Harnessing immunotherapy for liver recipients with hepatocellular carcinoma: a review from a transplant oncology perspective

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***Abstract*:** Without stringent criteria, liver transplantation for hepatocellular carcinoma (HCC) can lead to high cancer recurrence and poor prognosis in the current treatment context. Checkpoint inhibitors can lead to long survival by targeting coinhibitory pathways and promoting T-cell activity; thus, they have great potential for cancer immunotherapy. Therapeutic modulation of cosignaling pathways may shift paradigms from surgical prevention of recurrence to oncological intervention. Herein, we review the available evidence from a therapeutic perspective and focus on immune microenvironment perturbation by immunosuppressants and checkpoint inhibitors. Partial and reversible interleukin-2 signaling blockade is the mainstream strategy of immunosuppression for graft protection. Programmed cell death protein 1 (PD-1) is abundantly expressed on human liver allograft-infiltrating T-cells, which proliferate considerably after programmed death-ligand 1 (PD-L1) blockade. Clinically, checkpoint inhibitors are used in heart, liver, and kidney recipients with various cancers. Rejection can occur after checkpoint inhibitor administration through acute T-cell-mediated, antibody-mediated, or chronic allograft rejection mechanisms. Nevertheless, liver recipients may demonstrate favorable responses to treatment for HCC recurrence without rejection. Pharmacodynamically, substantial degrees of receptor occupancy can be achieved with lower doses, with favorable clinical outcomes. Manipulation of the immune microenvironment is a therapeutic niche that balances seemingly conflicting anticancer and graft protection needs. Additional translational and clinical studies emphasizing the comparative effectiveness of signaling networks within the immune microenvironment and conducting overall assessment of the immune microenvironment may aid in creating a therapeutic window and benefiting future liver recipients with HCC recurrence.

Keywords: hepatocellular carcinoma, immunotherapy, liver transplantation, microenvironment

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Introduction

The management of hepatocellular carcinoma (HCC) recurrence after liver transplantation is an unmet need in therapeutics. This is because under immunosuppression, cancer develops early during the post-transplant period and has a higher chance of extrahepatic spreading, particularly if the pretransplant HCC status exceeds Milan or University of California San Francisco criteria.[1–3] In this scenario, locoregional therapy, which is the first-line therapeutic choice for recurrent HCC in nontransplant patients, may be ineffective; thus, effective management strategies are urgently required.[4] In liver recipients with disseminated HCC recurrence, sorafenib confers survival benefits but is associated with considerable drug toxicity.[5] Most immunotherapies for organ transplantation are intended to achieve sufficient immunosuppression to prevent organ rejection or limit autoreactivity without impairing

the host's ability to protect against opportunistic infections and malignancies. Thus, patients with new or recurrent malignancies after transplantation often have a relatively low chance of undergoing another surgery; however, in these patients, the effects of other treatment approaches may be nonsignificant.[6] The development of systemic therapy with sustained effectiveness is required urgently.

Cancer immunotherapy modulates the immune system to fight cancer. It includes adoptive cell transfer [chimeric antigen receptor (CAR)-T-cell engineering, T-cell receptor, and tumor-infiltrating lymphocytes], immune checkpoint inhibitors [programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1) inhibitor and CTLA-4 inhibitor], cancer vaccine, and general immunotherapy [interleukins (ILs), interferons, and colony stimulating factors]. Selective upregulation of B7-H1 and the resulting B7-H1/PD-1-mediated T-cell dysfunction in the tumor microenvironment were found to have major roles in impairing spontaneous immune responses and immunotherapy efficacy; thus, a conceptual breakthrough has occurred in understanding the limitations of immune responses to cancer.[7,8] Therefore, checkpoint inhibitors have become a major treatment option for cancers, including HCC, across different anatomic sites of origin. Thus, among cancer immunotherapies, immune checkpoint inhibitors have great potential because they may provide substantially longer disease-free survival than other current target therapies, such as sorafenib.[9–12] Checkpoint inhibitors currently approved as systemic treatments for HCC include nivolumab and pembrolizumab. In the phase I/II study of nivolumab, in which 30% of patients were sorafenib-experienced, the objective response rate was 20% in patients treated with 3 mg/kg nivolumab in the dose-expansion phase and 15% in the dose-escalation (0.1–10 mg/kg) phase, but the exact dose–response relationship was not clear in the latter phase.[7] In the single-arm phase II trial of pembrolizumab, a 17% objective response rate was reported, including complete response in 1% and partial responses in 16% sorafenib-experienced patients.[8] However, no biomarker in both studies could predict potential responders before checkpoint inhibitor therapy was initiated.

