Review

HCC: Evolving Role of Systemic Therapies as a Bridging Treatment to Liver Transplantation

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Simple Summary: Hepatocellular carcinoma (HCC) is a common cancer and a leading cause of cancer-related deaths worldwide. However, HCC can be effectively treated in selected cases with liver transplantation representing one of the limited options for potential cure. Unfortunately, many patients are ineligible for liver transplantation due to either advanced tumor at initial diagnosis or due to disease progression while awaiting liver transplantation. Our review discusses the role of systemic therapies as a bridging treatment to liver transplantation, thereby enabling more HCC patients to undergo potentially curable liver transplantation.

Abstract: Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths. Classically, liver transplantation (LT) can be curable for HCC tumors within the Milan criteria. Bridging strategies to reduce the dropouts from LT waiting lists or/and to downstage patients who are beyond Milan criteria are widely utilized. We conducted a literature-based review to evaluate the role of systemic therapies as a bridging treatment to liver transplantation (LT) in HCC patients. Tyrosine Kinase Inhibitors (TKIs) can be used as a bridging systemic therapy to LT in patients with contraindication to locoregional liver-directed therapies. Immune Checkpoint Inhibitors (ICIs) treatment can be utilized either as a monotherapy or as a combination therapy with Bevacizumab or TKIs prior to LT. Acute rejection post liver transplantation is a concern in the context of ICIs treatment. Thus, a safe ICI washout period before LT and cautious post-LT immunosuppression strategies are required to reduce post-LT rejections and to optimize clinical outcomes. Nevertheless, prospective clinical trials are needed to establish definitive conclusions about the utility of systemic therapy as a bridging modality prior to LT in HCC patients.

Keywords: Hepatocellular carcinoma (HCC); systemic therapies; bridging; liver transplantation (LT); Tyrosine Kinase Inhibitors (TKIs); Immune Checkpoint Inhibitors (ICIs)

1. Introduction

Liver cancer is the sixth most common diagnosed cancer and the third most common cause of cancer-related deaths worldwide [1]. Hepatocellular carcinoma (HCC) accounts for approximately 80% of liver cancers, most often, in the background of liver cirrhosis secondary to multiple risk factors including hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, alcohol-related liver disease (ALD) or metabolic dysfunction-associated steatotic liver disease (MASLD) [2,3].

Liver transplantation (LT) is the treatment of choice for HCC patients in early stages if the tumor is unresectable [4,5]. There are various criteria used to assess eligibility for LT in HCC. Classically, the Milan criteria is utilized to define eligibility for LT with a goal of achieving 4-year overall survival

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(OS) of more than 75%. The Milan criteria is included in the Barcelona Clinic Liver Cancer (BCLC) staging system, the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver-European Organization for Research and Treatment of Cancer (EASL-EORTC) guidelines [5,6]. The University of California-San Francisco (UCSF) criteria represents another important expanded criterion for LT in HCC patients with comparable outcomes to the Milan criteria. The UCSF criteria is adopted in countries such as Australia and New Zealand [7–9]. Moreover, The United Network of Organ Sharing (UNOS) Down-Staging Criteria for LT is widely adopted in USA with a successful 5-year post-LT-OS rate of 74% [10].

The biggest challenge for LT is organ shortage and long LT waiting lists leading to patients' dropout due to disease progression or liver decompensation [11]. In the USA, the national dropout rate for HCC patients from the transplant list reaches up to 29% as per the UNOS figures [12]. Thus, there is a need to implement therapies that control HCC until the availability of liver transplantation. This "bridging therapy" approach to transplantation aims either to reduce the number of dropouts from LT waiting lists for patients within Milan criteria or/and downstage patients who are beyond Milan criteria [13]. Bridging strategies are either locoregional liver-directed or systemic therapies. Liver-directed therapies include Radiofrequency Ablation (RFA), Microwave Ablation (MWA), Transarterial Chemoembolization (TACE), Transarterial Radioembolization (TARE) and Stereotactic body radiotherapy (SBRT) [14,15].

