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Role of immunotherapy in downsizing hepatocellular carcinoma prior to liver transplantation

Ouranos K *et al*. Immunotherapy prior to liver transplantation

Abstract

Hepatocellular carcinoma (HCC) is an aggressive primary liver neoplasm that, according to tumor stage, can be treated with resection, transplantation, locoregional treatment options or systemic therapy. Although interventions only in early-stage disease can offer complete tumor regression, systemic therapy in advanced disease can significantly prolong overall survival, according to published clinical trials. The emergence of immunotherapy in the field of cancer therapy has had a positive impact on patients with HCC, resulting in atezolizumab-bevacizumab currently being the first-line option for treatment of advanced HCC. In light of this, application of immunotherapy in the pre-operative process could increase the number of patients fulfilling the criteria for liver transplantation (LT). Implementation of this approach is faced with challenges regarding the safety of immunotherapy and the possibly increased risk of rejection in the perioperative period. Case reports and clinical trials assessing the safety profile and effectiveness of neoadjuvant immunotherapy, highlight important aspects regarding this newly evolving approach to HCC management. More studies need to be conducted in order to reach a consensus regarding the optimal way to administer immunotherapy prior to LT. In this review, we summarize the role, safety profile and future considerations regarding the use of neoadjuvant immunotherapy prior to LT in patients with HCC.

Key Words: Hepatocellular carcinoma; Immunotherapy; Tumor downsizing; Liver transplantation; Neoadjuvant; Rejection

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Core Tip: Immunotherapy has been used in the treatment of advanced hepatocellular carcinoma (HCC) with promising results. Extending its use in the preoperative period prior to liver transplantation (LT), either alone or in combination with other locoregional treatment modalities, could increase the pool of potential LT candidates. Data from case reports and ongoing clinical trials assessing neoadjuvant immunotherapy prior to LT could revolutionize the current consensus regarding HCC downsizing practices and improve survival of patients with this type of malignancy.

INTRODUCTION

Hepatocellular carcinoma (HCC), the most common primary liver malignancy, constitutes the sixth most common cancer worldwide and the fourth most common cause of cancer-related mortality[1]. Incidence of HCC has been on the rise in parts of the world, such as Europe and the United States, where the main risk factors for HCC development include HBV and HCV infection, alcohol consumption and non-alcoholic fatty liver disease (NAFLD)[2-4]. Due to the fact that HCC has been the fastest-rising cause of cancer-related mortality[2], and that most patients present at an advanced stage at the time of diagnosis, multiple treatment approaches have been thoroughly investigated by the scientific community in an effort not only to detect the cancer at an earlier stage, when more treatment modalities are applicable, but also ensure complete eradication of the tumor.

Optimal treatment options for HCC depend on tumor morphological characteristics, liver functionality and overall physical status of the patient, as suggested by the Barcelona Clinic Liver Cancer staging system (BCLC), one of the most used staging

systems. According to BCLC, very early (0) and early (A) stages are potentially curative with radiofrequency ablation (RFA), surgical resection or liver transplantation (LT), with an overall survival (OS) greater than 60 mo. Patients with intermediate (B), advanced (C) and terminal (D) disease, however, who are not candidates for curative resection or transplantation, are best treated with transarterial chemoembolization (TACE), systemic therapy and supportive care, respectively, and face a grim prognosis with an OS of 20 mo for stages B and C and less than 3 mo for stage D[5-7].

Patients with early-stage disease who are not candidates for surgical resection can undergo liver transplantation (LT) as a curative option, given that they fulfill the respected criteria, with a 4-year survival rate of 75%. These criteria, widely known as the Milan criteria (MC), screen patients for liver transplantation eligibility based on morphological characteristics of the tumor. However, strict application of the MC can exclude many patients from receiving the potentially curative treatment of LT, solely on the basis of tumor size and number[8,9]. In an effort to include more patients within the MC and further utilize the clinical benefits of LT, the concept of downstaging has been introduced in the treatment of HCC. Downstaging refers to a decrease in the tumor burden to the point where patients meet the MC and can receive LT. Downstaging options include, but are not limited to, TACE combined or not with doxorubicin eluting bead (TACE ± DEB), RFA, microwave ablation (MWA), transarterial radioembolization (TARE), irreversible electroporation (IRE), high intensity focus ultrasound (HIFU), stereotactic body radiotherapy (SBRT) and systemic therapy[10]. Post-transplant survival rates in patients who had undergone LT after successful downstaging to MC have been shown to be comparable to that of patients undergoing LT and initially presenting within the MC[11].