In the transplantation setting, patients with HCC are already under immunotherapy, which generates an artificially immunosuppressive microenvironment for graft protection against rejection or other immune-mediated damage.[13] Although rejection is a major concern of checkpoint inhibitor therapeutics in transplant oncology, we aimed to concisely review this strategy from the transplantation perspective and elucidate approaches to modify the immune microenvironment for graft protection and tumor suppression by modulating cosignaling pathways to achieve patient survival. The schematic of the conceptual framework for this review is depicted in Figure 1.

Immunosuppressants create an artificially immunosuppressive microenvironment for graft protection in transplantation unless clinical tolerance develops

The major clinical immunosuppressants used for liver recipients are calcineurin inhibitors, which act dose-dependently and reversibly by partially blocking the IL-2 signaling pathway, which is critical for final T-cell activation. For graft protection in clinical transplantation, calcineurin inhibitors limit the activation of the immune system and thus antigen presentation, resembling *in vivo* partial T-cell anergy.[13] Chimerism can be observed in liver transplant recipients.[14,15] The recipient DNA in post-transplant liver biopsy specimens increased after liver transplantation as early as 1 week, peaked at approximately 30–40 weeks, and was detectable 63 weeks after transplantation.[15] Moreover, most recipient-derived cells showed macrophage/Kupffer cell differentiation, and only up to 1.6% of recipient-derived cells in the liver grafts demonstrated hepatocytic differentiation.[15] Although graft tolerance is the immunological holy grail in transplantation, it may not correlate with chimerism.[16] The major barrier to operational tolerance is the occurrence of allograft rejection, mostly mediated by effector T-cells.[17] Cosignaling pathways (detailed in Figure 1) coordinated by costimulatory and coinhibitory molecules are critical to optimal T-cell effector function.[18]

The PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathways contribute to the immune tolerance of a transplanted organ,[19] and the PD-1/PD-L1 pathway is critical in maintaining liver transplant tolerance in animal models.[20–22] In a human study, PD-L1 was expressed by hepatocytes, cholangiocytes, and cells along the sinusoids in post-transplant liver allografts, and PD-1 was abundantly expressed on allograft-infiltrating T-cells.[22] Moreover, PD-L1 blockade-enhanced the allogeneic proliferative responses of

