Adjuvant and neoadjuvant therapies in hepatocellular carcinoma

Josep M. Llovet[1,2,3†], Roser Pinyol[1], Mark Yarchoan[4,5], Amit G. Singal[6], Thomas U. Marron[2], Myron Schwartz[7], Eli Pikarsky[8], Masatoshi Kudo[9] & Richard S. Finn[10]

[1]Liver Cancer Translational Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Universitat de Barcelona, Spain.
[2]Mount Sinai Liver Cancer Program, Divisions of Liver Diseases, Department of Medicine, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
[3]Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.
[4]Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, USA.
[5]Bloomberg-Kimmel Institute for Cancer Immunotherapy, Johns Hopkins University School of Medicine, Baltimore, MD, USA.
[6]Department of Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA.
[7]Department of Liver Surgery, Recanati/Miller Transplantation Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
[8]The Lautenberg Center for Immunology and Cancer Research, Institute for Medical Research Israel-Canada (IMRIC), Hebrew University-Hadassah Medical School, Jerusalem, Israel.
[9]Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine, Osaka, Japan.
[10]Department of Medicine, Division of Hematology/Oncology, Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

[†]e-mail: josep.llovet@mountsinai.org

Abstract

Liver cancer, specifically hepatocellular carcinoma (HCC), is the sixth most common cancer and the third leading cause of cancer mortality worldwide. The development of effective systemic therapies, particularly those involving immune-checkpoint inhibitors (ICIs), has substantially improved the outcomes of patients with advanced-stage HCC. The ~30% of patients who are diagnosed with early stage disease currently receive potentially curative therapies, such as resection, liver transplantation or local ablation, which result in median overall survival durations beyond 60 months. Nonetheless, up to 70% of these patients will have disease recurrence within 5 years of resection or local ablation. To date, the results of randomized clinical trials testing adjuvant therapy in patients with HCC have been negative. This major unmet need has been addressed with the IMbrave 050 trial demonstrating a recurrence-free survival benefit in patients with a high risk of relapse after resection or local ablation who received adjuvant atezolizumab plus bevacizumab. In parallel, studies testing neoadjuvant ICIs alone or in combination in patients with early stage disease have also reported efficacy. In this Review, we provide a comprehensive overview of the current approaches to manage patients with early stage HCC. We also describe the tumour immune microenvironment, and the mechanisms of action of ICIs and cancer vaccines in this setting. Finally, we summarize the available evidence from phase II/III trials of neoadjuvant and adjuvant approaches, and discuss emerging clinical trials, identification of biomarkers and clinical trial design considerations for future studies.

[H1] Introduction

Liver cancer is the sixth most common cancer and the third leading cause of cancer-related mortality worldwide, after lung and colorectal cancer[1]. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Over the past two decades, the development of effective systemic therapies has substantially improved the outcomes of patients with advanced-stage HCC[2–6]. In particular, regimens including immune-checkpoint inhibitors (ICIs) are currently adopted as first-line therapies in clinical guidelines[7]. However, this remarkable progress in systemic therapy has not been paralleled by improvements in the treatment of early stage HCC, which typically involves administration of therapies with curative intent, such as resection, liver transplantation or local ablation[1,2]. Despite an impressive median overall survival (OS) beyond 60 months after resection or local ablation, up to 30-50% of patients have disease recurrence at 3 years, often resulting from intrahepatic metastases or de novo tumours arising in the underlying liver pathology[1,2].

In contrast with randomized clinical trials (RCTs) testing adjuvant treatments in patients with several other solid tumours such as breast[8], lung[9] or colorectal cancer[10], those testing such approaches in HCC after potentially curative resection or local ablation have not yielded positive results over the preceding decades[11]. In 2023, however, the treatment landscape for patients with early stage HCC evolved following positive results from IMbrave 050, in which adjuvant therapy with the ICI atezolizumab plus the anti-angiogenic agent bevacizumab significantly improved recurrence-free survival (RFS) over active surveillance in patients with a high risk of disease recurrence[12]. This result is a major advance in the management of HCC[7].

In parallel, early phase clinical studies testing ICIs, either as monotherapy or in combination, in the neoadjuvant setting have reported favourable outcomes[13–15]. While phase III studies are awaited, neoadjuvant treatment holds the promise of further improving outcomes for patients with early stage HCC, as has been the case for those with in melanoma[9,16], and colorectal[17,18] or lung cancer[19–21].

In this Review, we provide a comprehensive overview of the current management of patients with early stage HCC, describe the components of the HCC immune microenvironment along with the mechanisms of action of ICIs and cancer vaccines in this context, and present the results of phase II/III trials in the neoadjuvant and adjuvant [referred to as (neo)adjuvant from here onwards] settings. A proposed flowchart outlining treatment sequences —supported by evidence-based data from these trials — aims to facilitate navigation through these interventions. Finally, we conduct a critical analysis of emerging

clinical trials, biomarkers and trial designs for future investigations of (neo)adjuvant treatments in HCC, providing context from other tumour types.

[H1] Management of early stage HCC

(Neo)adjuvant therapies for HCC have been mostly considered in the context of resection and local ablation for early stages of HCC. Thus, we first analyse the current standard selection criteria and outcomes from these treatments globally.

[H2] Resection

Surgical resection is recognized worldwide as the preferred treatment for patients with early-stage HCC, whereas liver transplantation is indicated in those with early-stage disease who are not deemed suitable for resection[7,22]. However, the eligibility criteria for resection — which are based on clinical factors such as tumour extent, liver function, functional status and the availability of other therapies (e.g. ablation) vary considerably by location (TABLE 1)[7,22–27]. In general, European and USA guidelines recommend more restrictive criteria for resection than Asian guidelines[7,22–27]. Consequently, the reported outcomes differ widely depending on the selection criteria applied, with 5-yr survival rates ranging between 50% (China) to 70% (Europe)[28].

Overall, 40% of all HCCs occur in China, where around 85% of these cancers are related to hepatitis B virus (HBV)[29]. In Asia, the availability of screening and surveillance programmes is limited (less than 25% of all patients with HCC are diagnosed by surveillance[30]) and, consequently, the disease is detected at an advance stage in more than 70% of the cases[31]. Transplantation is also not widely available in many Asian countries. This is related to the limited acceptance of deceased-donor transplantation in Japan and Korea, and to the limited development of transplantation relative to the number of patients with HCC in China, with an estimated of 318,000 new HCC cases occurring annually in China, and 4,762 transplants in 2017, 44% of which were for HCC[32]. Liver function tends to be better preserved in HBV-related HCCs than in those related to other aetiologies, with up to ~20% of patients having non-cirrhotic disease[33]. Accordingly, Asian surgeons tend to adopt an aggressive approach to resection in terms of both tumour burden (including multinodular tumours involving 2–3 liver segments or segmented Vp1–Vp2 macrovascular invasion) and the degree of liver dysfunction[24]. These strategies, captured in Asian guidelines[34], have been associated with perioperative decompensation (i.e. deterioration in liver function) rates of ~20% and mortality rates of up to ~5%[22]. With these data in mind, some Asian countries, such as Japan

and Korea, have adopted more-restrictive practices, similar to those adopted in Europe and North America (TABLE 1).