In the modern era of cancer immunotherapy, alteration of signals that modulate the interaction between cancer cells and cells of the immune system, has led to many advances in the treatment of various cancer types, including HCC[12]. Although immune-modulating therapies are mainly used in advanced HCC, neoadjuvant immunotherapy is a promising approach as a means of downstaging the tumor prior to LT, yielding

positive outcomes in the post-transplant period[13,14]. The aim of this review is to summarize the role of immunotherapy as a downstaging technique and also highlight future considerations regarding its safety and clinically beneficial endpoints in the perioperative period and beyond.

ORTHOTOPIC LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA

The MC have been widely used as a tool for determining which patients are eligible for LT. According to these criteria, patients may undergo LT if the following requirements are met: (1) single tumor with a diameter ≤ 5 cm or (2) up to three tumors, each ≤ 3 in diameter and no extrahepatic spread or vascular involvement. Although patients with HCC transplanted within the MC have a 4-year survival rate of 75% and a recurrence-free survival rate of 83%, there are studies suggesting that patients not fulfilling the MC may still benefit from LT[15,16]. Overdependence on the MC may mask the true number of patients that would benefit from a transplant. In light of this, several expanded criteria have been proposed in an effort to include patients in the transplant process. What makes these criteria stand out to MC, is that they take into account not only morphological characteristics of the tumor, but also integrate biological aspects of the disease and response to locoregional treatment (LRT) in their algorithm[17]. One of the most commonly used biological parameter is alpha-fetoprotein (AFP). AFP serves as marker of HCC differentiation and can be used in the pretransplant period to identify patients at high risk for HCC recurrence after LT. AFP levels ≥ 1000 ng/mL are associated with poor outcomes following LT, although there are no established guidelines that indicate the optimal AFP threshold that accurately predicts post-LT outcomes[18,19]. Other well-studied biological parameters that can be taken into consideration include des-gamma-carboxyprothrombin (DCP) levels, neutrophil-to-lymphocyte ratio (NLR), the prognostic nutritional index, aspartate aminotransferase-to-platelet ratio index aspartate aminotransferase-to-neutrophil ratio index[18]. Evaluation of tumor response to LRT is a newly evolving concept in optimal selection of patients for LT, that aims to downstage

patients within the MC, promising comparable survival rates to patients with HCC receiving LT and already within the MC. Response to treatments that result in decreased tumor burden can be viewed as a complementary marker of the biological aggressiveness of the tumor and risk of HCC recurrence after LT[15]. All of the proposed expanded criteria that include the aforementioned parameters have 5-year survival rates that approximate that of MC, resulting in many institutions adopting them for the purpose of selecting patients with HCC for LT[18].

Application of the expanded criteria, however, requires an adequate reserve of available organs for transplantation, since more patients are included in the transplant process. And while this is not a problem for countries located in Asia, where living donor liver transplantation (LDLT) is the main organ source, countries of the Western world mainly depend on deceased donor liver transplantation (DDLT), which necessitates strict selection of eligible patients for LT[19]. Moreover, patients receiving DDLT typically have longer wait times when compared to patients receiving LDLT, raising concern for tumor progression in such circumstances. The above remarks highlight the importance of careful selection of patients for LT, in order to maximize the positive outcomes following LT. Downstaging therapy, ideally within the MC, is common practice nowadays and has a robust armamentarium of treatment approaches that serve to reduce tumor burden and make HCC amenable to transplantation. Also, bridging therapy aims to halt tumor progression and allow patients to receive curative treatment. Although there are no clear-cut indications for downstaging or bridging therapy, results from various studies suggest that patients presenting with tumor characteristics beyond the established criteria for LT, as well as patients with waiting times 6 mo or longer until LT, should receive neoadjuvant therapy[20,21]. Outcomes following implementation of pretransplant treatment modalities have been mixed. A study from Yao *et al*[8] revealed post-transplant survival and recurrence-free probabilities of patients with HCC successfully downstaged within MC to be comparable to those observed in patients with HCC and already within the MC at the time of diagnosis[22]. Other studies conducted by Lao *et al*[23], Chapman *et al*[24], and Gordon *et al*[25] have also reached to similar conclusions. However, several other studies

examining the effect of LRT on post-LT outcomes found out that neoadjuvant therapy is not associated with improved outcomes and may even increase recurrence of HCC following downstaging protocol implementation[26-30]. The lack of consistent outcomes following LRT application prior to LT has generated an extensive discussion of whether conventional LRT should be modified or enriched with the aim of enhancing the downstaging and bridging options for HCC[31]. Immunotherapy has been on the spotlight of HCC in recent years and is mainly used for late-stage disease when curative treatment is unfeasible, resulting in improved OS and progression-free survival (PFS)[32]. Neoadjuvant immunotherapy as a form of LRT prior to LT is a promising new approach that aims to leave behind the flaws associated with conventional LRT and increase the number of patients receiving curative treatment.