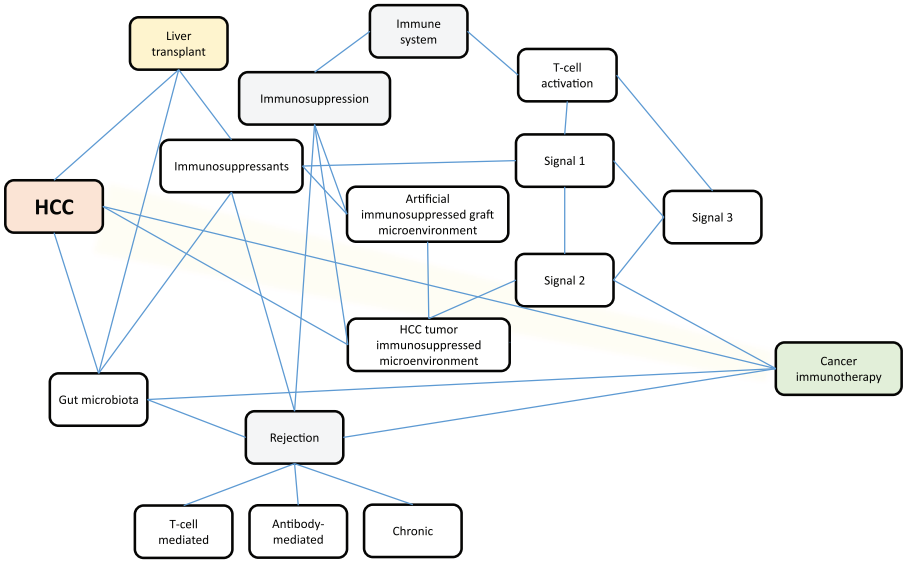

Figure 1. Schematic of the conceptual framework for immunotherapy in liver recipients with hepatocellular carcinoma. The three-signal model of T-cell activation: signal 1: antigen-specific (MHC/HLA-TCR/CD3) signaling; signal 2: cosignaling pathways; signal 3: IL-2-CD25/IL-2R signaling. Cosignaling pathways (including costimulatory and coinhibitory signals) are signals that accompany signal 1 to determine the final fate of T-cell activation. Optimal T-cell effector function requires costimulatory signals, and coinhibitory molecules contribute to immune suppression and exhaustion. Downstream pathways of complete T-cell activation include the IL-2-calcineurin pathway, the RAS-mitogen activated protein kinase pathway, and the IKK-NF-κB pathway.
HCC, hepatocellular carcinoma; IL, interleukin; NF-κB, nuclear factor kappa B
CD3, cluster of differentiation 3
HLA, human leukocyte antigen
IKK, I kappa B kinase
MHC, major histocompatibility complex
RAS, rat sarcoma virus
TCR, T cell receptor

these T-cells, and the interplay between donor- and recipient-PD-1-regulated rejection activity.[23] Although a cosignaling pathway is the intermediate stage in the three-signal model [signal 1 (antigen recognition, HLA-TCR/CD3), signal 2 (costimulation), and signal 3 (cytokine priming)] for T-cell activation,[13] PD-L1 blockade-enhanced allogeneic proliferative responses of graft-infiltrating T-cells may lead to breakthrough rejection under the low maintenance dosage of immunosuppressants in the transplant population undergoing anti-PD therapy for cancer. In summary, major clinical immunosuppressants target signal 1, and cancer immunotherapy targets signal 2.

Liver transplantation is a curative strategy for HCC: patient selection is the primary key to preventing post-transplant recurrence

HCC can be successfully managed through liver transplantation provided that the appropriate criteria are met to predict low extrahepatic dissemination risk before transplantation.[1,17] In previous studies, only 10% of patients meeting the Milan criteria showed HCC recurrence after liver transplantation, with high cure rates.[2,24] Many other criteria to further expand the inclusion of transplant candidates have been developed based on regional experiences; of them, few are superior to the Milan criteria.[17] HCC recurred in many liver transplantation patients who did not meet these criteria.[24] Moreover, the clinical course progressed rapidly even under current treatment modalities for nontransplant HCC patients.[25]

The immunosuppressant load might determine cancer recurrence.[26,27] Tumor-induced inflammation and reduced anticancer immune defense, expressed as a disturbed T-regulatory–CD8 lymphocyte balance, are responsible for increased recurrence after liver transplantation.[28] In addition, immunosuppressant drugs may stimulate cancer cell growth, accelerating tumorigenesis.[25]

The strategy of minimizing immunosuppression, mainly through calcineurin inhibitors, should be explored in the expanding field of transplant oncology.[29] Minimization strategies are justified by the intrinsic immunosuppressed status of cancer patients and the immunological privilege of the liver, which enables substantial reduction in the immunosuppressant load without compromising patient or graft survival.[30–32] By contrast, mammalian target of rapamycin (mTOR) inhibitors interfere with carcinogenesis by inhibiting the PI3K/Akt/mTOR pathway, the key regulator of cell proliferation and angiogenesis.[33,34] mTOR inhibiters are clinically applied for preventing transplant rejection (lower recommended dose, as they target signal 3) and for cancer treatment (higher recommended dose).[35] The combination of either sirolimus or everolimus with reduced-dose tacrolimus is well tolerated and effective in reducing recurrence.[5,36–38] However, there is inadequate evidence for this combination to recommend the optimal serum level of tacrolimus.[5] Whether increased exposure to mTOR inhibitors in liver recipients already exhibiting recurrent HCC exerts net survival benefits requires further investigation.[36–38] As the broadening of HCC indications for liver transplantation becomes the current trend in transplant oncology, minimized and individualized immunosuppressive strategies incorporating cosignaling pathway modulation (e.g. anti-PD therapy) are essential for managing HCC recurrence. In summary, accumulating evidence supports the contribution of immunosuppressants or costimulatory pathway modulation to T-cell activity, creating a therapeutic niche for the management of post-transplant HCC.