This review will focus on systemic therapies including: Tyrosine Kinase Inhibitors (TKIs) and Immune Checkpoint Inhibitors (ICIs) as bridging strategies to LT in HCC patients.

2. Systemic Therapies

2.1. Tyrosine Kinase Inhibitors (TKIs)

The therapeutic effect of TKIs is attained by binding to various tyrosine kinase receptors, thereby inhibiting downstream intracellular signaling, eventually resulting in apoptosis and anti-angiogenic effects which play a major role in the tumor micro-environment of HCC [16]. Sorafenib, a small molecule TKI, was approved by the FDA for the treatment of advanced HCC based on breakthrough results of the SHARP Trial in 2008 [17].

After approval for advanced HCC, TKIs were investigated as bridging treatments to LT. In 2010, Saidi et al. reported a case series of 7 HCC patients whose tumors met the Milan criteria. All patients were treated with Sorafenib and 6 of them successfully underwent LT without local recurrence or distant metastasis [18]. In 2013, Vitale et al. reported another case series of 6 HCC patients who had Child-Pugh class A and intermediate stage disease, these patients received Sorafenib before LT and the 4 patients who received Sorafenib for a period of ≥ 2 months before LT were disease-free 27-to-41 months after LT [19]. In 2018, Golse et al. reported a cases series of 5 HCC patients, three of them underwent hepatectomy or TACE then received Sorafenib as a bridging therapy before LT, the 2 other patients received Sorafenib as a down staging therapy before LT. There were no tumor recurrences after LT within a 27-month follow-up [20]. In 2022, an observational study from France found that 62 out of 327 HCC patients listed for LT were treated with Sorafenib, 50% of these patients received Sorafenib because of HCC progression after locoregional therapy (LRT), the other 50% received Sorafenib because of ineligibility to receive LRT. 26 patients could go to LT, the 5-year OS and RFS were 77% and 48%, respectively [21].

Combination therapy with LRT and Sorafenib as bridging strategy to improve outcomes was also investigated. In 2014, 20 HCC patients who met the UCSF criteria for LT were randomized in a prospective trial to Y90 radioembolization with or without Sorafenib (1:1 randomization ratio). Of the 20 patients, 17 underwent LT. Survival rates at 3-years were similar between the two groups. Combination therapy was associated with higher peri-transplant biliary complications and a trend towards more acute rejections [22]. In 2015, another prospective trial was published on 50 HCC patients who met the Milan criteria for LT were randomized to TACE plus either Sorafenib or placebo, 17 patients could proceed to LT, both groups had similar results in terms of time to progression (TTP), tumor response and time-to-LT [23]. In 2022, a retrospective study on 128 HCC patients, whose

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tumors were either within or beyond Milan criteria, found that those patients who received TACE plus Sorafenib before LT achieved significantly better 5-year-DFS compared to those who received TACE only, however there was no significant difference in the 5-year-OS rates between the two groups (77.8% vs 61.5%, p value: 0.51) [24].

Cabozantinib is another TKI that is approved as a subsequent-line of treatment for advanced HCC patients [25]. In 2022, Bhardwaj et al. reported 2 HCC patients who received Cabozantnib after prior treatments with Sorafenib and LRT. Both patients managed to go to LT. The first patient developed disease recurrence 5 months after LT and the other patient was disease free at 21 months follow-up post LT [26].

Summary and recommendations

Utilization of TKIs as bridging treatment to LT in HCC patients with contraindication to locoregional liver-directed therapies is reported in the literature (Table 1). Most published reports examined Sorafenib with encouraging survival rates. However, prospective clinical trials are needed to establish definitive conclusions about the utility of TKIs monotherapy as a bridging modality prior to LT in HCC patients.

Table 1. Overview of the studies on TKIs used as a bridging therapy to liver transplant.