IMMUNOTHERAPY FOR ADVANCED HEPATOCELLULAR CARCINOMA

Tumor microenvironment in hepatocellular carcinoma

The liver is an immunogenically active organ. Under normal conditions, antigen-presenting cells (APCs) take up, process and present the antigens that enter the hepatic sinusoids on T cells, in an effort to elicit a robust immune response and prevent tissue damage. Kupffer cells, which are liver-specific macrophages, liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HSCs) constitute the most important APCs in the liver parenchyma and, apart from their antigen-presenting role, complement the immunological repertoire of the liver by other means as well[33]. Kupffer cells produce anti-inflammatory molecules, mainly interleukin-10 (IL-10) and TGF-β, attracting regulatory T cells (Tregs) that possess immunosuppressive properties, whereas LSECs and HSCs express high levels of programmed cell death-ligand 1 (PDL1), contributing to attenuation of the immune response[34]. As a result, the liver can fight off antigens that could cause tissue damage and also maintain immune tolerance, thereby avoiding autoimmunity.

HCC development is governed by alterations in the normal liver environment that promote tumoral spread via upregulation of immunosuppressive molecules that hinder

the immune response against cancer cells[35]. Maintenance of this immunosuppressive tumor microenvironment (TME) is achieved not only by liver-residing immune cells, but also from migrating populations of lymphocytes, collectively referred to as tumor-infiltrating cells (TICs)[36]. According to the subpopulation being studied, TICs can elicit an antitumoral immune response or result in upregulation of immune evasion by cancer cells. Figure 1 depicts the dynamic and complex interactions of the components of the TME and their effect on tumor spread[35-38] (Figure 1).

Mechanisms of immune evasion are of special concern, since many cancer treatment modalities depend on them. Immune checkpoint molecules modulate T-cell activation and function, attenuate the immune response against cancer cells and allow for unchecked cellular proliferation[39,40]. More specifically, PDL1, expressed by cancer cells or cells of the TME, binds to programmed cell death protein-1 (PD1) on the surface of T-cells, leading to T-cell exhaustion and inability to mount an effective immune response. Also, cytotoxic T-lymphocyte -associated protein 4 (CTLA-4) on T cells outcompetes CD28 for B7 on the surface of APCs, leading to loss of the co-stimulatory signal necessary for T-cell activation[41]. In order to halt tumorigenesis, alteration of the signals that promote immune evasion was made possible with the introduction of antibodies known as immune checkpoint inhibitors (ICIs). Such antibodies that mainly target PD1 (cepilimumab, nivolumab, pembrolizumab), PDL1 (atezolizumab, durvalumab, avelumab) and CTLA-4 (ipilimumab), have been used in the treatment of various cancers, including HCC, and have been shown to correlate with improved OS in major studies assessing their efficacy[42].

The role of immunotherapy in advanced hepatocellular carcinoma

Although systemic therapy targeting signal conduction pathways appeared in the treatment of HCC in 2007, immunotherapy lagged for about a decade before making a debut in 2017[43-45]. Nivolumab, a PD1 immune checkpoint inhibitor, was the first monoclonal antibody to be assessed in the treatment of advanced HCC. The CheckMate 040 was a non-comparative, dose escalation and expansion trial that included 262 patients

(48 in the dose escalation and 214 in the dose expansion phase) and revealed that nivolumab had an objective response rate (ORR) of 15%-20% according to the mRECIST criteria and a median OS of 13.2 - 15 mo, findings that were comparable to the outcomes produced by sorafenib, the first-line treatment for HCC at that time. Due to the fact that no control arm was available in that trial, subsequent analyses comparing nivolumab to sorafenib were conducted. The CheckMate 459 phase 3 trial, assigning 743 patients with HCC to receive either nivolumab (intervention arm) or sorafenib (control arm), however, failed to show a statistically significant improvement in median OS [hazard ratio 0.85 (95%CI: 0.72-1.02); *P* value above the protocol-defined significance level] and PFS [hazard ratio 0.93 (95%CI: 0.79-1.1); *P* value above the protocol-defined significance level], but revealed a clinically significant median OS of 16.4 mo *vs* 14.7 mo in the intervention and control arms, respectively. Even more, grade 3-4 adverse effects were reported in 22% of patients treated with nivolumab *vs* 49% of patients treated with sorafenib, justifying the use of this immune-modulating therapy in patients who are not candidates for sorafenib[32,46-48]. Pembrolizumab, another PD1 immune checkpoint inhibitor, was also assessed in the KEYNOTE 224 study, yielding an ORR of 17% and median OS of 12.9 mo[49]. Phase 3 trials assessing the comparative efficacy of pembrolizumab to best supportive care, failed to show statistical significance in the primary endpoints of OS and PFS, albeit a clinically significant increase in OS[32,50,51]. Multiple other monoclonal antibodies have been thoroughly investigated as potential first-line treatment options for advanced HCC, including tislelizumab, durvalumab, avelumab, tremelimumab and atezolizumab. Results from these studies have revealed promising outcomes regarding the effect of these immunotherapies in OS and PFS when compared to currently established first-line options for HCC. Table 1 summarizes the major trials that harness immunotherapy, either alone or in combination with other modalities (*e.g.*, addition of a second ICI or systemic therapy), for the treatment of advanced HCC[32,33,39-42,46,47,49,52-54] (Table 1).