Post-transplant HCC recurrence: add-on immunosuppressive microenvironment

The immunosuppressive microenvironment of HCC is attributable to the abundant expression of immune checkpoint molecules, such as CTLA-4, PD-1, TIM3, lymphocyte-activating gene 3 protein, and B- and T-cell attenuator[39–41]; alterations in molecules and cellular pathways involved in antigen processing and presentation; and hypoxia-induced cytokine/chemokines [e.g. IL-10, transforming growth factor (TGF)-β, and arginase] and immunosuppressive molecules (e.g. PD-1 and PD-L1) from HCC and stromal cells that attract regulatory T-cells, cause defects of effector T-cells, and inhibit phagocytosis.[39,42–44] Cancer stem cells are a small subset of cancer cells with high capacity for self-renewal, differentiation, and tumorigenesis.[45] Given their central role in cancer initiation, metastasis, recurrence, and therapeutic resistance, liver cancer stem cells constitute a therapeutic opportunity for achieving cure and preventing the relapse of HCC.[45] Studies have reported that 28–50% of HCC cells express progenitor cell markers such as CK7 and CK19, suggesting that at least a portion of HCC cells have characteristics intermediate between progenitors and differentiated mature hepatocytes.[45,46] Moreover, cancer stem cells, if ever present in the microenvironment and their occurrence is rare in the nontransplantation setting,[45] often express a lower level of major histocompatibility complex class I molecules than do bulk tumor cells[47] and exhibit enriched PD-L1 expression through glycosylation regulation by the epithelial–mesenchymal transition/β-catenin/STT3/PD-L1 signaling axis[48]; this facilitates the immune escape of these cells. Responsiveness to checkpoint blockade immunotherapy is favorable when a local CD8+ T-cell-based immune response occurs in the tumor microenvironment.[49] Accumulating evidence is indicating that the activation of oncogenic pathways in tumor cells can impair the induction of local antitumor immune responses.[49] For instance, WNT-β-catenin signaling reduces T-cell recruitment, MYC function gain inhibits T-cell activation and infiltration, and PTEN loss reduces efficient T-cell priming.[49] As an immunosuppressive microenvironment has already established for HCC cells and stromal cells,[39] artificial immunosuppression achieved by partial T-cell activation suppression for liver graft protection leads to a more complex HCC tumor immunological microenvironment. Whether the selective modification of the tumor microenvironment can restore near-normal anticancer immunity while preventing graft rejection warrants further investigation. In summary, advocating immune capability to discriminate the microenvironment between HCC and liver allograft would be key to successful immunotherapy in this transplantation population.

Remote modulation of gut microbiota in liver transplant oncology

Accumulating evidence is suggesting that gut microbiota play remote roles in the liver microenvironment in transplantation, rejection, and HCC.[50–53] During liver transplantation, fecal microbial communities, such as those of *Actinobacillus*, *Escherichia*, and *Shigella*, demonstrate a substantial decrease, whereas those of Micromonosporaceae, Desulfobacterales, *Sarcina* (Eubacteriaceae), and *Akkermansia* demonstrate a considerable increase.[50]

In patients with acute T-cell-mediated rejection, *Bacteroides*, *Enterobacteriaceae*, *Streptococcaceae*, and *Bifidobacteriaceae* increased, but *Enterococcaceae*, *Lactobacillaceae*, *Clostridiaceae*, *Ruminococcaceae*, and *Peptostreptococcaceae* decreased.[51] Compared with healthy controls, patients with early HCC demonstrated fewer butyrate-producing bacteria but more lipopolysaccharide-producing bacteria.[52] Intervention with probiotics shifts the gut microbial community abundance toward certain beneficial bacteria, including *Prevotella* and *Oscillibacter*, producers of anti-inflammatory metabolites. This subsequently reduces Th17 polarization and promotes the differentiation of anti-inflammatory Treg/Tr1 cells in the gut; this in turn alters proinflammatory cytokine levels in the extra-intestinal tumor HCC microenvironment.[52] Furthermore, human studies have suggested that intestinal microbiota not only play a role in carcinogenesis but also determine the efficacy of chemotherapy and immune checkpoint inhibitors.[53–56] For instance, increased microbial diversity, irrespective of species identity, was associated with improved responses to checkpoint inhibitors in humans.[54,57] Furthermore, patients treated with antibiotics during the course of therapy had decreased antitumor responses.[54] In summary, during decision making for therapeutic strategies in transplant oncology in the future, gut microbial modulation may ensure favorable responses to immunotherapy.

Immunotherapeutics of HCC in the transplantation setting: is rejection the bottom line?