In North America and Europe, the predominant underlying HCC aetiologies are hepatitis C virus (HCV) infection, alcohol-related liver disease and metabolic-related fatty liver disease (MAFLD), which typically result in cirrhosis with synthetic dysfunction and portal hypertension[33,35–37]. Screening and surveillance programmes have variable levels of uptake across these countries. The estimates of early detection in Japan is 65%, whereas it range from 25-50% in Europe and is of 17.5% in China[30,37,38]. Moreover, the increased availability of transplantation in Western countries relative to Asia leads surgeons to adopt a more conservative approach to resection, selecting only optimal candidates[27]. Therefore, in Western countries surgical resection is only indicated for patients with cirrhosis who have a single tumour (regardless of size), and provided they have well-preserved liver function (Child–Pugh score of A (the most favourable) with total serum bilirubin levels <1 mg/dl) and an absence of clinically relevant portal hypertension (no ascites, platelet count >100,000 platelets/mm^3 or hepatic venous pressure gradient <10 mm Hg). In the past few years, patients who exceed one or more of these criteria (for example, those with 2–3 lesions) have also been considered for liver resection in highly selected patients. In a Japanese study, 5-year OS was better for patients with single vs multiple tumors (68% vs 58%), and for patients without vs with portal hypertension (71% vs 56%)[39]. Nevertheless, a consensus on extended criteria for liver resection in patients with cirrhosis has not been reached, in part because resection in patients who do not meet these endorsed criteria resulted in significantly lower OS relative to those who meet the criteria (5-year OS: 35% vs 65%, respectively)[26]. Application of the criteria from Western guidelines results in a perioperative decompensation rate of ~5% and a perioperative mortality rate of ~0.5–1%[22]. Notably, novel minimally invasive surgical techniques, such as laparoscopy or robotic-assisted hepatectomy, are now being used more frequently worldwide potentially leading to expansion of resection criteria by enabling patients with mild portal hypertension to safely undergo minor liver resection[7,40].

[H2] Local ablation

Local ablation offers a potentially curative treatment for small tumors (≤3 cm in largest diameter, maximum 3 nodules), providing excellent outcomes with minimally invasive procedures[41]. Local ablation is usually performed using needles introduced percutaneously under ultrasonography or CT guidance[41]. Local ablation can involve either chemical, thermal or electrical methods. Percutaneous ethanol injection was the original ablation technique, although this has been largely replaced by radiofrequency ablation (RFA) or microwave

ablation (MWA), which provide both superior OS and objective response rates (ORRs) with fewer sessions[42]. ORRs and RFS after local ablation are inversely proportional to tumour size, with optimal outcomes observed for patients with small-diameter HCCs in whom 3-year RFS and OS are approximately 45% and 75%, respectively[43,44], and the 5-year OS is around 60%[45–48]. Guidelines from the American Association for the Study of Liver Diseases (AASLD)[7,22] and European Association for the Study of the Liver (EASL)[7,22] both recommend RFA or MWA for the management of small and early stage HCCs, although MWA is increasingly used at Western centres.

Eligibility for local ablation is determined by tumour size, location and likelihood of a complete response, which is monitored using CT and/or MRI[49,50]. A meta-analysis of data from 23 studies concluded that patients with single tumours <2 cm in largest diameter (that is, with a Barcelona Clinic Liver Cancer Stage (BCLC) of 0) have similar OS outcomes with local ablation or resection[51], whereas the results of a retrospective study[38] and an RCT[43,52] showed that patients with larger-diameter but resectable tumours (BCLC A) have superior OS with resection relative to local ablation (median OS of 105 months vs 71 months, respectively)[47]. For patients diagnosed with solitary early stage HCC unsuitable for surgery, an ablation-first strategy is recommended (TABLE 1)[7,22,45]. For tumours exceeding 3 cm in maximum diameter, some studies suggest that combining local ablation with transarterial chemoembolization (TACE) might improve OS[53,54].

Overall, current guidelines recommend thermal ablation (RFA or MWA) as the treatment of choice for patients with small, early stage HCC who are ineligible for or decline surgery. Alternative local therapies (TACE or stereotactic body radiotherapy) can be used for patients with BCLC A HCC who are not candidates for resection or with tumours in locations that preclude a percutaneous approach, including those with tumours >3 cm in largest diameter[7].

[H2] Unmet clinical needs

The likelihood of disease recurrence after resection or local ablation remains substantial, ranging from 50% to 70% at 5 years (TABLE 1); the risk of recurrence is highest during the first 12 months following curative treatment[55]. These early recurrences usually present as extrahepatic metastases or as intrahepatic metastases far distal from the resection margin[56,57], that are presumed to be related to occult micrometastases already present at the time of resection. Tumour characteristics (including size, number, grade of differentiation, vascular invasion, differentiation and serum α-fetoprotein (AFP) levels) are all risk factors for early HCC recurrence[58]. By contrast, late HCC recurrence (>12 months)

likely reflects new primary tumours (also known as de novo HCCs) and is related to the underlying liver disease. Age, sex, aetiology of the underlying liver disease and cirrhosis are all risk factors for late recurrence[59]. Overall, the development of effective peri-operative (neo)adjuvant therapies is urgently needed to mitigate this high risk of HCC recurrence.

[H1] Tumour immune microenvironment in HCC

[H2] Immune cell types, tumour neoantigens and mechanisms of immune response and escape

Cancer immunosurveillance is a dynamic process involving the elimination of malignant cells, with the interplay between innate and adaptive immune responses being intricately shaped by the tumour microenvironment (TME). In the liver, the immune microenvironment primarily comprises immunosuppressive cells and signals that create a tolerogenic niche[1,5,60]. Key cells involved in immune evasion in HCC include tissue-resident macrophages (Kupffer cells), regulatory T (T_{reg}) cells, monocyte-derived macrophages and immature granulocytic cells often collectively referred to as myeloid-derived suppressor cells (MDSCs)[5] (FIG. 1). These cell types are largely immunosuppressive, and thus hinder the development of effective innate and adaptive antitumour immunity, alongside dysfunctional dendritic cells (DCs) and regulatory B cells.

Macrophages –mostly tumour-associated macrophages (TAM)- contribute to hepatocarcinogenesis and immune evasion through various mechanisms, including secretion of immunosuppressive cytokines, expression of the immune-checkpoint ligand PD-L1, recruitment of T_{reg} cells and T_H17 cells, promotion of angiogenesis and downregulation of pro-inflammatory cytokines[61]. High numbers of TAMs are associated with a poor prognosis in patients with HCC[62]. Neutrophils can also drive tumour progression, likely by promoting immunosuppression, tumour cell survival, extracellular matrix remodelling and angiogenesis[63].

The liver also contains an abundance of MDSCs that produce factors suppressing T cell activation[64]. Furthermore, patients with HCC have increased numbers of both T_{reg} cells and MDSCs in blood relative to individuals without cancer. Circulating regulatory DCs contribute to systemic immunosuppression through the production of IL-10[65]. B cells have a dual role in HCC immunobiology, promoting tumour development but also enhancing the response to immunotherapy by producing antitumour antibodies and activating T cells[66].