Author/year	Milan Criteria	Treatment	No. of transplanted patients	Post-LT DFS	Post-LT OS	Post-LT follow-up (months)
Minoux et al. (2022) [21]	In: 69.4% Out: 30.7%	Sorafenib	26	48%	77%	60
Abdelrahim et al. (2022) [24]	In: 74% Out: 26%	TACE +/- Sorafenib	128	100% vs 67.2%, p=0.07	77.8% vs 61.5%, p=0.51	60
Bhardwaj et al. (2022) [26]	In: 100%	Cabozantinib	2	50%	50%	21
Golse et al. (2018) [20]	In: 60% Out: 40%	3/5: hepatectomy or TACE then Sorafenib 2/5: Sorafenib	5	100%	NA	27
Hoffmann et al. (2015) [23]	In: 100%	TACE + Sorafenib vs TACE+ Placebo	17	HR: 1.259 (95%CI: 0.486, 3.270)	NA	10
Kulik et al. (2014) [22]	up to UCSF criteria: 100%	Y90 radioembolization +/- Sorafenib	17	NA	72% vs 70%, p=0.57	36
Vitale et al. (2013) [19]	Out: 100%	Sorafenib	6	66%	66%	(27-41)
Saidi et al. (2010) [18]	In: 100%	Sorafenib	7	85%	NA	NA

Abbreviations: NA: not available, OS: overall survival, Post-LT: post liver transplantation, TACE: Transarterial Chemoembolization, UCSF: University of California San Francisco.

2.2. Immune Checkpoint Inhibitors (ICIs)

2.2.1. Efficacy & Safety

Over the past few years, immune checkpoint inhibitors (ICIs) revolutionized the field of oncology, leading to prolonged survival with manageable side effects in various cancers [27]. In 2020, the combination therapy of Atezolizumab (ICI) with Bevacizumab was granted FDA approval for first-line treatment of advanced HCC patients based on superior survival outcome compared to the classical treatment with Sorafenib [28].

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The mechanism of action of ICIs involves activation of the suppressed innate immune system which is contradictory with functions of the immunosuppressant agents that are typically used post-LT. Consequently, the use of ICIs may increase the risk of rejection of the transplanted liver, a formidable potential consequence of using ICIs after liver transplantation. In a retrospective study by Wang et al., 16 HCC patients received ICIs treatment before LT, the study showed that the median time of acute rejection was 7 days post-LT, a shorter Time-Interval (TI) between last dose of ICI and LT increased the risk of postoperative rejection (Median TI: 21 days vs. 60 days, p = 0.01) however there were no immune-related graft losses [29]. Therefore, it is essential to establish a safe washout period which is defined as the period between the last ICI dose and LT. In 2023, Kuo et al. concluded in a retrospective study that 42 days is a safe washout period for bridging ICI therapy with either Atezolizumab, Nivolumab or Pembrolizumab before LT [30].

Data on the efficacy and safety of ICIs as bridging treatment to LT is accumulating. In 2021, Tabrizian et al. reported a single center case series of 9 HCC patients whose tumors met either the Milan or UCSF criteria, all patients received Nivolumab ICI therapy before being successfully bridged to liver transplant. Interestingly, 80% of patients underwent LT within 4 weeks of last dose of Nivolumb and there were no instances of severe allograft rejections, tumor recurrences, or deaths at a median follow-up of 16 months post LT [31]. Moreover in 2021, Chen et al. reported a case series on 5 HCC patients whose tumors were beyond the Milan Criteria, they all received bridging/downstaging treatment with Nivolumab, the mean washout period was 63.8 days, none of the patients developed biopsy-proven acute rejection (BPAR) however 2 of them had HCC recurrences on follow-up [32]. In 2022, Schnickel et al. reported a case series of 5 HCC patients who received Nivolumab ICI therapy before LT. The two patients who underwent LT within 3 months from the last dose of Nivolumab developed BPAR and severe hepatic necrosis, however BPARs were observed in none of the patients who underwent LT >3 months from the last dose of Nivolumab [33]. Multiple case reports are published on ICIs monotherapy as bridging treatment to LT [34–37].

