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REVIEWS

Rethinking Immune Check Point Inhibitors Use in Liver Transplantation: Implications and Resistance

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SUMMARY

As immune checkpoint inhibitor use is expanded to liver transplant candidates, questions on their effectiveness, effect on graft rejection and impact on graft tolerance arise. With tumor cells' immune microenvironment modulation and development of resistance to therapy, liver tumor recurrence post liver transplantation can happen, which poses a challenge for systemic therapy treatment in the setting of immunosuppression. Current evidence on multimodal therapies and on balance with immunosuppression may make use of immune checkpoint inhibitors permissible after liver transplantation.

Immune checkpoint inhibitors (ICIs) have revolutionized cancer therapy, including the two most common liver tumors, hepatocellular carcinoma and cholangiocarcinoma, but their use in the peri-transplantation period is controversial. ICI therapy aims to heighten cytotoxic T lymphocytes response against tumors. However, tumor recurrence is common owing to tumor immune response escape involving ablation of CTL response by interfering with antigen presentation, triggering CLT apoptosis and inducing epigenetic changes that promote ICI therapy resistance. ICI can also affect tissue resident memory T cell population, impact tolerance in the post-transplant period, and induce acute inflammation risking graft survival post-transplant. Their interaction with immunosuppression may be key in reducing tumor burden and may thus, require multimodal therapy to treat these tumors. This review summarizes ICI use in the liver transplantation period, their impact on tolerance and resistance, and new potential therapies for combination or sequential treatments for liver tumors. ***(Cell Mol Gastroenterol Hepatol 2025;19:101407; https://doi.org/10.1016/j.jcmgh.2024.101407)***

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Since the promising results of immune checkpoint inhibitor (ICI) ipilimumab for treatment of advanced melanoma in 2010 and its United States Food and Drug Administration approval the following year, the field of systemic therapy in oncologic tumors has changed.[1] Not only melanoma, but also other solid organ tumors such as lung, bladder, and triple-negative breast cancer have also shown tumor burden reduction and increased overall survival with the use of ICIs.[2–4] In liver tumors, the story is no different; ICI therapy has replaced traditional tyrosine kinase-based therapy as first-line treatment for hepatocellular carcinoma, exemplified by atezolizumab administered with bevacizumab, and durvalumab and tremelimumab.[5,6] Additionally, durvalumab has becoming a major component in the standard-based therapy for cholangiocarcinoma (CCA) in combination with gemcitabine and cisplatin.[7]

Abbreviations used in this paper: **αKG, α-ketoglutarate; ACR, acute cellular rejection; AFP, α-fetoprotein; AMR, antibody-mediated rejection; APC, antigen presenting cell; Arg1, arginase-1; β2M, β2-microglobulin; CCA, cholangiocarcinoma; CAF, cancer-associated fibroblast; CAR, chimeric antigen receptor; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; CNI, calcineurin inhibitor; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CMV, cytomegalovirus; DAMPs, damage-associated molecular patterns; DC, dendritic cell; DNMT1, DNA methyltransferase 1; EBV, Epstein-Barr virus; EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; EZH2, enhance of zeste homolog 2; FAK, focal adhesion kinase; FasL, Fas ligand; FasR, Fas receptor; FAP, fibroblast activating protein-α; FGF, fibroblast growth factor; FMT, fecal microbiota transplant; Gal-9, galectin-9; G-MDSC, granulocytic myeloid-derived suppressor cells; GITR, glucocorticoids-induced TNF receptor; GPC3, glypican-3; HCC, hepatocellular carcinoma; HLA-I, human leukocyte antigen; HMGB1, high mobility group protein B1; H3K27, histone 3 lysine 27; ICI, immune checkpoint inhibitor; ICB, immune checkpoint blockade; IDO, indoleamine 2,3 dioxygenase; IFN, interferon; Ig, immunoglobulin; IGF1, insulin growth factor 1; IL, interleukin; iNOS, nitric oxide synthetase; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; JAK, Janus kinase; KIR3DL3, killer cell Ig-like receptor, three Ig domains and long cytoplasmic tail 3; Lag-3, lymphocyte activation gene-3; LOX, lysine oxidase; LPS, lipopolysaccharide; MDSC, myeloid-derived suppressor cells; MHC-I, major histocompatibility complex-I; MTA, 5-methylthioadenosine; NK, natural killer cell; mTOR, mammalian target of rapamycin inhibitor; Nktr1, natural killer cells triggering receptor 1; PAMPs, pathogen-associated molecular patterns; PD-1, programmed death 1; PDAC, pancreatic ductal adenocarcinoma; PDGF, platelet-derived growth factor; PDL-1, programmed death 1 ligand; PtdSer, phosphatidylserine; ROS, reactive oxygen species; S1P1, sphingosine 1 phosphate; SAM, S-adenosylmethionine; SOCS, suppressor of cytokine signaling; STAT3, Signal transduce and activator of transcription 3; TAA, tumor-associated antigen; TAM, tumor-associated macrophages; TCM, central memory T cell; TCMR, T-cell medicated rejection; TCR, T-cell receptor transduced; TEM, effector memory T cell; TEx, exhausted T cell; TGFβ, tumor growth factor beta; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domain; TIME, tumor immune microenvironment; Tim-3, T-cell immunoglobulin mucin-3; TLR, toll-like receptors; Tmem, memory T cell; TNF, tumor necrosis factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Treg, regulatory T cell; TRM, tissue resident memory T cell; VEGF, vascular endothelial growth factor; VISTA, V-domain Ig suppressor of T-cell activation.**

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Despite the major breakthroughs of ICIs in immune-oncology treatment, some patients present tumor resistance to immunotherapy. This resistant cancer cells often necessitate ICI rechallenge or alternative systemic therapy with second- or third-line treatments to reach clinical response.[8] Tumor progression or acquired resistance can still happen, either on initial ICI treatment, after stable disease, or after a period of durable clinical response.[9]

ICI therapy poses additional challenges concerning transplantation. Significant toxicity from immune-related adverse events such as colitis, skin reaction, and pneumonitis, to name a few, can happen at any point of therapy, even post-treatment regimen.[10] Degrees of toxicity presentation may vary in a dose-independent manner, and symptoms may persist beyond 12 weeks, especially if exhibiting arthritis, endocrinopathies, ocular symptoms, xerostomia, and neurotoxicities.[11] Even though ICIs are not metabolized by the liver, ICIs have limited utilization in patients with cirrhosis, and are namely reserved for those without advanced liver disease or with Child-Pugh A degree of hepatic dysfunction out of concern for decompensating events such as ascites and gastrointestinal bleed from esophageal varices. ICI use during the peri-transplantation period is controversial. Indeed, inflammatory effects of ICIs can affect graft function and overall post-transplant survival. However, efficiency of ICI therapy in cancer has the potential for preventing tumor dissemination before transplantation and improve recurrence-free survival post transplantation. This review will first summarize the use of ICIs before and after liver transplantation, and the mechanisms of tumor immune resistance elicited by ICIs as they relate to the two most common hepatic tumors, hepatocellular carcinoma (HCC) and CCA,[12] and the future therapies to reduce recurrence of these tumors.