In general, the liver TME is immunosuppressive; this may be counteracted by the presence of immune cells with the ability to effectively eliminate cancer cells[63,67]. Key

effectors of anticancer immunity include $CD8^+$ T cells as well as liver-resident and liver-infiltrating natural killer (NK) cells[5]. These cells can trigger an adaptive immune response against a wide variety of different tumour antigens, including tumour-associated antigens (TAAs) and tumour-specific antigens (also referred to as neoantigens) resulting from genomic alterations, abnormal RNA splicing or post-translational modifications, and integrated viral open reading frames (FIG. 1). In certain tumour types, the number of neoantigens in a tumour (or tumour mutational burden (TMB)) is correlated with responsiveness to ICIs[68,69]. However, in the IMbrave 150 study[4], which demonstrated an OS benefit with atezolizumab plus bevacizumab versus the tyrosine-kinase inhibitor (TKI) sorafenib as first-line treatment for patients with unresectable HCC no significant association between TMB and either response rates or survival was detected[70]. TMB clustered in a narrow range, with a low median of 4.4 mutations per megabase (mut/Mb); whether the small subset of patients with HCCs with a high TMB (>10 mut/Mb) derived a greater benefit from ICIs remains to be determined. HCCs typically have a low TMB. Furthermore, a high TMB does not correlate with increased immune infiltration[71,72]. This discrepancy might be explained by the presence of an impaired antigen-presenting machinery[71,73]. Indeed, in HCC the presence of large-scale copy-number alterations (CNAs) results in the loss of genes involved in antigen presentation, suggesting that CNAs contribute shaping of the TME[71].

Cancer cell-intrinsic signalling cascades can also affect the HCC immune microenvironment. In a mouse model of HCC, activation of WNT–β-catenin signalling promotes immune escape by impairing recruitment of DCs and interfering with recognition by NK cells[74]. TGFβ signalling contributes to an immunosuppressive cancer field effect[75]. *MYC* overexpression leads to PD-L1 overexpression, whereas *TP53* mutations promote the recruitment of immunosuppressive cells[76]. Mutations in epigenetic writers increase TMB, yet are associated with downregulated IFNγ signalling[77,78].

[H2] HCC immune types

Tumours can be categorized as inflamed or non-inflamed on the basis of immune microenvironment-related features (FIG. 2). Inflamed tumours constitute approximately 30% of HCCs, and exhibit extensive immune cell infiltration and immune activity, detected as increased expression of immune checkpoints (such as PD-1 or its ligand PD-L1), activation of interferon signalling and a low burden of large chromosomal alterations[71,72,79]. On the basis of previously described mRNA-based gene signatures[72,79], inflamed HCCs can be further subdivided into immune active, immune exhausted and immune-like tumours. Immune active HCCs present high levels of cytolytic activity and high activation of interferon

signalling, whereas in immune-like tumours interferon signalling coexist with *CTNNB1* mutations. Conversely, immune-exhausted tumours are characterized by exhausted T cell infiltrates and activation of TGFβ signalling[72,79]. Overall, patients with inflamed HCCs tend to have a favourable prognosis and are the most likely to have better outcomes when receiving ICIs owing to the presence of responsive immune cells[73,79]. Several gene signatures capturing the inflamed components of the TME have been associated with a favourable response to ICIs[70,73,79–82], but none have been clinically validated thus far.

Conversely, non-inflamed tumours have limited immune cell infiltration and low immune activity within the TME[72,79,83]. These tumours are characterized by T cell exclusion and can be subdivided into immune-intermediate tumours, with *TP53* mutations and a high degree of chromosomal instability, or immune-excluded tumours, with *CTNNB1* mutations that result in activation of canonical WNT signalling[79]. Patients with HCCs classified as non-inflamed tend to have a low likelihood of benefiting from immunotherapies[73,84].

[H2] Immune-checkpoint inhibitors

Immune cells have ligand–receptor immune checkpoints that can either inhibit or stimulate their effector function, resulting in modulation of the length and magnitude of immune responses, and minimize tissue damage. Immune checkpoints that promote T cell activation and expansion include CD28, GITR and OX40[5], and inhibitory immune checkpoints include PD-1, CTLA4, LAG3, TIGIT and TIM3[5] (FIG. 1).

Currently, the main immunotherapeutic approach for patients with HCC — regardless of tumour stage — involves restoring antitumour immunity with ICIs, which are monoclonal antibodies that block inhibitory checkpoints or their ligands[5]. In this sense, adjuvant administration of atezolizumab plus bevacizumab in patients at high risk of recurrence after effective resection or local ablation in patients with HCC has demonstrated improvement in RFS[12].

[H1] Adjuvant therapies in HCC

[H2] Past and ongoing phase III trials in HCC

The prevention or delay of HCC recurrence after hepatic resection or local ablation with adjuvant therapies has been an unmet medical need for decades[1] (TABLE 2). Two systematic reviews identified several RCTs assessing the effect of adjuvant therapies on RFS after local, potentially curative therapies[11,85]. The most recent of these studies analysed data from seven trials deemed to be of high quality and with results reported between 2002 and 2020[11]. In summary, most of the studies failed to identify any clinical benefit, and among

the positive studies validation in Western trials is awaited. For instance adjuvant administration of retinoids[86], vitamin K2[87], IFNα[88,89] and ^{131}I-lipiodol embolization[90] failed to demonstrate efficacy. Similarly, the phase III STORM trial compared sorafenib versus placebo after resection or local ablation in 1,114 patients with HCC and did not show improvement in RFS (33.3 months versus 33.7 months)[91]. Similarly, the mTOR inhibitor sirolimus did not significantly impacted in RFS in the SiLVER trial, were 525 patients with HCC undergoing liver transplantation were studied[92]. Conversely, RCTs conducted in China have reported clinical benefits with (neo)adjuvant therapies. Two studies reported improvements in RFS in patients with early/ intermediate stage disease receiving adjuvant hepatic intra-arterial chemotherapy (HAIC) with folinic acid, 5-fluorouracil and oxaliplatin (FOLFOX) versus placebo[93] or resection followed by adjuvant TACE versus no intervention[94]. These results would need further confirmation to be sufficient to support the use of HAIC or TACE in the (neo)adjuvant setting in patients with HCC.

In a phase III trial of adjuvant adoptive cell therapy with cytokine-induced killer (CIK) cells generated by incubation of patients' peripheral blood mononuclear cells with IL-2 and an anti-CD3 antibody, median RFS was improved with CIK cell therapy (44.0 months versus 30.0 months with placebo; HR 0.63, 95% CI 0.43–0.94; *P* = 0.010)[95]. However, owing to issues with trial design (insufficient power, unbalanced prognostic characteristics among treatment arms) and the lack of external confirmation, this approach is not recommended by any clinical guidelines.

In the past few years, the prevention of de novo HCC recurrences has relied on treating the underlying liver disease. In this regard, effective antiviral therapy has substantially reduced the incidence of disease recurrence in patients with viral-related HCC[96]. Despite discouraging preliminary results[97], a retrospective analysis demonstrated that direct-acting antiviral therapy is safe and improves OS in patients with HCV-related cirrhosis and a history of HCC[98]. Similarly, the use of antiviral agents against HBV after resection seems to decrease RFS compared with no antiviral therapy, although we note that this was a two-stage longitudinal study[99].

[H2] IMbrave 050

Results from the global, open-label, phase III IMbrave 050 trial[12], have shown a significant RFS improvement in patients with HCC at high risk of recurrence after resection or local ablation who received adjuvant atezolizumab plus bevacizumab compared with those who underwent active surveillance in patients (BOX 1). This regimen was tested in the adjuvant setting as a logical consequence of its proven effectiveness in advanced-stage HCC[4].