The IMbrave150 trial was a cornerstone in the management of advanced HCC. This global, open-label phase 3 randomized trial compared atezolizumab-bevacizumab to

sorafenib in the treatment of advanced HCC. Atezolizumab is a PDL1 immune checkpoint inhibitor and bevacizumab is a vascular endothelial growth factor (VEGF) inhibitor. 501 patients were randomly assigned in 2:1 ratio to receive either atezolizumab-bevacizumab or sorafenib until there was clinical benefit or emergence of unacceptable side effects. The primary endpoints were OS and PFS, whereas secondary endpoints included ORR, duration of response, deterioration of quality of life, physical functioning, and role-functioning. According to the results, median OS was 19.2 moths (95%CI: 17.0-23.7) with atezolizumab-bevacizumab and 13.4 mo (95%CI: 11.4-16.9) with sorafenib [hazard ratio 0.66 (95%CI: 0.52-0.85), *P* value < 0.001], whereas PFS was 6.9 mo (95%CI: 5.7-8.6) with atezolizumab-bevacizumab and 4.3 mo (95%CI: 4.0-5.6) with sorafenib [hazard ratio 0.65 (95%CI: 0.53-0.81), *P* value < 0.001]. Results of secondary endpoints were also statistically significant and favored the atezolizumab-bevacizumab arm. Grade 3-4 adverse effects occurred in 56.5% and 55.1% of patients in the intervention vs control arm, respectively, with the most frequent severe adverse effect in the atezolizumab-bevacizumab group being high-grade hypertension (15.2% of patients)[55]. The overall outcome of this study resulted in atezolizumab-bevacizumab being the current first-line treatment option for managing advanced HCC[56-59].

Recently, the HIMALAYA study assessed the efficacy of combination tremelimumab and durvalumab in advanced HCC. This phase 3 study involved 1234 patients that were randomly assigned to receive durvalumab and tremelimumab or sorafenib or durvalumab monotherapy. The ORR was 20.1% in the durvalumab-tremelimumab group *vs* 5.1% and 17% in the sorafenib and durvalumab groups, respectively. The PFS and OS were 3.78 and 16.4 mo in the durvalumab and tremelimumab group, 4.07 and 13.8 mo in the sorafenib group, and 3.65 and 16.6 mo in the durvalumab group. Grade 3-4 adverse events occurred at a lower rate in the durvalumab-tremelimumab and durvalumab groups when compared to the sorafenib arm. Overall results of this breakthrough study open up new treatment options that could be integrated into the treatment algorithm of HCC management[60].

As suggested by the above remarks and Table 1, clinical trials assessing the combination of immunotherapy and systemic therapy or the use of two ICIs concurrently, have shown greater outcomes when compared to trials that use single-agent therapy (immune-modulating or systemic) in the intervention arm. An ambitious treatment approach is the combination of ICIs with LRT, the latter of which is traditionally used in early-stage disease or as a means of downstaging or bridging therapy prior to LT[61]. The idea behind this approach is that LRT can alter the TME by inducing a robust antitumoral immune response and reduce the number of immunosuppressive molecules. Although these effects could theoretically justify LRT as a single therapy to control tumor progression, evidence suggests that such responses are weak and transient and cannot completely control the tumor. The addition of immunotherapy could amplify the antitumoral responses produced by LRT, thus creating a synergistic interaction between ICIs and LRT that could effectively control tumor spread[62,63]. There are a few trials assessing the combination of LRT with ICIs, since most of them take advantage of immunotherapy in the form of adoptive cell and vaccine therapy. However, results from these studies have demonstrated favorable outcomes in terms of OS and safety, thus encouraging the implementation of this combination in case other first-line treatment modalities fail[62].