Combination therapies of ICIs with TKIs were also investigated as bridging treatment strategy to LT. In 2021, Qiao et al. reported a cohort on 7 HCC patients who received Lenvatinib in combination with either Pembrolizumab or Camrelizumab ICI therapy prior to LT. The biopsy-proven acute rejection rate was 14.3% [38]. In 2022, Abdelrahim et al. published a case report on an HCC patient whose tumor was beyond the Milan criteria. The patient received Atezolizumab plus Bevacizumab combination therapy prior to successfully going to LT with no HCC recurrence after 12 months of follow-up [39]. In 2023, Schmiderer et al. also published another case report on an HCC patient who was successfully transplanted after receiving the Atezolizumab plus Bevacizumab combination therapy [40].

Summary and recommendations

There is growing evidence in the literature regarding the role of ICIs as bridging treatment to LT in HCC. ICI treatment can be utilized either as a monotherapy or as a combination therapy with Bevacizumab or TKIs prior to LT (Table 2). Acute rejection post LT is a concern in the context of ICIs treatment. Thus, a safe ICI washout period before LT and cautious post-LT immunosuppression strategies are required to reduce post-LT rejections and to optimize clinical outcomes [29,30]. Furthermore, prospective clinical trials are needed to establish definitive conclusions about the utility of ICIs as a bridging modality prior to LT in HCC patients.

Table 2. Overview of the studies on ICIs used as a bridging therapy to liver transplant.

Author/year	Milan Criteria	Treatment	No. of transplanted patients	Washout Period (Days)	Post-LT IS-protocol included steroids	BPAR	HCC recurrence	Post-LT follow-up (months)
Schmiderer et al (2023) [40]	Out: 100%	Atezolizumab +Bevacizumab	1	42	Yes	No	No	12
Abdelrahim et al. (2022) [39]	Out: 100%	Atezolizumab +Bevacizumab	1	60	No	No	No	12
Dave et al (2022) [41]	In: 87%	Nivolumab	5/8	11–354	NA	Yes: 40%	No	NA

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	Out: 13%			(Median: 105)				
Schnickel et al (2022) [33]	NA	Nivolumab	5	10-330	NA: 40% No: 20% Yes: 40%	Yes: 40%	No	2–16
Tabrizian et al. (2021) [31]	up to UCSF criteria: 100%	Nivolumab	9	1-253 (80% of patients <= 30 days)	Yes	No	No	8-23 (median: 16)
Chen et al (2021) [32]	Out: 100%	Nivolumab	5	Mean: 63.80±18.26	No	No	Yes: 40%	NA
Sogbe et al. (2021) [34]	Out: 100%	Durvalumab	1	90	Yes	No	No	24
Chen et al. (2021) [35]	In: 100%	Toripalimab	1	93	Yes	Yes: 100%, fatal hepatic necrosis	NA	NA
Qiao et al. (2021) [38]	NA	(Pembrolizumab or Camrelizumab) + Lenvatinib	7	42	Yes	Yes: 14.3%	NA	NA
Nordness et al. (2020) [37]	In: 100%	Nivolumab	1	8	Yes	Yes: 100%, fatal hepatic necrosis	NA	NA
Schwacha-Eipper et al. (2020) [36]	In: 100%	Nivolumab	1	105	NA	No	No	12

Abbreviations: BPAR: Biopsy Proven Acute Rejection, IS: Immunosuppression, NA: not available, OS: overall survival, Post-LT: post liver transplantation, UCSF: University of California San Francisco.