Despite extensive characterization of the risk factors for HCC recurrence following resection[27], which include having a single tumour of >10 cm in largest diameter, multinodular nodules, high serum AFP levels, poor differentiation and/or the presence of microvascular invasion, the IMbrave 050 trial used a broader range of criteria to define risk (see BOX 1 for details), and might therefore have set a precedent for the design of future trials in this setting.

The primary end point was RFS assessed by an independent review facility (IRF) in the intention-to-treat population (ITT). A total of 668 patients were randomly assigned (1:1) to receive atezolizumab plus bevacizumab (intervention group) or were managed with active surveillance. At a median follow-up duration of 17.4 months, median RFS had not been reached in either group, although statistical analyses favoured intervention over active surveillance (HR 0.72, 95% CI 0.56–0.93; *P* = 0.012). IRF-assessed time to recurrence was longer in the intervention group (HR 0.67, 95% CI 0.52–0.88; *P* = 0.003). The median duration of treatment was 11.1 months for atezolizumab and 11.0 months for bevacizumab. The incidence of grade 3–4 adverse events (AEs) was 41% and 13% with intervention and active surveillance, respectively, and 8.7% of patients discontinued both atezolizumab and bevacizumab owing to AEs. The most common immune-related AEs (irAEs) were hepatitis (32% versus 15%), rash (20% versus 2%) and hypothyroidism (20% versus <1%), mostly of grade 1–2[12]. Most hepatitis-related events were abnormalities in the serum levels of aspartate aminotransferase and alanine aminotransferase. Additionally, 8.4% versus 1% of patients had irAEs requiring systemic corticosteroids.

In summary, adjuvant atezolizumab plus bevacizumab significantly improved RFS compared with active surveillance, albeit with an increased incidence of AEs (some of which were manageable). Whether this RFS benefit translates into an improvement in OS (a key secondary end point of the trial) remains to be determined. Longer-term follow-up data are awaited.

[H2] Prediction of response to ICIs

As discussed previously, IMbrave 050 included patients with an established high risk of HCC recurrence after resection and local ablation based on clinical and pathological features; however, biomarkers of response to ICIs (that is, predictive biomarkers) have not yet been identified or reported. In cancer, the prediction of response to ICIs is complex, and only TMB, mismatch repair deficiency and expression of PD-L1 (determined by immunohistological staining) are currently accepted by regulatory agencies as companion diagnostics for some solid tumours, including melanoma and non-small-cell lung cancer (NSCLC)[100]. In a meta-analysis with results published in 2021, researchers validated associations between

biomarkers of response to ICIs and survival outcomes in a pan-cancer panel[101]. The results confirmed 11 predictive factors associated with a response to ICI across cancers (including TMB, T cell infiltration and expression of *CD8A*, *CXCL9* and *CD274* mRNA) but the authors acknowledged that each tumour has its own specificities[101].

In HCC, the value of PD-L1 expression ≥1% or TMB as predictive biomarkers of response to ICIs has not been demonstrated[70]. Conversely, associations between transcriptome-based biomarkers, and ORR or OS have been reported. Inflamed HCCs are deemed to have a favourable response to ICIs[79]. These tumours are enriched in three gene signatures (referred to as inflammatory[79]; interferon and antigen presentation (IFNAP)[73]; and T cell inflammation signatures[70]), which predict response to ICIs. Similarly, analyses of samples from patients enrolled in IMbrave 150 led to the identification of molecular predictors of improved outcomes ($CD8^+$ T cell density, effector T (T_{eff}) cell signature and high expression of PD-L1) and also inferior outcomes (high T_{reg}:T_{eff} cell ratio) with atezolizumab plus bevacizumab[70]. Results from other studies suggest that an MAFLD-related aetiology might be related to a lack of response owing to unique immunological traits hampering antitumoural surveillance[11,102]. Whether these factors predict RFS in the adjuvant setting needs to be explored. If the described predictive biomarkers are validated, the question is how to translate these findings into clinical practice. Currently, approvals of companion biomarkers for systemic therapies are made on the basis of results from phase III trials in cohorts stratified using the candidate biomarker, or on prespecified analyses of biomarker-based subgroups from properly powered phase III trials. Nonetheless, the accelerated approval of treatments in the advanced-stage setting, based on data from single-arm trials aligning therapies with specific molecular alterations, has resulted in a situation in which current strategies for biomarker approval need to be revisited. Given the need to tailor ICI-based to patients most likely to derive benefit, conducting adequately powered post-hoc analyses in specific subgroups from phase III trials has been proposed as a pathway leading to biomarker approval[103].

[H1] Neoadjuvant ICIs in solid tumours

[H2] Mechanisms of action of neoadjuvant versus adjuvant approaches

The preponderance of preclinical and clinical evidence suggests that response and resistance to ICIs in early-stage cancers are dependent upon similar principles as advanced stage disease[104]. Adjuvant ICIs stimulates anti-tumour immunity against micrometastases after the primary tumour is removed, whereas neoadjuvant immunotherapies use the primary tumour as a source of antigens to stimulate such responses; in both situations those

micrometastases can eventually lead to disease recurrence. Antitumour immune responses with immunotherapy depend upon interactions between T cells, antigen-presenting cells, and tumour cells. Such interactions are more likely to occur when a large burden of primary tumour (containing the antigens targeted by the immune system) are still present, providing a potential mechanistic rationale for why neoadjuvant immunotherapies may be preferable to adjuvant immunotherapies (FIG. 3). In this regard, studies in other tumour types have demonstrated that T cell expansion is greater when ICIs are administered prior to complete surgical removal of the tumour as opposed to after surgery[105]. In addition, micrometastases, which can be present during adjuvant therapy, are believed to be less immunogenic than macroscopically detectable lesions. Consequently, when the primary tumour is present (neoadjuvant setting) ICIs can promote de novo induction of T cell-mediated immunity, expansion of pre-existing antitumour T cells, and development of a more diverse tumour-specific T cell repertoire more efficiently than after tumour removal (adjuvant setting) (FIGS 3A and 3B)[106]. Data from a landmark study using an orthotopic model of breast cancer showed that neoadjuvant ICIs might outperform adjuvant ICIs[107]. Delivering ICIs precisely surgery enabled T cell expansion, yielded the best antitumour activity while also decreasing toxicity[107]. These favourable outcomes were associated with increased numbers of tumour-specific $CD8^+$ T cells.

In a mouse model of triple-negative breast cancer, neoadjuvant induction and activation of DCs in primary tumours enhanced systemic antitumour immunity and improved survival[108]. In a similar model, depletion of T_{reg} cells potentiated the effect of ICIs when applied to primary tumours[109]. Also, in mouse models of colon and prostate cancer, combined administration of anti-CTLA4 and anti-PD-1 antibodies in the low tumour burden state (following resection of the primary tumour) provided improved control of established tumours but compromised antitumour immunity[110]. This impaired response was attributed to IFNγ-mediated depletion of tumour-reactive T cells owing to activation-induced cell death. Finally, neoadjuvant but not adjuvant administration of ICIs preserves T cell clones reactive to less-common immunogenic clones in mouse models of head and neck squamous cell carcinoma[111]. This study highlights the concept of immunodominance, whereby T cells targeting a dominant clone are primarily expanded at the expense of T cells reactive to subdominant clones. Although the aforementioned mechanisms support neoadjuvant administration of ICIs, additional preclinical and clinical studies are required to optimize the use of drug combinations in this setting.