Liver Tumors and Immune Checkpoint Inhibitors

Primary liver cancer is the third leading cause of death worldwide, despite being sixth in cancer incidence.[12] HCC is the most common type of liver cancer followed by CCA. Early detection of these two tumor types is challenging and often requires systemic therapy to control tumor progression in the advanced stages. Although both tumor types can grow in nonfibrous environments, HCC is often associated with advanced hepatic fibrosis. Tumor resection in the presence of high fibrosis can lead to decompensations of liver disease such as ascites and hepatic encephalopathy, for which liver transplantation is a therapeutic option for early and nonresectable disease. Systemic therapy with ICIs is reserved for patients with evidence of local spread or distant metastasis in the setting of preserved liver function.[13] However, given concerns of portal hypertension-related events driving mortality, ICI clinical trials have been limited to those with Child-Pugh A disease, and tolerance of ICIs in more advanced hepatic dysfunction has not been fully explored.[14] Only 3% to 6% of those receiving first-line therapies atezolizumab-bevacizumab or tremelimumab-durvalumab achieve full response, whereas at least 20% of patients show progression of HCC.[5,6] For patients with a high risk of HCC recurrence after resection or ablation, atezolizumab-bevacizumab improved recurrence-free survival, but did not eliminate progression of disease.[15] Similarly, patients with CCA treated with the combination of therapy plus durvalumab or pembrolizumab display overall improvement, but only 25% to 27% of patients achieved partial response.[16] This suggests ICI resistance or acquisition of resistance to ICI therapy overtime.

Immune checkpoint is an important tumor immune resistance mechanism that suppresses anti-tumor immune response. ICIs target the major immune checkpoint pathways such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death 1 (PD-1), or its ligand programmed death 1 ligand (PDL-1). Both CTLA-4 and PD-1 are transmembrane glycosylated proteins of the CD28 family member. Their expression at the cell membrane represses cytotoxic T lymphocytes (CTLs), which are central in anti-tumor response.[17]

The purpose of ICIs is to unleash the CTL response. Tumor cells display a distinct antigen signature from their normal counterpart called tumor-associated antigens (TAAs). These TAAs are recognized and internalized by antigen presenting cells (APCs), which subsequently present the antigen to naïve $CD8^+$ T cells via major histocompatibility complex I (MHC-I).[18] In the meantime, APCs provide a second signal through CD80/86 that binds CD28 expressed on $CD8^+$ T cells to stimulate their proliferation and differentiation into CTLs. Anti-tumor response efficiency relies on the capacity of the TAAs to activate CTLs and the ability of these activated CTLs to infiltrate the tumor. CTLA-4, an immune checkpoint protein constitutively expressed on naïve T cells and on FOXP3 regulatory T cells (T_{reg}), is upregulated in activated T cells, and competes with CD28 for the accessibility of CD80/86 on the APC to repress CTLs stimulatory signal.[19] PD-1 is a monomer expressed on the surface of T lymphocytes, and natural killer (NK) cells. Similar to its homologous receptor, PD-1 cell surface expression is upregulated on CTLs, and induces T cells exhaustion when bound to its cognate ligand PD-L1 expressed on APC and tumor cells. T cell exhaustion induced by PD-1 activation is characterized by the phosphorylation of the N-terminal immunoreceptor tyrosine-based inhibitory motif (ITIM) and C-terminal tyrosine in an immunoreceptor tyrosine-based switch motif (ITSM).[20] In response to this phosphorylation, SHP1 and SHP2 phosphatases are recruited to dephosphorylate and block ZAP70 and CD3ζ proteins, which are involved in TCR signal transduction of T cells. This results in inhibition of CTL activity, interferon (IFN)-γ and tumor necrosis factor (TNF)-α secretion, and proliferation while promoting CTL apoptosis via PI3K/AKT pathway inhibition.[21]

The characterization of these immune inhibitory pathways led to the development of ICIs for CTLA-4, PD-1, and PD-L1 immunoglobulin (Ig) to foster CD28 costimulatory signal and restore CTLs differenciation.[1] Interestingly, immune checkpoint blockade (ICB) does not only restore CTLs differentiation, but also reactivate previously "exhausted" CTLs, induce T_{reg} depletion, and increase memory T cells

(T_{mem}).[21] Though monotherapy can achieve response such as nivolumab in melanoma,[22] combination ICI therapy has begun to be explored. The phase III clinical trial, HIMALAYA, showed efficacy in HCC by targeting both CTLA-4 and PD-1 with durvalumab-tremelimumab to enhance antitumor immune response in unresectable HCC.[5]

ICI Use in Liver Transplantation

Liver transplantation is a treatment strategy for HCC and CCA.[13,23] The Milan criteria and University of California San Francisco (UCSF) scores were proposed to better select patients with HCC for liver transplantation based on tumor size and findings on liver explant.[24,25] Achieving Milan criteria and an α-fetoprotein (AFP) target allows patients with HCC to get exceptions and place them at a higher priority for liver transplantation.[26] Although the utilization of these scores has led to successful outcomes in liver transplantation, the HCC recurrence rate has been reported of at least 15%.[23,27] Recently, data has shown 12.5% HCC recurrence with 17-month median time to recurrence and a 10-year post-transplant HCC recurrence-free survival of 13.3% based on patients who underwent successful downstaging pre-transplantation in 5 academic centers in the United States.[28] Thus, the risk estimation of tumor recurrence after transplant (RETREAT) score has been proposed to detect earlier post-transplant HCC recurrences and enable earlier treatment through local regional therapy, surgical resection or systemic therapy.[29] With regards to CCA, there are no predictive scores for recurrence pre- or post-transplant yet. Recurrence rate in the literature have been reported as low as 18% 5-year recurrence risk for early intrahepatic CCA, and 13% for perihilar CCA after undergoing neoadjuvant chemoradiation therapy followed by transplantation.[23] However, recent data also demonstrated 5-year disease-free survival of 50.2% in those undergoing liver transplantation for perihilar CCA after undergoing chemoradiation protocol regardless of underlying presence of primary sclerosing cholangitis.[30]

ICI use as an adjuvant therapy prior liver transplant has been more permissible to control tumor burden and maintain transplant eligibility in accordance with Milan criteria.[24] Case reports have demonstrated their success in downstaging to meet Milan criteria and enable subsequent liver transplantation.[31] Addition of ICI therapy is being considered for this patient population in the peri-transplant period to achieve early remission, control micrometastasis, and avoid tumor recurrence post liver transplantation. As a result, the Organ Procument & Transplantation Network (OPTN) has revised its bylaws to enable the utilization of ICI systemic therapy for HCC downstaging or the reduction of tumor board to fit with exceptions criteria.[26]

However, safety data is limited, with significant heterogeneity among studies regarding effects on long-term graft tolerance and ICI-related adverse events, although these events can occur at any time, even post therapy. Indeed, hepatic necrosis post-transplant was observed in 2 patients treated with toripalimab 93 days and 8 days prior to liver transplantation, respectively. Though autopsy was not pursued in either case, expression of PDL-1 on the grafts was noted post-transplant suggesting ICI-related graft injury.[32,33] Both of these cases had used sorafenib and other modes of local regional therapy prior to ICI treatment, questioning if additional therapies augmented the immune response induced by ICI. Although studies on biomarkers have found to be correlated with ICI response, and others like interleukin (IL)-6 and IL-17 to be correlated with ICI-related adverse effects, there are no reliable markers to predict adverse effects following a solid organ transplant.[34,35]

Data on the use of ICIs in the post-transplant period to treat tumor recurrence is lacking. Most of the literature on the interaction between ICIs and immunosuppression therapy comes from managing inflammatory adverse events caused by ICI therapy in non-transplant patients. Inflammatory toxicities induced by ICIs are characterized by high CTL expansion and infiltration into different tissue sites causing inflammation.[36] In patients with ICI-induced colitis, CTL recruitment is aided by CXCL9 and CXCL10 myeloid cells. Recruited CTLs express activation markers such as granzyme B and IFN-γ, as well as CTLA-4 and PD-1 receptors, which also play immunosuppressive functions.