2.2.2. Response Assessment

The classic radiologic disease assessment criteria such as response evaluation criteria in solid tumors (RECIST) may not adequately evaluate response in HCC. These criteria rely on tumor size, which can remain unchanged in locally or systemically treated HCC due to multiple factors, such as treatment-induced necrosis, the presence of ascites and reactive lymph nodes [42]. This results in an underestimation of HCC tumor response. Therefore in 2001, the European Association for the Study of the Liver (EASL) criteria were put forward to evaluate viable lesions on abdominal magnetic resonance imaging (MRI) [43]. Using this method, the arterially enhancing tumor burden is calculated in 2 dimensions. In 2008, the RECIST criteria was modified to (mRECIST) that includes changes in tumor arterial enhancement [44]. The mRECIST can be applied to contrast-enhanced, multiphasic computed tomography (CT) or MRI. One of its advantages over the EASL criteria is that it provides recommendations for new lesions and non-target lesion selection, such as portal vein thrombosis, lymph node at the porta hepatis, ascites or pleural effusion [45]. The objective response rate was evaluated as a surrogate endpoint for overall survival (OS) in a systematic review of 14056 patients with HCC treated with ICIs. The results of the meta-analysis showed that objective response predicted by mRECIST (OR-mRECIST) was an independent predictor of OS, and that OR-mRECIST correlated better with OS than RECIST [46].

With the addition of immunotherapy to the armamentarium of treatment of HCC, pseudo-progression on imaging became a concern, but was dismissed as it is rarely seen in HCC [47]. Therefore the immune response evaluation criteria in solid tumors (iRECIST), designed to assess pseudo-progression, may not be required in HCC treated with immunotherapy [47]. On the contrary, tumor shrinkage is seen with immune checkpoint inhibitors, and thus the RECIST criteria can be applied in this setting, as was the case in several clinical trials on immunotherapy in HCC.

Summary and recommendations

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Both RECIST and mRECIST criteria (Table 3) are recommended by the guidelines for disease evaluation after systemic treatment [48].

Table 3. The RECIST and mRECIST criteria for response assessment in HCC.

RECIST criteria	mRECIST criteria
CR: Disappearance of all target lesions	CR: Disappearance of any intra-tumoral arterial enhancement in all target lesions.
PR: ≥30% reduction of the sum of the diameters of target lesions.	PR: ≥30% reduction of the sum of the diameters of viable (enhancing) target lesions.
SD: features classified as neither PR nor PD.	SD: features classified as neither PR nor PD.
PD: ≥20% increase of the sum of the diameter of target legions.	PD: ≥20% increase of the sum of the diameter of viable (enhancing) target legions.

Abbreviations: CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

2.2.3. Biomarkers

Companion diagnostic tests, such as PD-L1 expression, can help identify patients who would benefit the most from ICIs and are widely used in various malignancies. However this approach does not apply to HCC where ICIs are administered to all patients regardless of the PD-L1 expression knowing that some cases will be resistant to ICIs. For example, in the Atezolizumab/Bevacizumab arm of the landmark IMbrave 150 study, 19% of the patients were refractory to treatment [49]. This highlights the need for predictive markers that can help select patients who are most likely to benefit from ICIs. This is particularly relevant in the context of graft rejection risk when utilizing ICIs as bridging treatment to liver transplant.

PD-L1 expression

In the Checkmate-040 trial, which compared Nivolumab monotherapy to combination therapy with Ipilimumab in advanced HCC patients who previously received Sorafenib, an objective response was observed regardless of PD-L1 status [50]. Similarly, in the HIMALAYA trial, treatment with Durvalumab and Tremelimumab showed OS benefit compared to Durvalumab or Sorafenib as single agents in advanced HCC regardless of PD-L1 expression [51]. In the Checkmate-459 trial that compared Nivolumab vs. Sorafenib for advanced HCC, patients with PD-L1≥1% achieved a higher response rate with Nivolumab compared to those with PD-L1 expression <1% (28% vs. 12%). However, this did not translate into a survival benefit, and both subgroups had a similar median OS (16.1 months for PD-L1≥1% and 16.7 months for PD-L1<1%) [52]. The KEYNOTE-224 trial aimed to assess the safety and efficacy of Pembrolizumab in patients with advanced HCC previously treated with Sorafenib. The study found that PD-L1 expression assessed by combined positive score (CPS) ≥1 was associated with response to Pembrolizumab in a subgroup of patients. The tumor proportion score (TPS) ≥1% was not significantly associated to response, which could imply that the combination of immune and tumor cell scoring might improve the predictive value of PD-L1 testing [53]. A systematic review and meta-analysis by Yang et al. evaluated the predictive value of PD-L1 in patients with HCC treated with ICIs, and found that patients with a positive PD-L1 expression had better ORR (pooled odds ratio, 1.86, 95% CI, 1.35–2.55). However, there was no difference in the disease control rate compared to those who were PD-L1 negative [54].