[H2] Pathological response to ICIs in the neoadjuvant setting

Several studies have shown that T cell infiltration in solid tumours is a predictive biomarker of response to ICIs[70,83,101,112]. Moreover, analyses of samples derived from patients with melanoma who received neoadjuvant ICIs[113,114] have revealed the presence of the three primary patterns of T cell infiltration identified in solid tumours. According to these solid tumours can be classified as T cell-rich (or hot), with high levels of T cell infiltration within the tumour core; T cell-excluded (or excluded), with T cell infiltration limited to stromal regions; and T cell-low (or cold), with a generally low presence of T cells (FIG. 2). Although the T cell-rich infiltration pattern is the most favourable in terms of response to ICIs, this pattern is not a definitive predictor of response. In this regard, patterns of immune cell infiltration have been linked with distinct levels of pathological response. In the context of neoadjuvant therapy for patients with melanoma, pathological response is defined as the fraction of residual viable tumour cells in the treated tumour area as determined by a pathologist[106,114–116], which encompasses both viable tumour cells and signs of tumour regression, such as necrotic cells, pigmented macrophages, fibrosis and fibro-inflammatory stroma. In these tumours, the percentage of viable tumour cells is used to define the following response categories: pathological complete response (pCR), near-complete pathological response, pathological partial response and pathological non response, which occur when 0%, >0% to ≤10%, >10% to ≤50% and >50% of the tumour material, respectively, remains viable[115]. Furthermore, analyses of data from clinical trials of neoadjuvant ICIs in patients with melanoma[113] have revealed that patients with a higher TMB have improved pathological responses relative to those with lower TMB. The highest pathological response rates were reported in patients with tumours enriched with IFNγ-related signatures along with a high TMB, suggesting that these two mechanisms are required for a favourable response.

The histopathological features of response to neoadjuvant ICIs in patients with NSCLC have also been described[112]. These features include increased infiltration of lymphocytes and macrophages in tumours, presence of tertiary lymphoid structures, proliferative fibrosis and neovascularization. As per today, biomarkers to stratify patients therapeutically that are based on pre-treatment tissue have yet to be clinically validated.

In certain tumour types, such as melanoma and breast cancer, pathological response has been proven to be superior to radiological assessment[116,117] and is currently an accepted surrogate for RFS. For other cancer types, such as pancreatic ductal adenocarcinoma (PDAC)[118], the results from clinical trials are contradictory.

In a phase II clinical trial with results published in 2022, patients with early stage HCC received neoadjuvant treatment with the anti-PD-1 antibody cemiplimab[13]. Significant

tumour necrosis, the prespecified primary end point, was 20%. This end point was defined as >70% of necrosis within the pathological specimen, a cutoff extrapolated from another study including patients who had received TACE and subsequently underwent liver transplantation[119]. In parallel, another study evaluating perioperative treatment with the anti-PD-1 antibody nivolumab and the anti-CTLA4 antibody ipilimumab similarly used 70% necrosis as the cutoff to define a pathological response (indicative of <30% viable tumour cells), whereas a third trial of a longer course of neoadjuvant cabozantinib and nivolumab defined exploratory major pathological response as >90% necrosis (<10% viable tumour cells)[14]. All of these end points were defined arbitrarily owing to the paucity of data from studies testing neoadjuvant approaches in HCC, underscoring the need for more comprehensive studies to establish appropriate surrogate end points.

Additionally, in-depth histological analysis of blood and tissue samples could help to identify and define biomarkers of response that correlate with survival. In the trial of neoadjuvant cemiplimab[83], the analysis of samples taken at the time of resection -after treatment- revealed that responsive tumours had high levels of T cell infiltration, although some nonresponsive HCCs also had T cell enrichment (FIG. 2). Deeper analyses revealed a robust correlation between pathological responses to neoadjuvant cemiplimab and the presence of intratumoral triads comprising regulatory DCs, PD-1^{hi} progenitor CD8^{+} T cells and CD4^{+} T cells expressing features of T follicular helper cells (such as CXCL13 and IL-21)[83]. Of note, these niches were more frequent in tumours from responders even before undergoing treatment. Despite these results, more data from large cohorts of patients are required to substantiate a robust surrogate role for this cellular infiltration pattern. Currently, guidelines for HCC trial design have not adopted pathological response as a surrogate of RFS. In fact, guidelines on trial design for patients with HCC[120] recommend the use of modified Response Evaluation Criteria In Solid Tumors (mRECIST) for assessing responses to therapies in patients with early and intermediate-stage HCC, and both RECIST and mRECIST to evaluate responses to systemic therapies in the advanced-stage setting. Nonetheless, some of the preoperative trials with results published to date have highlighted a discordance between pathological response, significant tumour necrosis (quantified using high-resolution MRI) and standard RECIST[13] and thus, the rules for assessing responses to neoadjuvant therapies in patients with HCC remain to be established. Considerations for (neo)adjuvant ICIC trial design, derived from these trials, are discussed in Box 2.

Pathological response to neoadjuvant BRAF–MEK inhibitors seems to have less predictive value in melanoma than to checkpoint blockers. Complete responders had a significantly higher 1-year and 2-year RFS than those without a pCR (88% versus 63% and

79% versus 13%, respectively, although no significant difference in RFS was observed between patients with a pathological partial response and those with a pathological non-response[106]. Thus, further studies are required to establish the value of pathological response-based end points in predicting benefit from neoadjuvant approaches.

[H2] Neoadjuvant trials with ICIs in several cancer types

The interest in testing neoadjuvant ICIs has been growing rapidly in various solid tumour types. Since results from one of the first clinical trials of such a therapeutic approach in patients with NSCLC were reported in 2018[19], the efficacy and safety of neoadjuvant ICIs has been firmly established in multiple tumour types, with FDA approvals for two indications (NSCLC[20,121] and triple-negative breast cancer[8]). The hypothesis that neoadjuvant–adjuvant ICIs can translate into superior clinical outcomes than adjuvant ICIs was directly tested in the Southwest Oncology Group S1801 clinical trial[9]. In this study, patients with stage III–IV melanoma were randomly assigned to receive the anti-PD-1 antibody pembrolizumab before and after resection (neoadjuvant–adjuvant strategy; *n* = 154), or after surgery only (adjuvant (standard of care) strategy; *n* = 159). Both groups received the same number of doses of pembrolizumab for a total treatment duration of 1 year: patients in the neoadjuvant–adjuvant group received 3 doses (approximately 9 weeks) before surgery and 15 doses after surgery whereas those in the control group received all 18 doses after surgery. Event-free survival was significantly improved among patients who received pembrolizumab both before and after surgery (72% versus 49% with adjuvant-only pembrolizumab at 2 years; HR 0.58, 95% CI 0.39–0.87; *P* = 0.004, median follow up 14.7 months)[9]. These findings are from a study in a tumour type that tends to be responsive to ICIs and thus, their relevance to less responsive tumour types, such as HCC, remains to be demonstrated. Nonetheless, these data provide evidence that (neo)adjuvant administration of ICIs results in superior antitumour immune responses than adjuvant only administration.