Although combination immunotherapy is a superior approach than single-agent immunotherapy for the treatment of HCC, there are a few remarks that need to be pointed out. The need of combining various immunotherapeutic drugs in specific dosages may come as a challenge for smaller hospitals that are neither readily equipped, nor familiar with the specific combination regimens used to treat HCC. The lack of availability of highly efficacious drugs in resource-limited hospitals prevents the widespread application of immunotherapy, leaving healthcare providers with a restricted panel of drug options, mainly systemic chemotherapeutic agents, that, although effective, do not demonstrate the superiority of immunotherapy in treating HCC. Unfortunately, this hurdle inevitably affects pre-transplant ICI use for the same reasons mentioned above.

IMMUNOTHERAPY AS A DOWNSTAGING THERAPY PRIOR TO LIVER TRANSPLANTATION

It seems evident that immunotherapy has an integral role in the management of advanced HCC. The success of ICI use in the long-term survival of patients with HCC has brought up into question whether immunotherapy could also produce significant outcomes in early-stage disease and mainly as neoadjuvant treatment modality prior to LT. Although data on this topic are scarce, valuable information can be extracted regarding the future applications of ICIs in HCC management.

Goals of neoadjuvant immunotherapy

Delivery of immunotherapy prior to LT serves the same goals as application of conventional LRT, and, at the same time, establishes new perspectives in terms of prediction of post-LT outcomes and survival following transplantation. Bridging and downstaging ICI therapy is a novel approach of maintaining or even increasing the pool of transplant HCC candidates able to undergo curative LT. Beyond that, ICIs may have additional benefits post-LT, since they may be able to decrease disease recurrence by treating micrometastatic disease that was not detected prior to LT[14]. The basis behind the already mentioned promising benefits of neoadjuvant immunotherapy stems from the ability of ICIs to reconstitute the immune response towards an antitumoral microenvironment that halts disease progression. More specifically, histologic analysis of a specimen from a subject enrolled in a study evaluating the perioperative use of ICIs in patients with HCC revealed an increase in the number of cytotoxic CD8+ T-cells and levels of interferon-γ (IFN-γ), which are both known to mitigate the immunosuppressive TME seen in HCC and at the same time mount an effective antitumoral, inflammatory response that controls tumor spread. Also, although the cluster of regulatory T-cells, which are well known to induce an immunosuppressive environment and promote cancer spread, was increased, there was an eventual complete pathologic response observed in the analyzed specimen. This could be due to the high CD8+ T-cell /

regulatory T-cell ratio, favoring the antitumoral immune response, or to the presence of a mixed population of regulatory T-cells that serve to halt disease progression[64]. Other studies have also evaluated the mechanisms responsible for producing favoring outcomes following periprocedural ICI administration and have concluded that the overwhelming infiltration of tumor-specific CD8+ T-cells, the release of inflammatory cytokines, such as IFN-γ and TNF-α, the elevated number of tumor neoantigens that attract T cells and the relative decrease in the number of immunosuppressive and regulatory T-cells, all contribute to the positive immune-modulating outcomes of neoadjuvant ICI use[65-68]. Overall, neoadjuvant immunotherapy prior to LT in HCC serves three main goals: 1. preventing patients from waitlist dropout, when the time interval to LT is substantial (bridging therapy), 2. increasing the number of patients eligible for transplantation by including them in established LT criteria (downstaging therapy) and 3. ensuring micrometastatic spread eradication after LT, thereby increasing the chances of prolonged survival after surgery.

Considerations regarding the safe use of neoadjuvant immunotherapy prior to lt in patients with HCC

When contemplating ICI administration prior to LT, one has to take into account the time interval between the last dose of ICI therapy and LT, factors that predict response to ICI therapy, in order to prevent graft rejection, and the possible adverse events associated with ICI and how they could be effectively managed.