High CTL population in inflamed tissues can be controlled with steroid therapy, which triggers T cell apoptosis. Taking advantage of the binding of $\alpha4\beta7$ integrin CTL protein and mucosal vascular addressing cell adhesion molecule-1 (MAdCAM-1) expressed on intestinal epithelia, the use of anti-integrin such as vedolizumab has shown efficacy in steroid-refractory ICI-induced colitis.[37] For ICI-induced hepatitis, steroids are also first-line therapy. Immunosuppression medications such as azathioprine, mycophenolate mofetil, or tacrolimus used as anti-rejection medications in post-liver transplant period are second-line medications for steroid-refractory ICI-related hepatitis. Similarly anti-thymocyte globulin, used in cases of severe liver allograft rejection, has also been proposed as third-line medication in fulminant ICI-induced hepatitis.[38] Although sequential use of these therapies have proven to be safe pre-transplant, this existing data ought not to be extrapolated to the use of ICIs after liver transplantation given different immune microenvironment.

Transplant Immunology

The liver is one of the most important immunological organs of the human body containing a rich innate immune cell microenvironment that aims to achieve immune homeostasis, balancing antigen tolerance and inflammatory response in the presence of harmful antigens.[39] Its unique anatomic localization continuously exposes it to different antigens from the gastrointestinal tract through the portal vein flow. Upon liver transplantation, donor graft hematopoietic cells are transferred to the recipient where they undergo chimerism or replace the host's immune cells. This promotes long-term tolerance and improves graft survival of simultaneous organ transplant.[40] After allograft is placed into the recipient, hepatocytes that have undergone transient ischemia release pathogen-associated molecular

patterns (PAMPs) and damage-associated molecular patterns (DAMPs), inducing toll-like receptors (TLRs) activation on donor's APCs, which results in an inflammatory response in lymph nodes. This ischemic reperfusion injury occurring when blood flow is reestablished to the graft, augments the inflammatory response leading to host neutrophil infiltration followed by other APCs, cytokine secretion, and massive activation of T cells, either by recipient's or donor's APC that have processed the antigens.[41,42] This results in a majority of host T cell death. However, a subset of T cells proliferates and migrate to the allograft, while the remaining differentiate into T_{mem}. APCs become saturated by antigen release, which dampens the inflammatory response.[40] Different studies have, however, found that this is achieved by activation of different pathways, such as TIR and IRAK-M signaling, angiogenesis, and oxygen tissue restoration via vascular endothelial growth factor (VEGF) secretion, inhibitory cytokine secretion such as IL-10, and T_{reg} production. Additionally, removal of apoptotic material by phagocytic cells, and expression of anti-inflammatory lipid mediators such as aslipoxins, resolvins, and protectins promote a reduction of neutrophil recruitment and inflammatory response, thereby creating a tolerant environment.[43,44]

Failure to regulate this initial immunologic response will result in the release of hyaluronan, glycosaminoglycan, and uric acid, as well as pro-inflammatory cytokines secretion in the microenvironment by injured hepatocytes that play a role in the early acute T-cell medicated rejection (TCMR).[45] The uptake of antigens by recipient APCs to present to T cells are characteristic of late acute TCMR. In the attempts to repress this early rising inflammatory response, immunosuppressants like calcineurin inhibitors (CNIs), steroids, mycophenolate mofetil, and sometimes IL-2-depleting therapies are administrated during the early stage of liver transplantation. Immunotherapy at this critical stage would antagonize the efforts of controlling the inflammatory response by blocking T-cell apoptosis and reinforcing T cell activation, risking graft survival and thereby recipient's survival.[46]

The use of ICIs in clinical trials has been excluded in patients who have received an organ transplant, emphasizing the lack of data in their safety, efficacy, duration, and administration window from transplantation. In mice, ICI use induced rejection in already tolerant mice,[47] and scarce literature of their use in liver transplant recipients have demonstrated high rates of rejection. Case reports of ICI use in post-liver transplant patients with solid tumors including HCC and melanoma demonstrated rejection in 36% of them, regardless of Ipilimumab or pembrolizumab use.[48] Those on calcineurin monotherapy had the lowest rejection rate happening in a median time from transplantation of less than 5 years. High-dose steroids, discontinuation of ICI therapy, and dialysis was required to treat this TCMR. Despite attempts to control rejection with these interventions, graft loss was reported in 81% of patients.[46,48] Interestingly a cohort of 17 patients who had undergone kidney transplantation showed a lower rate of rejection (2 of 17 cases); one of these cases was salvaged by plasma exchange and anti-thymocyte therapy.[49] This suggests that the type of baseline immunosuppression therapy at the time of ICI therapy initiation may provide protection against graft rejection.

Memory T Cells in Tolerance

After initial inflammatory insult, remaining memory T cells are divided into highly proliferative central memory (T_{CM}) T cell, effector memory (T_{EM}), and tissue resident memory (T_{RM}) T cells. T_{CM} express high levels of CCR-7 and CD62-L and are located in secondary lymphoid organs, whereas T_{EM} reside in the peripheral compartments. T_{RM} express CD69, which interferes with cell surface expression of sphingosine 1 phosphate (S1P1), required for exiting lymph node and for blood circulation, thereby ensuring tissue retention.[50] T_{RM} reside in barrier tissue epithelia such as skin, intestine and liver sinusoidal endothelial cells in the liver with high expression of CD103 and CD69 retention markers and low CD62L. In the liver, T_{RM} upregulate E-cadherin ligand and express CXCR3 involved in tissue homing, and CD14 that mediates TLR-4 activation upon exposure to lipopolysaccharide (LPS).[51] T_{RM} readily responds to relapsing injury upon antigen presentation, releasing IL-2 and IFN-γ to recruit circulating T cells, activate DCs and NK cells, and maintain effector T_{RM} response even if they express exhaustion markers CD39 and PD-1.[51]

Clonal rearrangement and pathway analysis has demonstrated that CTLs share identical TCR than T_{RM} in patients with colitis following ICI therapy suggesting differentiation of these T_{RM} into CTLs, explaining colitis as adverse event during ICI therapy.[52] In tumor immune microenvironment (TIME) of hepatobiliary cancer, T_{RM} induction and maintenance is less understood. Patients with HCC harbored higher population of $CD103^{+}CD69^{+}T_{RM}$-expressing immune checkpoint receptors PD-1, T cell Immunoreceptor with Immunoglobulin and ITIM domain (TIGIT) and/or T-cell immunoglobulin mucin-3 (Tim-3) compared with patients with CCA, suggesting better HCC response to ICI therapy.[53] Indeed, patients with prostate, melanoma, lung, and kidney cancer responding to ICI therapy have a higher T_{RM} population.[54]

In liver transplantation, majority of T_{RM} are derived from donor allograft and can remain years after transplantation. As the liver graft is exposed to new antigens via its portal blood flow, lymphocytic population is replaced over time with *de novo* regeneration, in which T_{RM} subpopulations differ by their CXCR3 levels whereby $CXCR3^{high}$ T_{RM} are donor derived.[55] $CD69^{+}CD103^{+}$ T_{RM} and tissue resident NK cells have been detected in allografts and draining lymph nodes a decade after liver transplantation.[55,56] Lung transplantation studies have shown that preservation of donor T_{RM} have been associated with less episodes of allograft rejection.[57] However, it is unclear how T_{RM} plays an immunomodulatory role in liver transplant recipients and their response to recurrent malignancy.

