone or in conjunction with sorafenib. Tolerability evaluation was the main goal. There was just one grade 3 adverse event noted. Enhanced tumour-specific $CD8^+$ T cells were seen in the peripheral blood of 73% of the 15 evaluable patients, indicating at least a successful immune-provoked response[147].

Peptide vaccines

An alternate choice for eliciting a powerful immune response is peptide vaccinations. However, despite immunological surrogates, like the generation of GPC-3 reactive cytotoxic T cells in one phase I trial, being successful, this has not led to clinical results[148]. GPC-3 and MRP3 have shown any efficacy in inducing a T cell response rate over 70%, while other TAAs such as SSX-2, NY-ESO-1, hTERT, and MAGE-A all induce considerably lower rates. This is despite a plethora of TAAs being found in HCC trials using AFP[149].

Oncolytic viruses

A more recent development in the arena of tumour vaccines is the use of oncolytic viruses. These therapeutically useful viruses are targeted to preferentially replicate in cancer cells. To date, they have been predominantly introduced by intra-tumoral injection. The modified poxvirus JX-594 remains the lead oncolytic virus of interest in clinical trials about HCC. As an immunotherapeutic agent, it piqued considerable interest when it conferred a dose-related survival benefit (median of 14.1 mo compared to 6.7 mo) in phase II dose-finding trial of 30 patients[150,151]. The global, randomized, open-label, phase III study of Pexa-Vec (JX-594; an oncolytic vaccinia virus which selectively targets cancer cells) is currently recruiting patients with advanced HCC to two arms of vaccination with sorafenib *vs* sorafenib alone[152]. We excitedly await the results of this, particularly as a combination therapy.

Immunotherapy toxicities and management

Immune-related adverse events: Immune checkpoint molecules serve an important function in immune homeostasis. Inhibitory immune checkpoint molecules, like PD-1 and CTL-4, are particularly important for balancing activation of T-cell and self-tolerance[155]. ICIs targeting PD-1 or CTL-4 may thereby produce a range of immune-related adverse events (IrAEs) by increasing self-immunity. IrAEs can affect every organ system and can range from a minor rash to life-threatening consequences. IrAEs caused by PD-1/PD-L1 inhibition are dose-independent[156]. Unlike anti-PD-1/PD-L1

agents, the risk of antiCTL-4-related adverse events is dose-dependent[157]. The gastrointestinal tract and skin were the most often affected organ systems by both anti-CTLA-4 and PD-1/PD-L1 inhibitors, whereas the endocrine systems and liver were less frequently affected[156,157]. In meta-analysis of phase 2/3 trials patients treated with ICIs, found that rash was the most prevalent all-grade IrAE, with colitis and increase in aspartate aminotransferase level being the most common high-grade IrAEs. Ipilimumab was related with a significantly greater incidence of rash and colitis when compared to anti-PD-1/PD-L1 drugs[158].

Management of IrAEs: Because IrAEs are linked with a wide range of aggravating factors in the context of HCC, hepatologists faces significant hurdles in detecting and managing them. First, liver cirrhosis causes progressive immunological dysfunction, including immune deficiency as well as systemic inflammation[159]. Thus, in these patients, the liver-related immune homeostasis is already significantly disrupted. Second, cirrhosis-related hepatic and extrahepatic problems may overlap with or intensify symptoms caused by IrAEs, hampered early and rapid detection, which is compulsory concerning the consequence of potentially life-threatening episodes. Thus, before starting ICI therapy, individuals with HCC should be carefully selected and evaluated. In general, IrAE management is built on three pillars. First, close monitoring is required, including weekly clinical controls and, depending on the severity of the incidents, hospitalisation. Importantly, individuals with severe IrAEs should be referred to a specialised centre as soon as possible. This is especially important in patients with liver cirrhosis because distinguishing between cirrhosis-associated complications and IrAEs can be difficult, and premature discontinuation of an effective antitumor therapy or starting of steroid therapy in cirrhotic patients can have serious consequences[160]. Second, depending on the type and severity of IrAEs, ICI therapy may need to be either interrupted or permanently discontinued. Apart from PD-1/PD-L1-driven rash, adrenal insufficiency, nephritis and hypothyroidism, which recover after one month of cessation, permanent termination of ICI therapy should be

considered for higher grade IrAEs[160]. Re-exposure to ICI therapy after withdrawal, on the other hand, is associated with a significant risk of IrAE recurrence[161]. Third, for IrAEs of greater than grade 2, glucocorticoids (0.5 to 2 mg/kg/d prednisone oral or intravenous depending on the type and severity of IrAEs) may be needed. Cutaneous IrAEs are treated with topical, oral, or intravenous glucocorticoids and topical or oral antihistamines, depending on the severity of clinical presentation. Cutaneous IrAEs range from commonly observed rash or pruritus to very less common but more severe disorders such as Stevens-Johnson syndrome[160]. Steroids should be continued for at least three days and then tapered over a period of one to four weeks[162,163].