[H2] Phase I/II neoadjuvant trials in HCC

Some researchers have argued that administration of ICIs in the adjuvant setting is preferable owing to the inverse correlation between tumour burden (theoretically minimal in this setting) and efficacy[31,122,123]. However, neoadjuvant therapy, even if not continued in the adjuvant setting, can be considered as part of a perioperative approach (comprising both neoadjuvant and adjuvant phases) given that most anti-PD-(L)1 antibodies have a half-life of >3 weeks and remain in circulation well into postoperative recuperation. Additionally, neoadjuvant ICIs can be used to downstage patients' tumours and thus improve surgical

outcomes for some patients[14,124]. In addition, as discussed, neoadjuvant ICIs can induce a more robust immune response than adjuvant ICIs given the immunosuppressive systemic effects of invasive surgery[125–127]. One of the first published reports of neoadjuvant immunotherapy in HCC was the aforementioned phase I study in which patients with locally advanced disease received a combination of nivolumab plus the TKI cabozantinib for 3 months with the aim of downstaging tumours to enable curative-intent resection[14]. Out of 15 patients enrolled, 12 successfully underwent resection and 5 had a major pathological response defined as ≥90% tumour necrosis.

A similar trial, but with a shorter duration of the neoadjuvant intervention, assessed the effects of neoadjuvant cemiplimab on treatment outcomes in patients deemed to be candidates for resection[13]. These patients received just two doses of cemiplimab and underwent resection as early as 22 days after starting therapy, after which they received up to 8 additional cycles of treatment. Out of 20 patients who underwent resection, 20% had significant tumour necrosis. In another study, patients received nivolumab, with or without a single dose of ipilimumab, for 6 weeks before surgery and up to 2 years post-operatively[15]. Significant tumour necrosis occurred in 33% of 9 patients treated with nivolumab and 27% of 11 patients treated with nivolumab–ipilimumab.

These three initial studies highlight the utility of the neoadjuvant 'window-of-opportunity' setting and provide insight on the mechanisms of action of novel immunotherapies, given the potential to use resected tumour tissue for in-depth immune analysis rather than relying on scant on-treatment biopsy samples[128]. Despite the small sample sizes of these trials, the study of nivolumab–ipilimumab revealed that ipilimumab is more effective in patients with cold tumours, supporting the theory that CTLA4 blockade might be most beneficial in patients lacking pre-existing antitumour immunity[15].

Multiple trials are now exploring additional combinatorial preoperative approaches in patients with HCC (TABLE 2 and Supplementary TABLE 1). Moving forward, trials with larger cohorts are needed to validate the benefits of preoperative therapy in terms of RFS and OS, in comparison to the new benchmark set by IMbrave 050. Given the lack of successful trials in the perioperative space in HCC, no surrogate end point has been validated yet, as is the case for pCR in patients with NSCLC[20,121] and breast cancer[8]. Larger trials (some of which are currently underway) will help to establish and validate end points. The analysis of specimens obtained from patients receiving neoadjuvant ICIs in large trials will provide further insight into the differences in immunogenicity between HCCs of a viral aetiology versus those arising from metabolic-associated steatohepatitis, as the latter has been predicted to be associated with unique clinical responses[102]. Finally, larger trials will

be needed to explore the utility of continuing treatment with ICIs in the adjuvant setting, given that profound pathological responses can occur as little as 22 days prior to surgery[13] and that long-term PD-1 blockade predisposes patients to a higher risk of irAEs and increased financial burden without a proven effect on outcomes[129].

[H2] (Neo)adjuvant clinical trials with cancer vaccines

Cancer-prevention vaccines, such as those for human papillomavirus and HBV, have greatly reduced the incidence or certain virally-associated cancers, including HBV-related HCC[130]. By contrast, despite decades of intensive research efforts, vaccines designed to treat cancer have largely failed to improve outcomes for patients with cancer. At the time of writing this Review, only sipuleucel-T, a cancer vaccine for castrate-resistant metastatic prostate cancer, has conclusively provided a survival benefit in a large randomized clinical trial[131]. Many reasons might explain why prior cancer vaccines have failed. Most of these vaccines targeted TAAs, namely shared antigens expressed on cancer cells that are also expressed at lower levels on non-malignant cells. A novel generation of cancer vaccines targeting mutation-associated neoantigens has reinvigorated hope that this therapeutic class could become widely used to treat patients with cancer. Given that mutation-associated neoantigens are not expressed on any non-malignant cell, vaccines targeting them might avoid central or peripheral tolerance mechanisms, resulting in robust immune responses[131]. Nevertheless, given that mutation-associated neoantigens tend to be unique to each tumour, such vaccines need to be personalized for each patient with cancer. The development of novel vaccine platforms, including mRNA-based vaccines, along with the rapid declines in tumour-sequencing costs have made personalized vaccines targeting neoantigens possible[132–137]. Cancer mRNA-based vaccines tend to be administered alone or in combination with ICIs, and after resection, although they have also been used in neoadjuvant–adjuvant treatment approaches[136,138,139] (FIGS 3C and 3D).

In the randomized phase IIb KEYNOTE-942 trial, patients with resected, high-risk stage III–IV melanoma received pembrolizumab plus mRNA-4157, an mRNA-based personalized cancer vaccine consisting of a single synthetic mRNA encoding for up to 34 patient-specific tumour neoantigens[130]. This approach resulted in improved RFS compared to pembrolizumab alone (78.6% versus 62.2% at 18 months; HR 0.56, 95% CI 0.31–1.02), with no major increases in toxicity. The vaccine received Breakthrough Designation from the FDA in February 2023, representing a milestone for the era of cancer mRNA vaccines. Subsequently, the results of a phase I trial investigating the use of chemotherapy plus atezolizumab and a personalized mRNA vaccine were presented in May 2023. In this trial,

8 of 16 patients with resected PDAC remained free of cancer after a median follow-up of 18 months[136]. Consistent with the proposed mechanism of action of vaccine-induced antitumor immunity, patients enrolled in the trial with an immunologic response against the vaccine had a longer median relapse-free survival than patients without such a response[136]. Although further confirmatory studies are needed, these results provide initial clinical evidence that personalized therapeutic cancer vaccines can enhance responses to ICIs, with many other studies are planned. In HCC, the initial results of a phase I/II trial of the DNA-based therapeutic cancer vaccine GNOS-PV02 in combination with pembrolizumab demonstrated an ORR >30%, higher than the ORR of 14–17% observed in pivotal trials of anti-PD-1 antibodies in this context; larger confirmatory studies are needed to confirm benefit[140].

Overall, the feasibility of identifying tumour-specific neoantigens in resected specimens, the technological advances in the production of mRNA-based and DNA-based vaccines, and the results of the aforementioned trials[136,139] offer exciting prospects for further development of personalized neoantigen-based anticancer vaccines[132,141], which could improve the outcomes of patients with cancer types known to have high post-resection recurrence and mortality rates such as HCC.

[H1] Role of adjuvant therapy in HCC management

The management of patients with HCC has improved dramatically since the first BCLC classification was proposed in 1999[142]. In particular, the median OS of patients with early HCC has been substantially extended (beyond 60 months) as a result of the use of resection, liver transplantation and local ablation. In addition, locoregional therapies have extended the median OS of patients with intermediate-stage HCC to 25–30 months. For patients with advanced-stage HCC, the current availability of ~10 systemic regimens[1,2,5,36,41] has resulted in a shift from median OS durations of 3–4 months to 19–20 months after first-line treatment and 10–14 months after second-line treatment (FIG. 4A). Now the positive results of IMbrave 050[7] have led to a revised management algorithm for patients with early stage HCC by incorporating adjuvant therapies in this disease setting (FIG. 4). However, subsequent treatments for patients with disease progression during or after adjuvant atezolizumab plus bevacizumab are under debate and has not yet been tested in clinical trials.