Use of ICI after solid organ transplant showed mixed results. Liver transplant recipients exhibited acute cellular rejection (ACR) in 28.8%.[58] Of recipients, 13.4% died due to graft loss, whereas 44.2% of them achieved tumor control

associated with longer overall survival of 26.4 months compared with 3.4 months in non-responders. Tumor expressing PD-L1 was associated with ACR rejection after ICI therapy in a small number of patients. However, this effect may be salvaged by high quantity of tumor infiltrating lymphocytes.[59,60] Rejection has been observed with a higher frequency when receiving anti-PD1 therapy compared with anti-CTL4 agents, suggesting use of CTL-4 in patients with tumor PD-L1$^+$. Nevertheless, the number of reported cases treated with anti-CTL-4 therapy was lower compared with the patients treated with anti-PD-1 therapy.[48,61] The mechanism of ICI-mediated rejection is unclear and the immune phenotype of infiltrating T cells that are protective against ACR episodes has not been characterized.

TCR engagement with MHC is not enough to induce a full T cell activation and requires a costimulatory signal such as CD28 activation. CD28 is constitutively expressed on T cells and provide the costimulatory signal to full activated T cells when it is bound to CD80/CD86 expressed on APC. CTLA-4 competes with CD28 for the binding of CD80/CD86. However, CTLA-4 has a higher affinity for CD80/CD86 than CD28. Thus, CTLA-4 binding to CD80/86 inhibits the costimulatory signal from CD28 by binding competition, resulting in downregulation of CTL inflammatory response.[19] Consequently, blocking CTLA-4 with ICI risks to induce graft rejection after liver transplantation. Pre-clinical models of mice treated with anti-CTLA-4 antibodies on day 0, 4, and 6 post liver transplant demonstrated acute rejection within 15 days post-transplantation. These mice showed a significant increase of host splenic T cells, graft donor CD8$^+$ and CD4$^+$ T cells, and less apoptotic graft infiltrating T cells, leading to focal necrotic areas and severe liver injury. CD3$^+$ T cells purified from both graft and spleen of treated mice exhibited higher secretion of inflammatory cytokines IL-2 and IFN-γ, whereas secretion of the anti-inflammatory cytokine IL-4 was decreased compared with control mice.[47] Similar findings have been described in mice undergoing cardiac transplant and in CD28-deficient mice treated with anti-CTLA-4.[62] CD28 antagonist treatment in the first 2 weeks of cardiac transplant mouse model in combination with CNI or anti-CD157 prolonged heart allograft tolerance associated with an increase of CD4$^+$FOXP3$^+$T$_{regs}$ and regulatory DCs. Alloantibodies were also observed to be suppressed, suggesting a protection against chronic rejection.[63] Although the therapy was given in an early stage post-transplantation compared with current clinical care, blocking CTLA-4 in the early liver transplantation period can jeopardize the induction of graft tolerance.

CTLA-4 agonists such as abatacept have been investigated in their potential role in transplant tolerance. Cardiac transplant mice treated with abatacept showed less alloantibodies associated with a longer graft survival, but no increase in T$_{regs}$ population was observed.[63] CTLA-4 agonists have shown to be beneficial in kidney transplant recipients presenting a high risk of antibody-mediated rejection (AMR) in presence of donor-specific antibodies. Moreover, studies in non-human primates, treated with calcineurin inhibitor and belatacept after kidney transplantation, demonstrated that alloantibodies and germinal centers were reduced and graft survival increased compared with standard care, using calcineurin inhibitor, mycophenylate, and steroids. However, CTLA-4 agonist-CNI combination therapy was associated with higher cytomegalovirus (CMV) infections requiring chronic use of CMV prophylaxis, as therapy could not be tapered without risk of alloantibody recurrence or graft rejection.[64] Further clinical trials demonstrated efficacy and better kidney graft function when using CTLA-4 agonist-CNI combination for initial therapy or after conversion from CNI therapy. Transition to this therapy reduced immunosuppression-related side effects, particularly CNI-related nephrotoxicity, at a cost of more ACR episodes, and risk of Epstein-Barr virus (EBV)-associated post-transplant lymphoproliferative disorder in EBV$^+$ donor grafts or EBV unknown receipients.[65] CTLA-4 agonists are also used off-label for AMR in cardiac and lung transplant recipients.[66,67] In liver transplant recipients, its use has demonstrated mixed results, with reports of early graft loss and infections resulting in death, for which it is currently limited for use as salvage therapy.[68]

The PD-1/PD-L1 pathway plays a central role in post-transplant tolerance.[69] Indeed, early studies of cardiac transplant in double deficient CD28 and CD80/CD86, recipient mice treated with PD-L1 inhibitor showed an accelerated rejection, associated with a higher infiltration of CD4$^+$ T$_{EM}$ cells.[70] A fully MHC-mismatch cardiac transplant mouse model treated with a CTLA-4 recombinant protein fused with Ig H chain tail (CTLA4Ig) that blocked CD80/CD86 signal, exhibited ACR when anti-PD-L1 was administrated, regardless of the administration period compared with mice treated with CTLA4Ig alone. T cell population analysis demonstrated that infiltrated CD8$^+$ T$_{EM}$ population was augmented, where CD4$^+$ T$_{reg}$ was reduced in mice treated with combination therapy compared with the group receiving CTLA4Ig monotherapy. The group treated with combination therapy exhibited increased inflammatory infiltrate, vascular obliteration, and scattered tissue necrosis. To explore the mechanism of tolerance behind PD-1/PD-L1 pathway more deeply, the authors performed cardiac transplantation into PD-L1 deficient mice and observed a significant shorter graft survival compared with wild-type mice after CTLA4Ig therapy with a similar histology observed in double CD28 and CD80/CD86 deficient mice. In addition, to determine whether host or recipient PD-L1 is more important in tolerance, they performed a transplantation of PD-L1 deficient heart into wild-type mice treated with CTLA4Ig. Although graft was accepted, severe chronic rejection was observed 3 months post transplantation in PD-L1-deficient recipient compared with wild-type graft, which showed protection against chronic rejection. This study highlights the importance of host PD-L1 and PD-L1$^+$ APCs being critical in the induction and maintenance of graft tolerance, whereas PD-L1 in the graft prevents chronic rejection and local inflammatory.[71] In liver transplantation, however, graft PD-L1 is critical in the induction of graft tolerance. Indeed, PD-L1-deficient liver transplanted in allogeneic recipient mice resulted in an acute graft rejection.[69] This observation can be explained by its unique tolerogenic features and specific immune microenvironment composed of a large population of resident

macrophages that allow it to immunosuppress unnecessary inflammatory response.[72]

Immunosuppression in Post-transplant Tumor Recurrence

ACR episodes have been correlated with higher post-transplant survival, particularly in patients with hepatitis B virus-related HCC, whereas ACR patients treated with steroids were associated with higher HCC recurrence.[73] Mammalian target of rapamycin (mTOR) inhibitors such as sirolimus both interfere with cancer cell metabolism and proliferation and serve as an immunosuppressant agent. In patients with active HCC associated with elevated AFP at the time of transplant, administration of mTOR inhibitor for at least 3 months showed higher overall survival, delayed HCC recurrence, and improved survival after tumor recurrence.[74] The recurrence-free and overall survival advantage in patients receiving sirolimus was only seen in the first 3 to 5 years after transplant compared with patients receiving mTOR-free inhibitors.[75] In patients with HCC recurrence post-transplant, higher concentration level of mTOR inhibitor in combination with sorafenib lead to higher overall survival compared with non-mTOR inhibitor use as immunosuppressant agent.[76] Despite these known benefits, mTORs are not uniformly used in malignancy-related liver transplantations.