Differential diagnosis is required for gastrointestinal IrAEs, notably colitis and/or diarrhoea, in order to rule out infectious disorders and pharmacological side effects[160]. In the event of glucocorticoid failure, immunosuppressive medication (such as vedolizumab or infliximab) should be started as soon as possible[164]. The diagnosis and management of immune-related hepatitis in HCC patients receiving ICI therapy is particularly difficult[160]. As a result, seeking the advice of an experienced hepatologist as soon as possible is strongly advised. Intrahepatic tumour growth, hepatitis B virus and/or hepatitis C virus or newly acquired viral hepatitis, cytomegalovirus reactivation, hepatotoxic medication side effects, cholestasis, and ascites should all be ruled out before diagnosing immune-related hepatitis[160]. A liver biopsy should also be considered prior to steroid therapy. For severe cases of immune-related hepatitis, oral or intravenous steroids may be used[160]. After the toxicity has subsided, tapering should be done over a period of 4-6 wk[162,163].

Pneumonitis is a potentially life-threatening IrAE. As a result, the pneumonitis should be followed by a prompt and thorough differential diagnosis, including the elimination of viral etiologies, hepatopulmonary syndrome and porto-pulmonary hypertension[160]. Steroids should be started, and tapering should take 4-6 wk[163]. Following glucocorticoid failure, infliximab or mycophenolate mofetil may be administered[160]. Thyroid-related IrAEs include hypothyroidism and hyperthyroidism caused by thyroiditis. A progressive reduction in thyroid-stimulating hormone in

conjunction with normal or decreasing thyroxine levels should trigger repeated cortisol assessments to rule out immune-related hypopituitarism. In the case of hypothyroidism, thyroxine substitution is only recommended in symptomatic patients[162]. Thyroid antibodies and uptake should be assessed in symptomatic hyperthyroid patients, and beta-blockers and/or carbimazole should be investigated. Asymptomatic patients do not require any special treatment, and ICI therapy should be continued. Patients on ICI therapy should have regular thyroid stimulating hormone and free thyroxine tests. If central hypothyroidism is suspected, each pituitary hormone axis should be examined[160]. Cortisol, corticotropin-releasing hormone, adrenocorticotropic hormone, luteinizing hormone, thyroid stimulating hormone, free thyroxine, follicle-stimulating hormone, oestradiol (premenopausal women), testosterone (men), insulin-like growth factor 1, and electrolytes are examples of these hormones[162,163].

CONCLUSION

Finally, HCC is a multifaceted illness with multiple faces. It avoids early discovery, which provides the best chance of cure by resection/transplant, and systemic treatments are only of marginal efficacy at best, despite recent therapeutic advances. Current advances in immunotherapy and its combinations have altered the HCC treatment landscape, and clinical studies are continuing to pave the path forward. Immunotherapy increases survival rates and provides long-term cancer control in subsets of HCC patients while also minimising side effects. Further research into immunotherapy in combination with current treatments for HCC in the early and intermediate stages may assist a greater spectrum of patients. Continued research into PD-1/PD-L1, TMB, ctDNA, microsatellite stability, DNA mismatch repair, neutrophil/lymphocyte ratio, cytokines, and cellular peripheral immune response will hopefully identify the most reliable marker for selecting and sequencing systemic treatments to achieve the best outcome in HCC patients. Despite such significant treatment advances in HCC, numerous hurdles remain. The scientific community must

figure out how to appropriately sequence these medicines for the best potential response, how to control toxicities, and how to develop indicators to monitor for response and relapse.

Table 1 Immunotherapy with immune checkpoint inhibitor in hepatocellular carcinoma (clinical trial with reported results)

Treatment	Patients (*n*)	ORR%	OS (mo)	Ref.
Atezolizumab	59	17 (5)	NA	[44]
Nivolumab	371	15(4)	16.4	Yau *et al*, 2022
Camrelizumab	217	15 (0)	13.8	Qin *et al*, 2020
Pembrolizumab	278	18 (2)	13.9	Finn *et al*, 2020
Durvalumab	104	11 (0)	13.6	Kelley *et al*, 2020
Tremelimumab	69	7 (0)	15.1	Kelley *et al*, 2020
Durvalumab and Tremelimumab	159	9.5-24.0 (1-2)	11.3-18.7	Kelley *et al*, 2020
Pembrolizumab and Levantinib	100	36 (1)	22	Finn *et al*, 2020
Nivolumab and Ipilimumab	148	31-32 (0-8)	12.5-22.8	Yau *et al*, 2020
Atezolizumab and Bevacizumab	336	27 (6)	NE	Finn *et al*, 2020
Nivolumab and Cabozantinib	36	14 (3)	21.5	Yau *et al*, 2020
Nivolumab, Ipilimumab and Cabozantinib	35	31 (6)	NE	Yau *et al*, 2020

OS: Overall survival; NA: Not available; NE: Not evaluable; ORR: Overall response rate.