Summary and recommendations

In the absence of substantial evidence from clinical trials, PD-L1 expression is not recommended for routine use as a predictor of response to ICI in advanced HCC. Taking into consideration that there is a significant inter-assay heterogeneity in detecting PD-L1 in HCC as was evident in the Blueprint-HCC study [55], further efforts are needed to standardize the measurement of PD-L1 expression in HCC to improve its consistency as a potential biomarker of response to ICIs.

Microsatellite instability

Microsatellite instability (MSI) is the result of a deficiency in DNA mismatch repair (MMR) mechanisms. MSI-High tumors accumulate somatic mutations that lead to neoantigen formation,

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which in turn activates an immune inflammatory cascade, thus making these tumors sensitive to ICIs. The efficacy of ICIs in different types of MSI-High malignancies has been demonstrated in the KEYNOTE-016, -164, -012, -028, and -158 trials [56–60]. However, the prevalence of MSI high in HCC is low, and found to be between zero and 2.9% in a review of the literature [61]. Despite the limited evidence to supporting the use of immunotherapy in MSI high HCC [62,63], the low occurrence of microsatellite instability in this cancer diminishes the value of MMR status as a predictive biomarker.

Tumor mutational burden

Tumor mutational burden (TMB) refers to the number of DNA mutations per megabase (Mb) within the coding genome of tumors [64]. Elevated TMB (>10 mutations/Mb) leads to an increased neoantigen expression, which can trigger an immune response, making it a potential predictor marker for the effectiveness of immunotherapy. A high TMB was significantly associated with better objective response rates (ORR) with Pembrolizumab in the Keynote-158 trial, which led to the FDA approval of this drug in advanced solid tumors with high TMB. However the improved ORR did not translate into a survival benefit and none of the included patients with biliary cancers had a high TMB [65,66]. Indeed, compared to other types of malignancies, HCC has a relatively low TMB (a median of 5 mutations/Mb) [67]. Additionally, the TMB in HCC can fluctuate depending on the type of pathology sample between paraffin-embedded or fresh frozen section, and on the geographic origin of the patient [64]. In summary, the methodological variations and low TMB in HCC render the use of this predictive tool limited.

Tumor infiltrating lymphocytes

ICIs exert their anti-tumor effect by activating the immune cells in the tumor microenvironment. This includes tumor-infiltrating lymphocytes (TILs) such as cytotoxic CD8+ cells and natural killer (NK) cells. A post-hoc analysis of the CheckMate 040 trial showed that increased CD3+ and CD8+ cell infiltration led to an improvement in OS in patients treated with Nivolumab, but it did not reach statistical significance [68].

Inflammatory markers

Systemic inflammation, often triggered by a local pro-inflammatory response in the tumor microenvironment, can lead to poor survival outcomes in patients with cancer. Two inflammatory markers were studied in HCC treated with ICIs: the neutrophil to lymphocyte ratio (NLR) and the platelet to lymphocytes ratio (PLR). A study found that patients with HCC treated with Nivolumab who achieved a partial or complete response had significantly lower post-treatment NLR and PLR ($P < .001$ for both) compared to those with stable disease or progression. Both ratios were significantly associated with survival in multivariable analysis [69]. In a retrospective analysis of 362 patients treated with ICIs for HCC, patients with NLR ≥ 5 had significantly worse OS (7.7 vs. 17.6 months, $p < 0.0001$), PFS (2.1 vs. 3.8 months, $p = 0.025$), and ORR (12% vs. 22%, $p = 0.034$). Patients with PLR ≥ 300 had similar results, with significantly shorter OS (6.4 vs. 16.5 months, $p < 0.0001$) and PFS (1.8 vs. 3.7 months, $p = 0.0006$) [64].