Immunosuppression with CNIs such as tacrolimus has been extensively debated to increase risk of malignancies particularly with long-term exposure.[77] And though retrospective analysis of liver transplant recipients showed that ACR episodes were less frequent in patients with HCC undergoing liver transplantation, the occurrence of malignancy post-transplant often reflexes providers to decrease immunosuppression to enable anti-tumor response. Earlier studies demonstrated high liver graft rejection rates following ICI therapy of 37.5% in the setting of no immunosuppression or steroid-only therapy. Survival after ICI exposure from rejection was higher in patients who had an additional immunosuppressive agent to steroid therapy.[78,79] Indeed, recent analysis has suggested that at least a combination of 2-immunossupressor regimen is associated with a lower risk of rejection after ICIs. Moreover, rejection rate decreased with longer post-transplantation.[80] A delay in ICI initiation upon tumor diagnosis was also associated with decrease in efficacy, suggesting consideration of earlier ICI use as opposed to using it as salvage therapy. These recent analyses also demonstrated disease progression as a leading cause of death as opposed to graft failure demonstrated by earlier studies, and demonstrated better clinical outcomes when ICIs were used for non-HCC diagnosis. Still, these conclusions are taken from case series and systematic reviews, for which prospective and randomized studies are needed to draw more certain conclusions.[80]

Mechanisms of Resistance to ICIs

Tumor Intracellular Resistance Mechanisms

Despite the promise of ICI use in liver tumors and cancer treatment prior to liver transplantation, tumor recurrence and progression can still be observed owing to the tumor's ability to evade the immune system. Although mechanistic studies of tumor resistance have been done in non-transplant settings, one can extrapolate tumor recurrence post-transplant by the mechanisms outlined below. Cytotoxicity from anti-tumor immunity and ICIs induce a pressure selection on tumor cells that promote cells with survival advantages, fostering the emergence of immune resistant tumor cells and resulting in the tumor relapse[81] (see Figure 1). Although ICI treatment aims to unleash CTLs response, efficiency of the CTLs cytotoxicity depends, in part, on their capacity to identify tumor or the presentation of TAAs by the MHC-I complex at the surface of the tumor cells.

MHC-I-peptide complex or human leukocyte antigen I (HLA-I) is composed of the antigen and a glycopolypeptide that is non-covalently associated to the β2-microglobulin (β2M) subunit, which is essential in the transport of MHC-peptide to the surface.[82] One of the strategies employed by tumor cells to overcome ICI-induced T cell cytotoxicity is to reduce antigen presentation by frameshift deletion in the β2M exon, resulting in impaired T cell activation.[83] Epithelial cancers, such as HCC, frequently overexpress epidermal growth factor receptor (EGFR), which can be mutated into a constitutively active state, or be continuously activated by EGFR ligand such as TGFα and epidermal growth factor (EGF) that are abundant in the TIME. In vivo and in vitro studies described a reduction of MHC-I expression by EGFR, which can be reversed by EGFR inhibitor gefitinib.[84]

During cancer progression, tumor cells undergo rapid proliferation, resulting in accumulation of genetic mutations. Neoantigens are the byproduct of mutations, and transcription and post-transcriptional alterations specific to tumor cells. Neoantigens are associated with inflammatory response and overall, with higher and durable effector T cell response. In addition to reducing MHC membrane expression to avoid CTLs recognition, cancer cells can reduce their neoantigen production to evade immune response.[85]

Studies have demonstrated that tumor cell DNA hypermethylation silences genes and facilitates T cell evasion. DNA methyltransferase 1 (DNMT1) and enhance of zeste homolog 2 (EZH2), the catalytic subunit of histone 3 lysine 27 (H3K27) methyltransferase system, is upregulated in melanoma cell lines treated with anti-CTLA-4 and correlate with inhibition of genes related to antigen processing, antigen presentation, chemokine expression, and increase of exhausted T cells (T_{EX} and FOXP3^{+}Tregs).[86] Inhibition of DNMT1 and EZH2 by GSK1260 and 5-AZA-dc restored CXCL9 and CXCL10 chemokine secretion, and reconstituted T cell migration to the tumor in mice with ovarian cancer.[87] Moreover, methylation of PD-L1 promoter, PDCD1LG1, has been associated with resistance to PD-1 inhibitor nivolumab in patients with non-small-cell lung cancer, whereas low methylation on CTLA-4 promoter in patients with melanoma has been correlated with ICI treatment response and increased overall survival.[88,89] The combination of ICI with DNA demethylating agents achieves significant higher tumor growth inhibition compared with ICI therapy alone.[90]

Cytotoxic T cell function is also impaired by high levels of circulating IL-6, correlating with poor ICI response. Janus

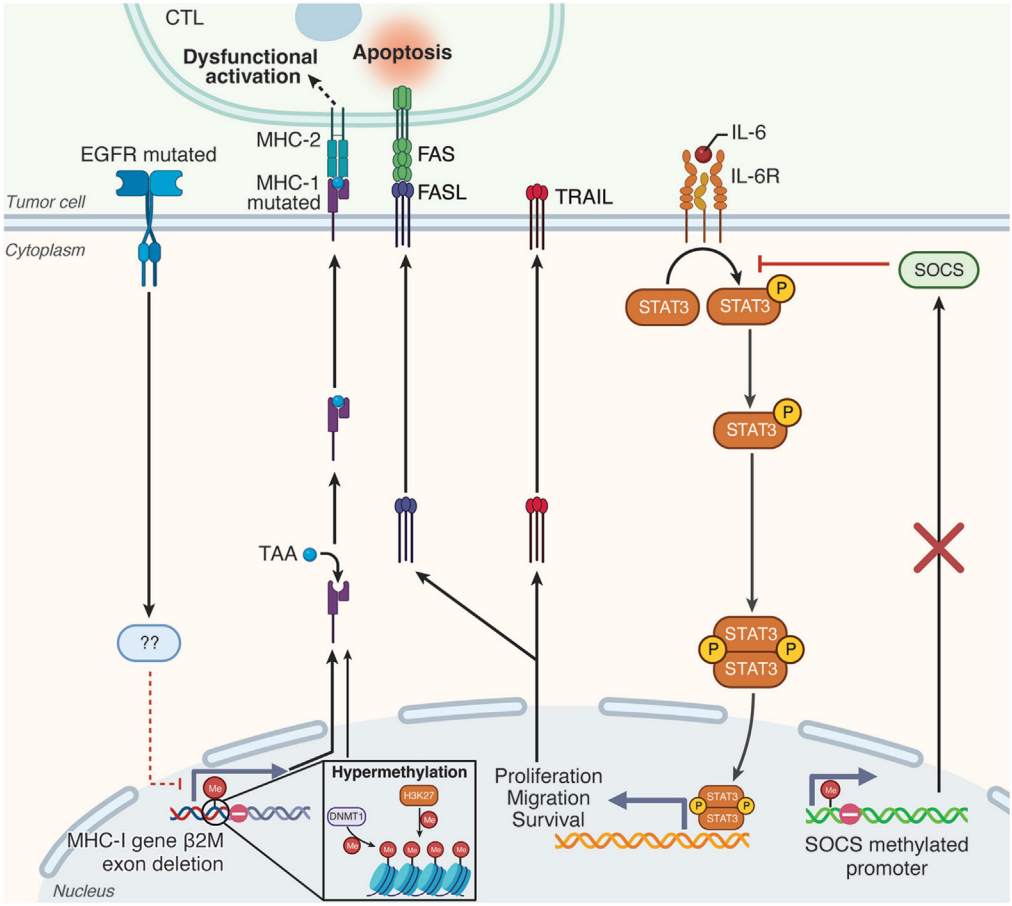

Figure 1. Intracellular mechanism of tumor cells to evade anti-tumor immune response. Tumor cells lead to dysfunctional MHC-I expression via: (1) Constitutive EGFR activation reduces MHC-I expression through an unclear mechanism; (2) Upregulation of DNMT1 and H3K27 methyltransferase complex that silences MHC-I gene via hypermethylation; and (3) Exon deletion of β2-microglobulin gene results in the loss of β2-microglobulin subunit of MHC-I, resulting in abnormal TAAs presentation and dysfunctional T cells activation. Tumor cells also induce tumor-infiltrated lymphocyte (TIL) apoptosis by overexpression of FAS ligand, which bind FAS receptors on TIL. Tumor IL-6 receptor activation leads to phosphorylation and nuclear translocation of STAT3, which promotes transcription of genes involved in migration, proliferation, and tumor cell survival. STAT3 phosphorylation is facilitated by SOCS gene silencing via DNA hypermethylation.