Post-LT ICI administration has been linked to donor allograft rejection[69]. Indications for using immunotherapy after transplant include recurrence of malignancy or emergence of a new tumor that is responsive to ICI therapy. When a transplant process takes place, immunosuppression typically follows to prevent the host's immune response against the transplanted allograft. ICI administration, by upregulating the T-cell response and dampening the signals that create a state of relative immunosuppression that is desirable post-LT, can result in T-cells attacking the graft, resulting in dysfunction, subsequent rejection, and eventual graft and/or patient loss. Despite this feared outcome,

studies evaluating graft function after ICI administration in patients undergoing LT have been mixed, and no consensus has been reached regarding the safety profile of immunotherapy in the perioperative period[70]. A case series study evaluating 13 HCC patients who received ICI post-LT revealed that 4 patients (31%) developed graft rejection[71]. Another study identified a cohort of 14 patients who received ICIs post-LT, with 4 of them (29%) experiencing graft rejection[72]. Moving to the downstaging setting, it is important to consider a washout period between the last dose of immunotherapy and LT in order to downregulate the immune response that was accentuated during ICI therapy, thus allowing the allograft to be successfully transplanted. The ideal time interval until LT has not been decided, mainly due to the limited number of studies harnessing ICIs as a downstaging tool, but there are some important aspects to consider regarding this topic. The half-life of the immune-modulating agent could be used as an adjunctive parameter to calculate the time of immunotherapy discontinuation to LT. However, further understanding of the mechanism of action of ICIs may prove the above remark unreliable. Indeed, occupancy of drug-specific targets by these medications can be prolonged, resulting in a duration of effect that extends beyond the period one would calculate based on the half-life of the ICI[73]. For example, although the half-life of nivolumab is about 25 days, it has been observed that its effects may last for up to 2 mo following a single infusion of the drug, due to sustained occupancy of PD1 on the surface of T cells. Although a short washout period would theoretically correlate with increased risk of graft rejection, there are notable examples that prove this point wrong. A study by Tabrizian *et al*[13] assessed the outcome of 9 HCC patients who were transplanted in a single center between 2017-2020 after receiving nivolumab 240 mg every two weeks as downstaging therapy. Washout period did not exceed 30 days for any patient after discontinuation of treatment and, notably, two patients discontinued nivolumab 1 and 2 days prior to LT. Following transplantation, no severe graft rejection, tumor recurrence or death occurred, with one patient developing mild rejection that was appropriately managed with an increase in the dose of tacrolimus. Interestingly, intraoperative blood transfusion was administered in the two patients who received LT within 2 days of

nivolumab discontinuation, which could have accelerated the rate of drug washout[13]. In another study by Chen *et al*[74], a patient who underwent LT and discontinued preoperative toripalimab 93 days before the procedure, suffered ICI-induced acute hepatic necrosis. Results of these studies could indicate that half-life of a drug could not by itself predict the optimal time to LT after downstaging therapy implementation. Other potential parameters or markers should be investigated in order to attain a more precise estimate of the washout period.

Predicting if a liver graft is suitable for transplantation after ICI administration is a promising feat that could smooth out the perioperative process. PDL1 molecule expression on the transplanted graft could act as surrogate biomarker of the safety of ICIs in terms of inducing or not graft rejection. The idea behind this approach is that PDL1-negative grafts will have fewer rejection rates when compared to positive ones, since ICIs won't be able to mount an inflammatory immune response in the absence of drug-binding molecules on the cells of the transplanted parenchyma, thus maintaining the immunosuppressive environment required for LT. A study by Shi et al. was conducted to compare the graft rejection rate in 5 cancer patients who received PDL1-negative allografts when compared to controls with an unknown PDL1 status in their transplanted liver, after receiving the immune-modulating agent toripalimab. Results showed that none of the 5 patients who received PDL1-negative grafts experienced rejection, whereas another patient treated off-record who received PDL1-positive graft, experienced rejection after ICI administration. In another study conducted by Friend *et al*[75], graft rejection was detected in two HCC patients who received nivolumab after being transplanted with PDL1-postive allografts[76]. DeLeon *et al*[77]. conducted a retrospective evaluation of seven cancer patients undergoing LT to assess the safety of post-transplant ICI use. Five out of seven patients in the study were assessed for PDL1 expression and two of them were positive. One of the two patients who received PDL1-poisitive grafts also demonstrated high levels of tumor-infiltrating lymphocytes in the transplanted liver. The results of the final study indicate that apart from PDL1 status, other potential biomarkers should be assessed to predict the outcomes of ICI use in the operative period.

Although no major studies have been conducted up to date that could reliably emphasize the role of miscellaneous biomarkers that predict the safety of ICI use during LT, immunohistochemical analysis of the transplanted allograft could be used as a surrogate parameter that aims to better delineate the outcome of LT following ICI administration.