[H2] Recurrence at intermediate stages

According to the current evidence, atezolizumab plus bevacizumab is indicated as adjuvant therapy for patients with a high risk of recurrence after resection or local ablation. In this

context, no other treatment has demonstrated improved RFS in a phase III study. Locoregional therapy would be recommended for patients with liver-localized recurrence after adjuvant therapy[7] and liver transplantation should be considered for those with recurrences that meet the Milan criteria (FIG. 4B). in a salvage liver transplantation study involving 110 patients, the 5-year OS in the ITT was 69%, with 55% of patients achieving cure after resection or successful salvage liver transplantation[143].

Patients with disease recurrence beyond the Milan criteria, with liver-only disease (intermediate stage) should be considered for locoregional therapies, including TACE or transarterial radioembolization (TARE). In patients with successful downstaging, which indirectly reflects more-favourable tumour biology, transplantation can be considered[144,145]. As is true for patients who initially present with BCLC B disease, those with a large intrahepatic tumour burden (such as bilobar multifocal disease) might be considered unsuitable for TACE and therefore as candidates for systemic therapies, given a lower likelihood of objective responses and a higher risk of liver injury with embolic therapies[146].

Although the concept of unsuitability for TACE has gained widespread recognition, currently no consensus exists regarding the threshold at which upfront systemic therapy should be used, particularly in patients with disease recurrence after adjuvant therapy. Finally, patients with disease recurrence in the advanced-stage setting should be considered for systemic therapies. The AASLD guidelines[7] recommend the anti-PD-L1 antibody durvalumab plus the anti-CTLA4 antibody tremelimumab, or the TKIs lenvatinib or sorafenib in patients with disease recurrence during or <6 months after atezolizumab plus bevacizumab. If recurrence occurs >6 months after stopping therapy, rechallenge with atezolizumab plus bevacizumab is advised.

[H2] Recurrence after (neo)adjuvant therapy: advanced stages or TACE unsuitable

The goal of adjuvant therapy in early stage disease is to increase the chance of cure after definitive therapy. To date, IMbrave 050 is the only phase III trial that supports such an approach in the setting of HCC[12]. For patients with disease recurrence and disease deemed unsuitable for TACE or with features indicating advanced-stage disease (such as extrahepatic spread or macrovascular invasion), systemic therapy should be considered.

At present, atezolizumab plus bevacizumab[4], durvalumab plus tremelimumab[147], lenvatinib[148] and sorafenib[3] are approved globally for the first-line treatment of advanced-stage HCC, although clearly no patients in the studies that led to these approvals received prior adjuvant systemic therapy[1,2,6]. One factor to consider when selecting a regimen for patients with disease recurrence after receiving these agents is the time between resection

or local ablation, and recurrence (FIG. 4B). For those with a long disease-free interval since completing (neo)adjuvant therapy (≥12 months), offering the same regimen they received for early stage disease, as is done in other malignancies (such as breast cancer) might be a reasonable approach. Conversely, patients with disease recurrence during or within 12 months of completing adjuvant treatment can have inherent resistance to such a regimen and a change of treatment is warranted. Of note, further studies are needed to assess whether this 12-month threshold or other time frames are the most suitable in determining the need for treatment change. Clinically, this is a similar scenario to that of patients with disease progression while receiving first-line therapy. Again, limited data are available to guide the 'best choice' in such a situation but many clinicians would probably favour other approved first-line therapies. Given that recurrence is occurring on an ICI-based regimen, the consideration of lenvatinib or sorafenib seems appropriate; however, the benefit of other ICI-based regimens, such as the FDA-approved combinations of durvalumab plus tremelimumab or ipilimumab plus nivolumab, after atezolizumab and bevacizumab is not known. Small-cohort studies have suggested that patients receiving ipilimumab plus nivolumab after prior therapy with ICIs have an ORR of ~16%[149]. Given that disease recurrence in this setting is incurable, local ablative therapies can be considered only in certain situations such as patients with oligometastatic disease and recurrence after a very long disease-free interval (years).

[H1] Conclusions

The past 5 years have seen remarkable changes in the treatment landscape of HCC. Most notably, the approval of numerous new systemic agents for advanced-stage HCC has left substantial knowledge gaps in choosing the optimal first-line regimen and the subsequent sequencing of these agents after disease progression[6]. Despite advances in the development of biomarkers predicting response to ICIs, no companion biomarker that enables identifying subgroups of patients with HCC who are most likely to benefit from these therapies has been approved. This situation has prompted some initiatives calling for the development of a specific biomarker approval pathway[103]. Currently, clinical decisions are based on clinical factors including, but not limited to, performance status, tumour burden, liver function and comorbidities[7]. As current ongoing phase III trials in early stage HCC mature, patients will have disease recurrence after receiving systemic therapy in this setting and the same questions will need to be addressed, initially by extrapolating data from studies involving patients with advanced-stage disease. Over time, prospective clinical data and from real-world experiences will be needed.

Perhaps the greatest opportunity from trials of systemic therapy in early stage HCC is the chance to perform detailed, relevant translational studies given that paired tissue samples (at the time of diagnostic biopsy and resection) are usually available. Clinical studies must mandate tissue and blood collection for these purposes. Currently, decisions to use these agents are guided by clinical and pathological considerations of recurrence risk but, ultimately, a biomarker-based approach is preferable[103]. Such biomarkers include not only tissue-based assays but also those based on circulating tumour cells, cell-free DNA and other liquid biopsy approaches, which have received increasing interest in the past decade[150]. Importantly, clinicians must bear in mind that some patients will be cured with resection or local ablation alone, and therefore the long-term safety of adjuvant regimens needs to be established to determine their true risk:benefit ratio. Given that HCC recurrence after resection has a bimodal distribution, the question of whether or not a predefined course of adjuvant therapy prevents late recurrences remains to be answered. Finally, whether or not the use of systemic therapy in the adjuvant setting improves OS remains to be established; such a question is difficult to address owing to the numerous lines of effective therapies available. Now the demonstration of a role for an ICI-based regimen in the adjuvant setting opens the door to studies evaluating these regimens in the neoadjuvant setting before locoregional curative approaches. The next wave of studies need to determine whether preoperative treatment results in similar or increased levels of clinical benefit through the direct comparison of adjuvant-only versus neoadjuvant–adjuvant approaches. The estimated duration of neoadjuvant approaches — aimed to achieve a balance between exposure to ICIs and prevention of tumour progression — is estimated to be around 6–8 weeks, but longer time-frames may be justified based on clinical activity of a regimen[13,15]. These studies will provide a framework to assess the efficacy of new regimens, not only on the basis of imaging responses but also of biological and pathological responses. In this novel scenario, several approaches can be considered, including ICIs, targeted therapies and cancer vaccines. Of note, the use of systemic therapies in the neoadjuvant setting has raised valid safety concerns, including the risk of inducing irAEs, that potentially delay or preclude potentially curative surgery. Thus, these aspects will need to be comprehensively monitored in trials together with their effect on event-free survival. Finally, an alternative pathway for biomarker-based approval of immunotherapies has been proposed; future trials testing ICIs in the (neo)adjuvant setting should follow these principles in order to enable a more-precise use of this important therapeutic class in early stage HCC[147].