kinase/signal transduce and activator of transcription 3 (JAK/STAT3) is an IL-6R downstream pathway activated in response to high circulating IL-6. In normal tissue, STAT3 is inhibited by the expression of suppressor of cytokine signaling (SOCS) proteins. In tumor, SOCS promoter undergoes hypermethylation, silencing SOCS gene expression and leading to abnormal activation of STAT3, resulting in cell proliferation, migration, and survival via anti-apoptotic genes Bcl-2, Bcl-xL, and Mcl-1.[91,92] Tocilizumab, an IL-6R inhibitor, and ruxolitinib, an inhibitor of JAK1/2, reduced tumor cell survival and proliferation in liver tumor cell lines.[92,93]

CCA cells overexpress Fas ligand (FasL) and have dysregulation of Fas receptor (FasR).[94] FasL-FasR is a cell death pathway playing a central role in tumor modulation and cancer progression. CCA cells are able to induce lymphocyte and NK cell apoptosis via the Fas pathway and increase their expression of anti-apoptotic protein c-FLIP and BCl-2 to avoid their own cell death.[95] TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis by binding its cognate receptors TRAIL-R1 or TRAIL-R2, which are overexpressed on tumor cells. TRAIL agonist therapy raised hope in immune oncology but failed in human clinical trials. Recently, a study showed that CCA cells overexpressed TRAIL and bind TRAIL-R on myeloid-derived suppressor cells (MDSCs) to promote MDSC survival through a non-canonical activation of the NFκB pathway.[96] This mechanism promoting MDSCs survival could explain the failure of TRAIL agonists in human clinical trials.[97]

Cancer Cells Modulate the Tumor Immune Microenvironment to Evade CTLs

TIME is complex, continuously evolving and restructuring the stroma, endothelial, and immune cells. Cancer cells modulate these different TIME parameters to evade the immune cells (Figure 2). Cancer-associated fibroblasts (CAFs) play a central role in stroma and cancer cell support by secreting growth factors, cytokines, and extracellular matrix proteins, which promote tumor proliferation and therapeutic immune resistance.[98] Although fibroblasts are

wound-healing specialized cells activated by an inflammatory response, CFAs are recruited and activated by both cancer cells chemokine secretion such as tumor growth factor beta (TGFβ), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), and by the inflammatory response at the tumor site.[99,100]

CAFs are sources of numerous cytokines and chemokines involved in the modulation of the TIME, such as IL-6.[101] Activation of STAT3 by IL-6 increases IL-10 secretion by the liver resident macrophages Kupffer cells, preventing NK cell activation. It also lowers CD80 and CD86 expression on APCs, suggesting poor APC activation, and switches dendritic cell (DC) progenitors from having antigen-presenting ability to a phagocytic-like action in cholangiocarcinoma.[102] This is associated with poor response to both PD-1 and CTLA-4 inhibitors, although the mechanism is not fully understood. High levels of circulating IL-6 were associated with poor response to atezolizumab, but combining anti-PDL-1 and anti-ILR-6 therapy increased cytotoxic T cell response to achieve tumor growth inhibition.[103]

To stay undetectable by the immune system, cancer cells can induce T-cell exhaustion (T_{EX}), a state of CTL dysfunction characterized by poor effector function. T_{EX} have important expression of inhibitory receptors such as PD-1, Tim-3, lymphocyte activation gene-3 (LAG-3), TIGIT, and V-domain Ig suppressor of T-cell activation (VISTA).[104] T_{EX} is induced by a chronic inflammation, resulting in progressive loss of function, improper activation, expression of inhibitory receptors, impaired cytokine secretion, and inability to respond to IL-7 and IL-15 stimulation.[105] Interestingly, PD-1 blockade induces upregulation of natural killer cells triggering receptor 1 (Nktr1) in a lung cancer subtype with Kras/p53 mutation, which activates JAK/STAT3 pathway leading to PD-L1 overexpression and CTL exhaustion. Thus, Nktr1 upregulation provides a survival advantage to the tumor cells under immune pressure.[106] TIM-3 has been described to be regulated by PI3k/Akt pathway in the CTLs and can be co-expressed with PD-1. Anti-PD-1 therapy in a lung cancer mouse model upregulates TIM-3 expression in CTLs resulting in PD-1 ICI resistance.[107]

Tumor progression necessitates an important quantity of energy where it uses up large quantities of the available nutrient and oxygen, setting up a TIME poor in glucose and oxygen, and forcing the different cells to use different metabolism pathways.[108] Low glucose levels impaired CTL activation and restrained anti-tumor immune response. Pathway analysis of genes that correlated with T cell exhaustion in HCC revealed those involved in glycolysis and methionine degradation, leading to higher 5-methylthioadenosine (MTA) serum levels. The liver, being a prime organ for metabolism, allows upregulation of GLUT1 and GLUT2 for glucose uptake in HCC. Both HCC and CCA demonstrate overexpression of hexokinase to convert glucose to glucose-6-phosphate for glycolysis, and adopt de novo fatty acid synthesis to aid in membrane synthesis and to use as energy source in the form of carbon dioxide via oxidation.[109,110] Hence, dysregulation of lipid metabolism has been correlated with poor outcomes, and aberrancies have been noted in the pentose pyrophosphate pathway, hexosamine biosynthesis pathway, TCA metabolites, and nucleotide metabolism.[111,112]

Immune cells must also adapt to lower aerobic respiration. T_{regs} switch to anaerobic metabolism under low glucose and hypoxia conditions as they increase GLUT1 expression, which continues to allow their proliferation in the tumor microenvironment.[113] Metabolites in the TIME also regulate the immune response. Glutamine depletion impairs T cell differentiation into memory phenotype through epigenetic remodeling. Similarly, low arginine levels impeded IFN-γ production, cytotoxic nitric oxide production, and NK cell differentiation.[114,115] Upregulation of indoleamine 2,3 dioxygenase (IDO) enzyme that catalyzes the conversion of tryptophan to its metabolite kynurenine, induce T_{reg} and MDSCs, while ablating effector T cell response and proliferation.[116] CAFs play a role in tryptophan depletion with accumulation of kynurenine, which induces a T_{reg} phenotype upon interaction with Wnt5a-ligand secreted by tumor cells.[117] CAFs upregulate expression of fibroblast activating protein-α (FAP), which aids in angiogenesis, epithelial-mesenchymal transition to allow tumor metastasis, and extracellular remodeling via collagen I cleavage to increase macrophage adhesion and prevent T cell tumor penetration.[118]

Cancer cells induce vessel growth and proliferation to facilitate their metastasis and removal waste via a process called angiogenesis. However, this neovascularization is abnormal as they are leaky and unevenly distributed, leading to a poor tumor perfusion with low pH and hypoxia, promoting tumor growth, spread, and apoptosis resistance, and impairs drug delivery to the tumor site.[119] In HCC, HIF1α, a hypoxia marker, mediated FAP expression and was associated with worse overall survival.[120]