Summary and recommendations

Although these results are encouraging, the utility of inflammatory markers as predictive biomarkers is limited by the fluctuation during the course of illness and treatment. Further validation in larger cohorts is necessary.

Gut microbiota

The commensal microbes of the digestive system is being highlighted as a key player in cancer pathogenesis and response to treatment in several types of malignancies, particularly in colorectal cancer [70]. A systematic review and meta-analysis assessed the relation of gut microbiota composition and response to ICIs in 775 patients with different types of solid organ malignancies. This effort resulted in identifying Faecalibacterium prausnitzii, Streptococcus parasanguinis, Bacteroides caccae, and Prevotella copri to be more commonly present in responders to ICIs and are associated with a better prognosis. In contrast, Blautia obeum and Bacteroides ovatus were associated with a poorer prognosis [71]. Zheng et al. examined fecal samples from eight patients with HCC receiving Camrelizumab and compared the microbiota of responders vs. non-responders at different time points during treatment. Responders were characterized by having higher taxa richness and

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more gene counts than non-responders. Furthermore, the study found a dynamic variation in the composition of microbiota over the course of treatment, such as increase in Proteobacteria in non-responders between weeks 3 and 12 of PD-1 blockade. Therefore, examining the variation in gut microbiota can be a promising predictor of response to ICIs in HCC, as early as within 3 to 6 weeks of treatment administration [72]. In a larger cohort of 65 patients with advanced hepatobiliary cancers receiving anti-PD-1 treatment, Lachnospiraceae bacterium-GAM79 and Alistipes sp Marseille-P5997 were significantly more enriched in responders, and their abundance was associated with better progression-free and overall survivals. In contrast, Veillonellaceae was significantly more abundant in non-responders and was associated with worse PFS and OS. Another observation from this study was that a more diverse gut microbiota is associated with a lower risk of immune-related adverse events [73]. Another study found that Lachnoclostridium enrichment conferred a survival benefit (median OS of 22.8 months vs. 5.6 months, p=0.032), while patients with enriched fecal Prevotella 9 had significantly worse OS compared to others (median OS of 8.6 months vs. 17.2 months, p=0.039). The best median OS was in patients with both Lachnoclostridium enrichment and Prevotella 9 depletion in the feces (22.8 months) [74].

Summary and recommendations

Overall, the gut microbiota composition and its dynamic variation throughout immunotherapy administration is a promising biomarker to predict response to treatment, but still needs validation in larger studies. Additionally, its clinical application may be challenging given the high susceptibility of the microbiome to external factors, such as antibiotic administration and dietary changes, which may complicate the interpretation and utility of this marker.

Genomic characteristics

HCC has a heterogeneous genomic profile, and several studies have been conducted to identify potential genomic biomarkers that can inform treatment decisions. HCCs can be categorized into two molecular types: proliferative and non-proliferative. Proliferative HCCs have a subclass characterized by high number of infiltrating CD4+ and CD8+ T cells, which respond well to ICIs [75]. On the other hand, non-proliferative HCCs are dominated by Wnt signaling, and tend to be less aggressive, have lower levels of AFP and are more differentiated [76]. The role of this molecular classification is not yet clear in clinical practice, and further evidence is needed to support its utility as a predictive biomarker.