Table 2 Advantages and disadvantages of vaccination strategies used in hepatocellular carcinoma

Vaccine type	Advantages	Disadvantages
Peptides	Easy to prepare; known target antigen	Adjuvants are needed; restricted antigen repertoire; restriction to human leukocyte antigen

Dendritic cells	Adjuvants are not needed	Individualized production
Peptide pulsed	Known target antigen	Restriction to human leukocyte antigen; restricted antigen repertoire
Protein pulsed	No restriction to human leukocyte antigen; known target antigen	Protein synthesis is more interesting; restricted antigen repertoire
Tumour lysate pulsed	Not human leukocyte antigen restricted full Ag repertoire; available	Tumour samples not always available; the predominance of self-antigens that may eclipse tumour antigens
Cell line pulsed	Not human leukocyte antigen restricted; unlimited Ag source	Responses against cell line-specific Ag

Table 3 Vaccination clinical trials in hepatocellular carcinoma with reported results

Vaccine	Patient inclusion criteria	Patients (*n*)	Immune response (%)	Observations	Ref.
AFP HLA-A*02 restricted peptides + IFA	AFP + tumours from (stage IV patients)	6	66	Increased CTL response	Butterfield *et al*, 2003
DCs pulsed with autologous tumour lysate	Unresectable HCC	8	62	Immune response generation	Iwashita *et al*, 2003
DCs pulsed with autologous tumour lysate	Advanced HCC	31	0	Improved survival	Lee *et al*, 2005
DCs pulsed with AFP HLA-A*02 restricted peptides	Stage IV patients with surgery chemotherapy	10	60	No clinical responses	Butterfield *et al*, 2006

DCs pulsed with hepatoma cell-line (HEP-G2) lysate	No other therapeutic option	35	11.4	Evidence of antitumor efficacy	Palmer *et al*, 2009
Gv1001 peptide + GM-CSF + cyclophosphamide	Advanced-stage HCC with no previous antitumor treatment	37	0	The immunological response is not detected	Greten *et al*, 2010
GPC3 HLA-A*24:02 and HLA-A*02-restricted peptides + IFA	Metastatic HCC or Advanced HCC	33	91	Antitumor efficacy	Sawada *et al*, 2012
DCs pulsed with fused recombinant proteins (AFP, MAGE-1 and GPC-3)	Surgical resection and locoregional therapy	12	92	Improved survival	Lee *et al*, 2015
GPC3 HLA-A*24:02 and HLA-A*02-restricted peptides + IFA	Vaccines as Adjuvant therapy	41	85	Improved recurrence rate	Sawada *et al*, 2016
AFP HLA-A*24:02 restricted peptides + IFA	Stage B/C tumours	15	33	Increased CTL response	Nakagawa *et al*, 2017

Clinical trials with no published results have been excluded from the review. Incomplete Freund's Adjuvant, *immunologic response measurement was not appropriate. HCC: Hepatocellular carcinoma; AFP: Alpha-fetoprotein; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; GPC-3: Glypican-3; IFA: Incomplete Freund's Adjuvant; MAGE: Melanoma-associated antigen.

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48	Min Yu, Hong Luo, Mingliang Fan, Xiuqi Wu, Bizhi Shi, Shengmeng Di, Ying Liu, Zeyan Pan, Hua Jiang, Zonghai Li. "Development of GPC3-Specific Chimeric Antigen Receptor-Engineered Natural Killer Cells for the Treatment of Hepatocellular Carcinoma", Molecular Therapy, 2018 Crossref	9 words — < 1%
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51 Omid Kooshkaki, Afshin Derakhshani, Negar Hosseinkhani, Mitra Torabi et al. "Combination of Ipilimumab and Nivolumab in Cancers: From Clinical Practice to Ongoing Clinical Trials", International Journal of Molecular Sciences, 2020

Crossref

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52 Qi Liu, Ye Tian, Yanyan Li, Wei Zhang et al. "In vivo therapeutic effects of affinity-improved-TCR engineered T-cells on HBV-related hepatocellular carcinoma", Journal for ImmunoTherapy of Cancer, 2020

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53 Uri Greenbaum, Ecaterina I. Dumbrava, Amadeo B. Biter, Cara L. Haymaker, David S. Hong. "Engineered T-cell Receptor T Cells for Cancer Immunotherapy", Cancer Immunology Research, 2021

Crossref

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54 Wen Bai, Peiwen Wang, Feng Yu. "CAR-T cells shed light on the treatments of fatal liver diseases", Biotarget, 2018

Crossref

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55 Zhicheng Du, Yu Yang Ng, Shijun Zha, Shu Wang. "piggyBac system to co-express NKG2D CAR and IL-15 to augment the invivo persistence and anti-AML activity of human peripheral blood NK cells", Molecular Therapy - Methods & Clinical Development, 2021

Crossref

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