Tumor-associated macrophages (TAMs) are regarded as the main tumor immune suppressive cells involved in angiogenesis, immune suppression, tumor invasion, and progression.[121] TAMs' infiltration and differentiation are driven by tumor cells through cytokine secretion such as CSF1, CCL2, or IL-4, but also by the TIME itself, such as extracellular matrix composition and hypoxia.[122] In CCA, TAMs are the main source of PD-L1, inducing CTL exhaustion and promoting CCA tumor progression.[123] In addition, TAMs express inducible nitric oxide synthetase (iNOS) and arginase-1 (Arg1), 2 enzymes involved in catabolism of L-arginine, an amino acid required in CTL activation and proliferation. L-arginine depletion in TIME causes reactive oxygen species (ROS), inhibiting CTLs.[124] TAMs also secrete cytokines such as IDO, IL-10, and CCL17, inducing T_{reg} differentiation and recruitment, which inhibits CTL infiltration.[125]

MDSCs also release reactive nitrogen species that activate and modify CCL2 chemokine, attracting monocytes and macrophages to the tumor microenvironment, but inhibiting T cell entry, trapping them in the stroma that surrounds the cancer.[126] MDSCs are immature myeloid cells from monocyte or neutrophil lineage with immune suppressive function, which promote tumor immune evasion and ICI

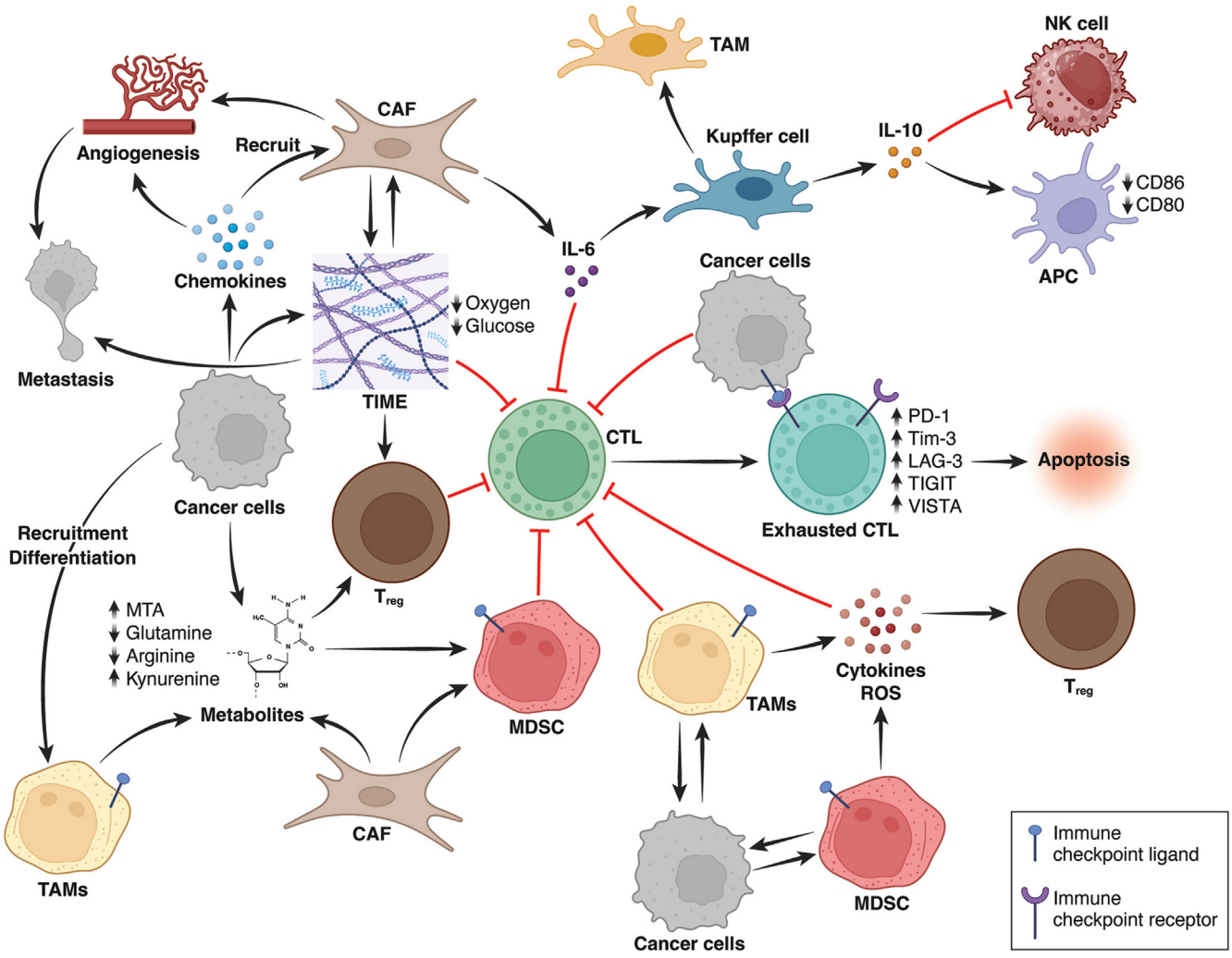

Figure 2. Complex interaction of tumor cells with the TIME to evade anti-tumor immune response. Tumor cells induce T cell exhaustion through: (1) immune checkpoint activation resulting in T cells apoptosis; (2) recruitment of immune suppression cells such as TAMs, MDSCs, Tregs, and CAFs, via cytokine and chemokine secretion; and (3) tumor metabolism, which induces hypoxia, low glucose, and depletion of metabolites in the TIME. Tumor cells preferentially uptake glucose, leading to accumulation of MTA and kynurenine, along with depletion of glutamine and arginine in TIME. These metabolites are also secreted by CAFs; they promote Tregs along with cytokines and ROS secretion by TAMs and MDSCs. IL-6, a central cytokine, induces differentiation of resident macrophages such as Kupffer cells into TAMs. and decreases CD80/86 on APCs. Tumor cytokine secretion stimulates angiogenesis, CAFs, TAMs and MDSCs, which promote tumor metastasis. CAFs secrete extracellular matrix, which form a dense physical barrier and limit T cells tumor infiltration.

resistance. In CCA, MDSCs are the second source of PD-L1 after TAMs and are recruited by CAF-derived CXCL2. Moreover, MDSCs' TIME infiltration is increased in response to immunotherapy targeting TAMs, highlighting their role in ICI resistance by a compensatory effect.[123]

The microbiome influences response to ICI therapy via crosstalk between immune system and micro-organism-associated molecular patterns.[127] Microbiome forms an additional layer of epithelial barrier, and microbial epitopes are often shared with neoantigens on tumor cells. When the epithelial barrier is disrupted, such as in dysbiosis in the setting of antibiotic treatment, microbes and their metabolites leak, increasing inflammation and contributing to ICI resistance. Fecal transplantation from ICI responders into germ-free mice demonstrated better tumor regression with ICI therapy compared with those receiving transplant from nonresponders.[128] Clinical trials of combination fecal microbiota transplant (FMT) with PD-1 therapy for metastatic melanoma have shown promise, but further studies are needed to elucidate how the microbiome impacts ICI response in HCC and CCA.[129]

Future Directions

Immunotherapy is a promising therapy for solid tumors, including HCC and CCA, and has the potential to revolutionize cancer care even in the peri-transplant period. However, tumors harbor and acquire resistance to ICI via complex and multiple mechanisms that highlight the

challenges to control cancer progression, stressing the importance of identifying new therapeutic targets and approaches to overcome resistance that can also be used in the peri-transplant setting. HHLA2, TIM-3, LAG-3, and TIGIT are cell surface proteins expressed on immune cells that are potential targets of immune-checkpoint blockade.