The importance of modulating cosignaling pathways is being recognized in transplant oncology. Selective enhancement of antitumor immunity without graft rejection is the primary contemporary goal. The 4-1BB/4-1BBL blockade is an inducible costimulatory pathway and a major component of CAR-T-cell engineering, which has a central role in CAR-mediated T-cell activation and subsequent tumor clearance. This blockade has potentially lower impact on solid organ transplant outcomes than pathway blocking.[58] The experimental therapeutic strategy involving the targeted delivery of PD-1-blocking single-chain variable fragments by CAR-T-cells can enhance antitumor efficacy *in vivo* and may provide another promising approach in the transplantation setting.[59] The combination of immunosuppressants (for graft protection) with cosignaling modulation (for HCC control) can be considered a strategy in the transplantation settings. Targeting mTOR pathways in combination with PD-1 blockade may increase antitumor efficacy in cancer.[60] The mechanism involves the binding of PD-1 to the downstream mTOR effectors eukaryotic initiation factor 4E and ribosomal protein S6, resulting in the promotion of their phosphorylation.[60] Combining IL-2 treatment with PD-1 blockade has considerable synergistic effects in enhancing virus-specific CD8+ T-cell responses and reducing the viral load.[61] Therefore, combined IL-2 therapy and PD-L1 blockade may be considered a regimen for treating chronic infections and cancer.[61] However, the target of calcineurin inhibition is the IL-2 signaling pathway. Thus, the aforementioned strategy should be applied very cautiously in transplant oncology. Moreover, inhibiting calcineurin using cyclosporine A increases PD-1 ligand expression in B-cells. PD-1^{high} B-cells are an immunosuppressive cell type specifically induced in the HCC microenvironment.[62] Anti-CD20 antibody (clinically used for preventing and treating antibody-mediated rejection) can be used to transiently reduce these cells and attenuate the immunosuppressive microenvironment contributed to by PD-1^{high} B-cells.[63] Experimental models of transplantation in both mouse and nonhuman primates have revealed that CD28-mediated signal blockade impairs the generation of donor-specific antibody, the presence of which is a prerequisite for antibody-mediated rejection.[64–66] Furthermore, transcriptome analyses before and during nivolumab therapy revealed increases in distinct immune cell subsets, activation of specific transcriptional networks, and more pronounced upregulation of immune checkpoint genes in melanoma patients exhibiting responses to nivolumab therapy.[67] When managing multiple medications targeting different immune druggable nodes, maintaining a balance is the key to therapeutic success in transplant oncology. In summary, meticulous titration of immune composition would achieve optimal patient outcomes in this field.

Dose optimization (low dose but within the therapeutic window) of anti-PD therapy as a strategy in transplant oncology

Anti-PD therapy doses lower than the recommended dose may be a practical solution for partially meeting the transplant oncology needs. Pharmacodynamic data of 39 patients with various cancers who received anti-PD-1 therapy indicated a sustained mean occupancy of PD-1

molecules of more than 70% on circulating T-cells at least 2 months after infusion, regardless of the dose.[68] Most studies on anti-PD therapeutic dose selection have investigated non-small cell lung cancer.[69,70] Peripheral receptor occupancy was saturated at the nivolumab dose of ⩾0.3 mg/kg, with no apparent relationship between tumor shrinkage rate and exposure.[69] Dosing of nivolumab and PD-L1 expression do not seem to lead to inferior overall survival.[70] The KEYNOTE-010 study reported no difference in the efficacy of 2 and 10 mg/kg pembrolizumab; thus, the United States Food and Drug Administration approved a lower dose of 2 mg/kg, enough to achieve antitumor activity, such that further dose increases were not necessary.[71] Thus, in summary, low-dose (but still within the therapeutic window) anti-PD therapy might be a feasible strategy, similar to the minimization strategy of immunosuppressants, in clinical situations where rejection is a major concern.