A promising predictive biomarker is the Wnt-β-catenin pathway, since its activation is one of the main cancer-driver gene mutations in HCC, causing the upregulation of oncogenes and favoring immune resistance. Activation of this pathway occurs in 30 to 50% of the cases of HCC, triggered by mutations in CTNNB1 that encodes β-catenin, and inactivation of AXIN1 or APC, which are inhibitors of Wnt pathway [11]. To test this in a clinical context, Harding et al. used next-generation sequencing to determine predictive and prognostic biomarkers for HCC. Among the patients treated with ICIs, activating alterations in the Wnt-β-catenin pathway were associated with worse outcomes in terms of disease control rate (0% vs. 53%), median PFS (2.0 vs. 7.4 months), and median OS (9.1 vs. 15.2 months) [77]. Morita et al. evaluated molecular and immunological features of HCC as predictive markers to ICIs response. The study concluded that absence of staining of the molecules in Wnt/β-catenin signaling, high infiltration of CD8+ cells, and high CPS of PD-L1 were significant contributors to response to PD-1 blockade [78].

Summary and recommendations

Additional research on larger group of patients is necessary to delineate the predictive value of HCC genomic characteristics and their correlation to treatments including ICIs.

3. Future Directions

Multiple prospective studies are ongoing to assess the safety and efficacy of bridging systemic therapies before LT.

The PLENTY202001 trial is investigating the safety and efficacy of Pembrolizumab in combination with Lenvatinib as neoadjuvant therapy in 192 anticipated participants with HCC exceeding the Milan criteria before LT. The study aims to determine whether this combination as a

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neoadjuvant treatment for advanced HCC could decrease postoper-ative recurrence and to analyze potential immune biomarkers of therapeutic response [79]. ESR-20-21010 is a single-arm, Phase II, multicenter clinical trial aiming to assess the safety and efficacy of Durvalumab and Tremelimumab for the treatment of 30 anticipated patients with HCC within the UCSF criteria who are listed for liver transplant and have cirrhosis or portal hypertension. The primary endpoint of this study is post-transplant rejection within 30 days of transplant [80]. The combinations of ICIs plus either Lenvatinib or Bevacizumab are currently under investigation in two other clinical trials too [81,82]. Finally, Sun Yat-Sen Memorial Hospital in China is studying single agent ICIs as bridging/downstaging treatment to LT in HCC patients who are beyond the Milan criteria [83].

Summary and recommendations

Ongoing clinical trials on bridging systemic therapies to LT are examining ICIs alone or in combination with either TKIs or Bevacizumab (Table 4). An important endpoint is rejection rates post LT. Publication of the results of these ongoing clinical trials will have a favorable impact on the management of this particular subset of HCC patients.

Table 4. Overview of the ongoing studies on bridging systemic therapy to liver transplant.

ClinicalTrials.gov ID	Bridging therapy	Trial phase	Primary Endpoints
NCT04425226 [79]	Pembrolizumab + Lenvatinib	NA	RFS
NCT05027425 [80]	Durvalumab + Tremelimumab	2	Cellular rejection rate
NCT05185505 [81]	Atezolizumab + Bevacizumab	4	Acute rejection rate post liver transplant
NCT04443322 [82]	Durvalumab + Lenvatinib	NA	RFS, PFS
NCT05475613 [83]	Anti-PD-1 inhibitors (Tislelizumab, Pembrolizumab, Nivolumab)	2	2-year event-free survival rate

Abbreviations: ID: Identifier, NA: Not available, PFS: Progression Free Survival, PD-1: Programmed cell death protein 1, RFS: Recurrence Free Survival.

4. Conclusion

Enhancing HCC patients' bridging to LT via systemic treatments is an evolving field, especially when locoregional liver-directed therapy is contraindicated. Investigations into single-agent TKIs, ICIs, or their combinations as bridging modalities are being conducted. ICIs combination with TKIs or VEGF inhibitors likely represent the most promising approach. However, concerns remain regarding post-LT rejection after bridging with ICIs, necessitating further prospective clinical trials to determine optimal pre-LT ICIs washout periods to ensure safety and efficacy. Concurrently, efforts to identify biomarkers, particularly to ICIs, are underway to better predict HCC tumor response to treatment and to mitigate risks associated with post-LT rejection.

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