HHLA2 is a transmembrane protein member of the B7 family expressed on APCs and stimulated B cells, also overexpressed in solid tumors including HCC and intrahepatic CCA, thus being a possible target for therapy. Its interaction with killer cell Ig-like receptor, 3 Ig domains, and long cytoplasmic tail 3 (KIR3DL3) reduces T cell proliferation and function in HCC via activation of JAK/STAT signaling pathway associated with immune tolerance and T exhaustion.[130] High HHLA2 expression has been associated with worse prognosis and poorly differentiated tumors, and its co-expression with PDL-1 correlated with higher mortality and worse tumor progression in both HCC and CCA.[130,131] HHLA2 is a promising alternative to PD-1/PD-L1 ICIs. Indeed, HHLA2 expression is typically inversely correlated with PD-L1 expression in cancer cells. Negative PD-L1 or PD-1/PD-L1 ICI-resistant tumors could be targeted via this new immune checkpoint pathway.[132] Thus, new ICIs targeting HHLA2 and another targeting KIR3DL3 are currently in phase Ia/Ib clinical trial for various cancers including CCA with anti-KIR3DL3 (NTC05824663 and NTC05958199).[133] LAG-3 and TIGIT are transmembrane proteins of the Ig superfamily expressed on $CD4^+$ and $CD8^+$T cells, T_{reg}, and NK cells. Although LAG-3 has higher affinity for MHC-II than CD4 T-cells, thereby decreasing T cell-activation, TIGIT exerts its effect by binding PVR ligand on tumor cells, decreasing TCR expression and cytotoxic response.[134] LAG-3 and TIGIT expression correlate with poor prognosis, but their blockade has shown to be useful in anti-tumor activity with infiltration of $CD8^+$ T cells, increased T cell proliferation, and longer progression-free survival in HCC.[135]

TIM-3 is a surface glycoprotein expressed on $CD4^+$ and $CD8^+$T cells, T_{reg}, NK cells, and myeloid and mast cells that halts T cell activation after binding to ligands on tumor cell surface or released by apoptotic tumor cells. These ligands include carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) and galectin-9 (gal-9), which are differently expressed on tumor cells, whereas high mobility group protein B1 (HMGB1) and phosphatidylserine (PtdSer) ligands bind TIM-3 as they are released from tumor cells undergoing apoptosis.[136] TIM-3 is upregulated on tumor cells after PD-1 therapy and in cells with acquired ICI resistance, for which dual PD-1 and TIM-3 therapy for cancer therapy is being explored in clinical trials. Interim analysis of phase II clinical trial of anti-TIM-3 cobolimab in combination with anti-PD-1 dostarlimab in patients with HCC showed objective response rate of 46% with mild adverse events.[137] Although there are several clinical trials ongoing for ICIs targeting LAG-3, TIGIT, or TIM-3 immune checkpoints, they show clinical efficiency in association with PD-1 or PD-L1 ICIs, thus limiting their potential efficacy in PD-1/PD-L1-negative patients.

Other potential targets for immunotherapy in HCC and CCA include CD112R and glucocorticoids-induced TNF receptor family-related (GITR) proteins, which have also had favorable preliminary results, although clinical trials are needed to test their efficacy in these tumors.[138,139] The infusion of patients' derived T-cells after they are engineered to target cancer cells and the use of cancer vaccines are additional treatment strategies aimed at raising an immune response against tumor cells; their efficacy in HCC still needs validation through clinical trials, although their application in CCA has been limited to pre-clinical models.[140]

Additional therapies to modulate TIME could reduce tumor progression in the pre transplant period with limited negative events compared with ICI therapy. ICIs may be used in combination with alternative treatments or as sequential therapy to maintain an anti-tumor response. ProAgio, an integrin $\alpha v\beta_3$ antagonist, targets CAFs and vascular endothelial cells to mediate their apoptosis by recruiting caspase 8 in mice with breast cancer. They also cause collagen breakdown, and reduce EGF, PDGF, and insulin growth factor 1 (IGF1). These effects decrease tumor proliferation, angiogenesis, and metastasis; the latter acting through lowering lysine oxidase (LOX) levels normally secreted by myofibroblasts and CAFs, which is responsible for collagen cross-linking.[141] Focal adhesion kinase (FAK) is a nonreceptor tyrosine kinase that is overexpressed in human pancreatic ductal adenocarcinoma (PDAC). FAK1 is associated with lower CTL tumor penetration, tumor proliferation, and immunosuppressive TIME, although the mechanism is not well understood. Reducing expression of FAK1 led to decrease in tumor collagen formation with reduction in profibrotic factors CXCL20, CCL20, and CCL6. Low FAK1 also decreased FAP-expressing fibroblasts and reduced infiltrating MDSCs, while it increased CTLs infiltration. Mice with PDAC treated with FAK inhibitor VS-4718, gemcitabine, and anti-PD1 demonstrated reduction in fibrosis, increased CTLs infiltration, and higher survival.[142] FAK1 inhibitor is currently undergoing clinical trial in those with advanced pancreatic cancer,[143] but may serve as additional therapy in other stroma-dense tumors such as intrahepatic CCA.

Chimeric antigen receptor (CAR) T cells are synthetically engineered T cells that have shown promise in HCC. These CAR T cells targeting glypican-3 (GPC3) overexpressed in HCC or in combination with CAR T targeting TGFβ, demonstrated partial response in 2 of 13 subjects with advanced HCC.[144] T-cell receptor transduced (TCR) T cells designed to recognize TAAs are also being considered, particularly in HCC tumors overexpressing AFP, with preliminary clinical trial results showing stable disease in 64% of the participants. Both types of engineered T cells have the potential side effect of activating an acute inflammatory cytokine response, leading to life-threatening multiorgan damage.[145] This suggests this therapy modality is an unsuitable application in the peri-transplant setting, at least in their present form.

Conclusion

As tumor biology becomes more clear, elucidating new targets for therapy, it is also becoming more evident that ICI

therapy alone may not be truly curative, given mechanisms of resistance as abovementioned, at least for HCC and CCA. Multimodal therapies for HCC and CCA such as ICI therapy and liver transplantation need consideration where addition of ICI as adjuvant therapy has the potential of changing the natural history of the disease and preventing tumor relapse. ICI use pre-liver transplantation has the benefit of addressing micro-metastasis pre-transplant, reducing tumor burden, avoiding patient wait-list dropout, and may prevent post-transplant recurrence. However, ICI therapy may achieve more impactful results and decrease tumor recurrence if combined with therapies targeting distinct mechanisms aside from immune checkpoints such as demethylating agents, CAFs, tumor metabolites, and even microbiome modulation.

Cancer cells acquire immune resistance by hijacking immune self-tolerance and inflammatory gate-keeper mechanisms, such as by expression of immune checkpoint proteins and modulation of their microenvironment. ICI use before and after transplantation requires a tight balance between tolerance and effective anti-tumor response. ICI use in the post-transplant period risks graft loss and overall survival, but promising data in post-kidney transplant suggests therapy is possible after liver transplantation, likely needing personalized regimens that do not interfere with immunosuppression therapy. Adjusting the type of ICI, dose, and timing based on drug half-life and type of patient may mitigate the risk of adverse events, including rejection post-transplantation. Further data is needed on the mechanisms of ICI effect on graft tolerance and ICI interaction with immunosuppression in the post-transplantation period to better ascertain safety of this class of drugs and efficacy on combination therapy to achieve disease remission.

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Conflicts of interest
This author discloses the following: Emilien Loeuillard works for Nextpoint Therapeutics. The remaining author discloses no conflicts.