Although rejection is an undesirable outcome of ICI therapy, other adverse events can also occur, collectively known as immune-related adverse effects (iRAEs). Such adversities can prolong or even terminate the transplant process, not only because iRAEs may make the patient ineligible for LT, but also because effective management of such outcomes may prolong the time interval to LT, resulting in progression of the malignancy and dropout from the transplantation criteria. Most iRAEs present within the first two weeks of treatment initiation, although they can occur at any time. Every organ can be involved, and severity can range from mild to life-threatening[78,79]. Results from major clinical trials have found that grade 3-4 adverse events occur at an acceptable rate that would justify their use in HCC treatment. In the IMBrave150 trial, grade 3-4 adverse effects occurred in 56.53% of patients who were treated with atezolizumab-bevacizumab when compared to 55.13% of patients in the control group who were treated with sorafenib. The percentage of high-grade adverse effects in the intervention group was not attributed solely on atezolizumab, since hypertension, the most common high-grade adverse event observed in the study, is most likely attributable to bevacizumab[47,58]. In the KEYNOTE 240 trial, grade 3-4 adverse effects occurred in 52% of patients treated with pembrolizumab *vs* 46,27% in the control arm[47].

It is not yet clear which class of ICIs is safer. While CTLA4 plays an important role in the induction of graft tolerance, PD1/PDL1 interactions result in both induction and maintenance of graft tolerance. Theoretically, this could imply that immunotherapy targeting PD1 and/or PDL1 molecules is more likely to cause organ rejection than agents that target CTLA4[80]. However, there are still no published studies that assess the comparative safety profiles of various classes of immunotherapy, so no definite conclusions can be drawn[71]. Regardless of which class will be chosen, treatment of iRAEs is the same, with glucocorticoids being the most common immunosuppressant agent that

can effectively ameliorate negative outcomes of ICIs[78]. Patients undergoing LT for HCC usually have compromised liver function. Nonetheless, ICI use is safe in this patient population, since these drugs are not metabolized in the liver.

As already mentioned before, the paucity of available donors for LT substantially limits this treatment approach for the management of HCC. Although currently not employed in the armamentarium of HCC management, autologous LT is a theoretically promising approach that could increase the number of patients receiving curative treatment. Data regarding autologous LT following immunotherapy are not yet available, but a hypothetical explanation of the mechanism behind this approach could ignite future discussions around this topic. Liver regeneration capabilities are well studied in the literature. The effects of immunotherapy in the TME have been extensively discussed above and generally promote an antitumoral immune response that aims to halt tumor progression and decrease tumor burden. As such, more liver parenchyma can be restored to its physiologic architecture. Such an occurrence can aid in the autologous LT process by increasing the available tissue for extraction and reimplantation following diseased liver removal. As ideal as this approach may sound, challenges along the way, such as remaining unidentified tumor burden, metastatic disease and recurrence of malignancy are all topics of concern that need further investigation. For the time being, autologous LT following immunotherapy requires more research in order to delineate the exact mechanisms that could result in positive outcomes.

Clinical trials and case reports assessing the use immunotherapy as a downstaging technique prior to LT in patients with HCC

Case reports: According to literature review, twenty cases involving patients with HCC receiving ICIs prior to LT have been published[13,73,74,81-83] (Table 2). The majority of the patients were male (85%) and the mean age was 58.4 years. The most common underlying liver disease was HBV-induced liver disease, while HCV infection, alcoholic liver disease and NAFLD were also observed. Notably, one patient had no underlying liver disease. The most commonly used ICI prior to LT was the PD1 inhibitor nivolumab (55% of cases).

Other immune-modulating agents used were toripalimab, durvalumab, camrelizumab and pembrolizumab. The time interval between the last dose of ICI and LT varied significantly among the cases, with one patient receiving the last ICI dose one day prior to LT and another one almost 29 mo prior to the operation. No recurrence of the tumor occurred in patients that had a successful LT after ICI use. Non-fatal perioperative complications, excluding rejection, occurred in only one patient, who developed bile leak that was appropriately managed without further consequences. Out of the 20 cases described, two patients had fatal rejection and two others experienced mild rejection that was adequately treated. The first patient with fatal graft rejection, described by Chen *et al*[74], had chronic HBV infection. He underwent DDLT due to recurrent HCC that was previously treated with resection, RFA, TACE, MWA, sorafenib, lenvantinib and toripalimab. The last cycle of ICI therapy was administered 93 days prior to LT. Following the procedure, the patient's liver function status deteriorated rapidly, and a liver biopsy performed on the second postoperative day revealed massive liver tissue necrosis that was attributed to toripalimab. The patient expired three days after the procedure[73]. The second patient with fatal graft rejection, described by Nordness *et al*[81], had chronic HCV infection. He underwent DDLT due to recurrent HCC previously treated with resection, sorafenib, RAE, TACE and nivolumab. The last dose of nivolumab was administered 8 days prior to LT. On postoperative day 5, rapid elevation of liver enzymes was noted, and the patient deteriorated clinically to the point where he was transferred to the ICU. A biopsy that was performed the next day revealed acute hepatic necrosis with a dense lymphocytic infiltration, findings that point towards a diagnosis of ICI-induced graft rejection. Reversible graft rejection that was observed in two patients was due to low levels of immunosuppressive medications and was appropriately treated with dose escalation, without inflicting any major damage to the graft recipients.