Real-world experiences

Checkpoint inhibitors for cancers, such as melanoma, cutaneous squamous cell cancer, non–small-cell lung cancer, HCC, and duodenal cancer,[18,72–77] have been used in heart, liver, and kidney recipients. Rejection can occur through acute T-cell-mediated, antibody-mediated, or chronic allograft rejection mechanisms.[72–74] CTLA-4 inhibitors, generally deemed less tolerable than PD-1 inhibitors, are associated with a significantly lower risk of allograft rejection than regimens containing a PD-1 inhibitor.[75] From limited reported HCC cases, one patient showed responses to nivolumab and demonstrated 10-month survival without graft rejection.[76] However, in Munker and colleagues' review of 14 liver transplant recipients treated with immune checkpoint inhibitors, graft rejection was reported in four cases, and in three cases, rejection occurred within 3 weeks since the initiation of therapy, with lethal outcomes.[76] Factors potentially affecting allograft rejection risk and treatment responses include the more integral role of the PD-1 pathway (compared with the CTLA-4 pathway) in organ acceptance, sequential implementation of different immune checkpoint inhibitor classes, time from transplantation to therapy, strength of immunosuppressive agents to prevent organ transplant rejection, and immunogenicity of the particular organ grafted.[19,77] However, additional relevant studies are needed before a concrete conclusion can be drawn. Notably, in addition to other rejection, immune-mediated hepatitis can occur in the liver graft after checkpoint inhibitor therapy.[78] In summary, a precision medicine approach involving cautious assessment of individualized rejection risk must be implemented before initiating immunotherapy against HCC in liver recipients.

Conclusions and future perspectives: toward overall immune assessment

Before formulating management strategies for liver recipients with HCC, the overall assessment of the immune microenvironment is essential. Donor immune cells (such as natural killer cells, natural killer T-cells, and lymphocytes) within liver graft are transplanted along with the graft into recipients,[79] and numerous cellular interactions and alternate binding partners characterize and complicate T-cell costimulatory pathways at the graft site. Further detailed understanding of the kinetics, cellular distribution, binding partners, and intracellular signaling networks of cosignaling molecules in alloimmunity may aid in the rational development of immunomodulatory strategies to prolong graft survival.[77] A therapeutic window for manipulation of cosignaling pathways in transplant recipients with cancer would enable the suppression of alloreactivity toward graft rejection while maintaining tumor-specific protective immune responses. Optimization of costimulation blockade-based regimens, including immunosuppressants, during and after transplantation could widely benefit liver recipients with HCC.[77] Table 1 summarizes potential application of major immunotherapeutic approaches for HCC in liver transplant recipients considering specificity, advantages, and limitations.[80]

Clinical and translational studies on the comparative effectiveness of immune perturbations, particularly cosignaling networks, are necessary for the rational formulation of therapeutics in transplant oncology. For instance, triple maintenance immunosuppression (calcineurin inhibitor + mycophenolate mofetil + corticosteroids) can efficiently block activation-induced upregulation of CD25 in CD8+ T-cells, but not CD4+ T-cells.[81] Another proof-of-concept example is that the ICOS/B7-H2 pathway is secondary to the CD28/B7 pathway in costimulating T-cell-mediated delayed-type hypersensitivity in mice, suggesting a functional hierarchy of CD28/B7

Table 1. Summary of potential application of immunotherapeutic approaches for HCC in liver transplant recipients: advantages and limitations.[76]

Immunotherapy for HCC	Specificity to kill HCC	Advantage	Limitation	Current clinical approval in nontransplant setting
Checkpoint inhibitor	No	High safety, simple administration, durable response	Lack biomarkers predicting responders	Yes
Adoptive cell therapy				
CAR T-cell	Yes	High efficacy	Lack HCC-associated tumor-specific antigens, risk of on-target, off-tumor toxicities	No
Other cells (cytokine-induced killer cells, tumor-infiltrating lymphocytes, natural killer cells)	No	Unclear	Difficulty of relevant immune cell extraction	No
Vaccine				
Tumor vaccine	Maybe yes	Unclear	Lack HCC-associated tumor-specific antigens	No
Dendritic cell vaccine	Maybe yes	Potent capacity of antigen presenting, safety	Unclear	No
Oncolytic virus	Yes	High efficacy	Safety	No

CAR, chimeric antigen receptor; HCC, hepatocellular carcinoma.

and ICOS/B7-H2 pathways and enabling the delineation of their relative contributions to costimulate T-cell immune responses.[82] Clinically, advancing age protects against acute cellular rejection.[83–85] Clinically guided minimization of immunosuppression is possible and safe.[86] Compared with grafts from deceased donors, lower acute T-cell-mediated rejection rates are noted after liver transplantation between biologically related living-donor–recipient pairs.[87] Therefore, in long-term surviving, elderly liver recipients with HCC, the risk of rejection under anti-PD therapy may be not substantial. In principle, translational studies should establish relevant therapeutic agents and combination strategies most likely to achieve patient benefits based on solid mechanistic and clinical justifications,[24] generating effective immunotherapeutic interventions with real-world benefits in clinical care.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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