Clinical trials: Currently, there is a limited number of clinical trials assessing the use of ICIs prior to LT in patients with HCC. However, there are multiple studies evaluating neoadjuvant administration of immunotherapy prior to liver resection in patients with

HCC[39] (Table 3). These are mainly phase 1-2 studies with no control arm that assess safety, efficacy, and tolerability of the immune-modulating agent, either alone or in combination with other therapies. Nivolumab is the most used ICI in these studies[84-88]. Other ICIs used include tislelizumab, cemiplimab, toripalimab and camrelizumab[89-92]. Most of these trials are ongoing, with the majority of them not having any published results. Analysis of completed studies, however, reveals satisfactory objective response rates and an acceptable rate of adverse events, setting the stage for the recommencement of phase 3, randomized studies that will provide us with valuable information regarding the benefits of neoadjuvant immunotherapy before resection or LT.

Up to date, there are two clinical trials contemplating neoadjuvant immunotherapy prior to LT in patients with HCC. The first trial (NCT04425226) is a randomized study that will assess the neoadjuvant use of pembrolizumab and lenvatinib as a downstaging and/or bridging therapy prior to LT in 192 patients with HCC. Participants will receive pembrolizumab 200 mg intravenously on day 1 of each 21-day cycle. Treatment will continue until unacceptable toxicity develops or until there are at least 42 days remaining to LT. Concurrently, study subjects will receive lenvatinib 8-12 mg orally at least 38 days every 6 weeks and until there are at least 7 days prior to LT. The primary endpoint will be RFS, whereas secondary endpoints include the disease control rate, the percentage of patients who will experience adverse outcomes and who will discontinue study treatment due to an adverse event, and the ORR. Results of the study are expected in December 2024[93]. The second trial (NCT04035876) is a phase 1-2, single-arm study that evaluated the use of camrelizumab and apatinib as downstaging and/or bridging therapy prior to LT in 120 patients with HCC. Participants received camrelizumab 200 mg intravenously every 2 weeks and apatinib 250 mg orally every day. Camrelizumab was discontinued 5 weeks before and apatinib 1 week before LT. Primary endpoints included objective remission rate and RFS, whereas secondary endpoints included OS, time to progress and rate of adverse events. Results of this study are not yet available[94].

CONCLUSION

Liver transplantation is a curative treatment approach for HCC. With respect to the current transplant criteria, conventional LRT has been widely used as downstaging and/or bridging therapy to increase the pool of potential LT candidates. Nevertheless, the benefits of immunotherapy in patients with advanced HCC have generated an extensive discussion whether ICIs could be used safely and effectively in the pretransplant process in order to yield favorable outcomes. When contemplating neoadjuvant immunotherapy, the risk of graft rejection after LT is a matter of concern; results from a limited amount of case reports, however, showed that the risk may not be as high, with fatal rejection presenting in only 2 out of 20 cases of LT after ICI administration. More studies need to be conducted in order to delineate the factors that could reliably predict outcomes after LT in patients receiving neoadjuvant immunotherapy. Determination of surface molecule expression, such as PD/PDL1, obtained *via* liver biopsy, is a tempting marker that could predict response to outcome, but, utilized alone, doesn't seem to accurately include all patients that would benefit or not from ICIs. More markers need to be taken into consideration, either alone or in conjunction with other aspects of disease treatment that focus on the pharmacokinetics of immunotherapy. Drug half-life could theoretically play an important role in determining the ideal time interval spanning from ICI discontinuation to LT. In practice, however, no fatal rejection was observed in patients with cessation of drug therapy even 1 day before surgery, emphasizing the fact that individualization of treatment regimen is a superior approach than strict adherence to the properties of the drug in order to allocate patients to the appropriate drug scheme. Patient comorbidities, availability of other neoadjuvant treatment options and the ability to timely treat emerging ICI-related adverse effects are all remarks that should be explored prior to initiating immunotherapy. Clinical trials that assess neoadjuvant ICI therapy, either before liver resection or transplantation, show promising results, both in treatment safety and efficacy, with primary and secondary study endpoints being met successfully. Insights from future studies, which are currently underway, are necessary to better understand the impact of neoadjuvant immunotherapy in the perioperative period and beyond.

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