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Competing interests

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Key points

1. Around 30% of patients with HCC undergo resection or local ablation as primary treatment.
2. The probability of recurrence at 3 years is of 30-50%, and is associated with size of the main tumour, microvascular invasion and poor differentiation degree
3. In the phase III IMbrave 050 trial, atezolizumab plus bevacizumab as adjuvant treatment for resection/local ablation in HCC patients at high risk of recurrence led to significantly better recurrence-free survival compared with active surveillance
4. Neoadjuvant exposure of immunotherapies allows a more efficient interaction between T cells, antigen-presenting cells, and tumour cells due to a larger tumour burden compared with the adjuvant approach
5. Immunotherapies delivered both as neoadjuvant and adjuvant to resection have shown significantly better results than adjuvant administration alone in melanoma and NSCLC
6. Cancer vaccines tested in phase II trials in combination with checkpoint inhibitors provided signals of efficacy in melanoma and pancreatic cancer. These approaches are currently explored in HCC.

FIG. 1 | Immune cells in the HCC tumour microenvironment. Key components of the tumour microenvironment of hepatocellular carcinomas (HCC), highlighting their protumoural and antitumoural roles. Cytokines triggering neutrophil recruitment include CCL5, IL-8, IL-17, CXCL1, CXCL2, CXCL3, CXCL5, CXCL8, CXCL12 and CXCL16. CAF, cancer-associated fibroblast; DC, dendritic cell; MDSC, myeloid-derived suppressor cell; NK, natural killer; T_{reg}, regulatory T cell.

FIG. 2 | Role of the HCC immune microenvironment in response to treatment. Spatial organization of the immune infiltrate in patients with hepatocellular carcinoma (HCC). These tumours can be classified as inflamed and non-inflamed on the basis of infiltration patterns and molecular traits. The correlation of these patterns with response to immune-checkpoint inhibitors (ICIs)[83] is shown schematically as well as with representative images of haematoxylin and eosin-stained resected lesions from patients who had previously received anti-PD-1 antibodies[83,128] *[unpublished images provided by Thomas Marron].* Patients with a response ('responders') can have either complete or partial pathological responses, as determined through histopathological examinations of the resected tumour bed. Similar to many tumour types, tumours from patients with HCC who have received ICIs can be classified as 'inflamed', with robust infiltration of lymphoid and myeloid cells; excluded, in which the lymphoid cell infiltrate is largely limited to the stroma; and 'cold', with a paucity of lymphoid infiltrate. Patients with tumours classified as hot can be responders, although a minority are non-responders. Postoperative samples from patients with excluded and cold tumours typically show little to no significant tumour necrosis.

FIG. 3 | Mechanism of action of immunotherapies and vaccines in the neoadjuvant and adjuvant setting in HCC. (A) Adjuvant approaches involve administration of immune-checkpoint inhibitors (ICIs) after surgery, leading to the activation of different subsets of T cells. **(B)** In neoadjuvant approaches, ICIs are administered before surgery, fostering the development of a broader range of T cell responses compared with adjuvant approaches **(C)** When used, mRNA-based cancer vaccines are administered after resection. To develop such vaccines, resected tumour tissues undergo targeted sequencing to identify specific tumour mutations. Peptides containing these mutations are then selected on the basis of their immunogenicity. Selected neoantigens are incorporated into plasmids as DNA fragments and subsequently transcribed into mRNA in vitro. Finally, these mRNAs are packed into nanoparticles. Neoadjuvant vaccine-based approaches can also be considered. **(D)** Mechanism of action of neoantigens-based vaccines. Nanoparticles containing mRNAs

encoding selected neoantigens are endocytosed by dendritic cells (DCs), where mRNAs are released and transcribed by ribosomes. Given that they are neoantigens, the resulting neoantigens are fragmented by the proteasome and presented on the cell surface through major histocompatibility complex (MHC) class I molecules. DC-mediated antigen presentation activates $CD8^+$ T cells, subsequently leading to cancer cell apoptosis. Alternatively, neoantigens produced within DCs can be secreted and internalized by other antigen-presenting cells, where they are degraded into fragments subsequently presented through MHC class II molecules, activating $CD4^+$ cells and inducing B cells to generate antibodies for cancer cell destruction.

FIG. 4 | Overview of updated management of HCC and proposed treatment approach after disease recurrence following adjuvant therapies. (A) The Barcelona Clinic Liver Cancer (BCLC) treatment algorithm incorporates new adjuvant agents for patients with early stage hepatocellular carcinoma (HCC) who are at high risk of recurrence after resection or local ablation. The management of patients with HCC follows a treatment strategy guided by the BCLC staging system, which classifies disease into five stages. Asymptomatic patients with a low tumour burden and good liver function (BCLC 0) should undergo local curative treatments, such as resection or local ablation. For those with BCLC A disease (patients with single tumors or up to three nodules each < 3 cm), transplantation or local curative treatments are considered on the basis of clinical factors, including presence of portal hypertension, number of nodules and liver function. In patients at high risk of recurrence atezolizumab plus bevacizumab is recommended as adjuvant therapy after resection/ablation. Asymptomatic patients with multinodular disease and adequate liver function (BCLC B) should receive chemoembolization, whereas those with portal thrombosis or extrahepatic spread (BCLC C) should be treated with systemic therapies. Regimens approved on the basis of results from phase III trials are shown in red. Drug combinations that have shown positive results in phase III trials but have not yet been approved are shown in yellow. **(B)** Proposed treatment approach after recurrence to adjuvant atezolizumab plus bevacizumab in patients with a high risk of recurrence after local curative treatment. [a]Based on guidance from the American Association for the Study of Liver Diseases (AASLD)[7]. ECOG PS, Eastern Cooperative Oncology Group performance status; LRT, locoregional therapy; LT, liver transplantation; M1, distant metastasis; N1, lymph node metastasis; q3mo, every three months; q6mo, every 6 months; SBRT, stereotactic body radiotherapy; TACE, transarterial chemoembolization; TARE, transarterial radioembolization.

1

Table 1 | Geographical differences in resection and ablation approaches for HCC

Region	Resection[1,7,48,151,22–28,47]			Ablation[1,7,22–25,28,47,48,152]		
	Tumour characteristics and liver function		5-year OS	Tumour characteristics and liver function		5-year OS
	Optimal candidates	Suboptimal candidates		Optimal	Alternative (ablation + TACE)	
Europe and North America	Single lesion of any size and preserved liver function	2-3 nodules <3cm or presence of portal hypertension	60–70% in patients with HCC ≤5 cm and no portal hypertension	Single lesion ≤2 cm (BCLC 0) or ≤3 nodules ≤3 cm (BCLC A); preserved liver function	NA	60–70% (with RFA, PEI or MWA)
Japan[a]	≤3 nodules ≤3 cm, single lesion ≤5 cm, 1–3 nodules >3 cm or Vp1/2, Vv1/2; Child–Pugh A/B	≥4 nodules of any size**,** portal hypertension, Vp3/4, Vv3/4 or single lesion >5 cm; Child–Pugh A/B	67% for patients with Child–Pugh A/B and portal hypertension, and 70% for patients meeting optimal criteria	≤3 nodules ≤3 cm; Child–Pugh A/B	Single lesion ≤5 cm or >4 nodules of any size**;** Child–Pugh A/B	62% for all patients, 71% for patients meeting optimal criteria
Korea	Single lesion of any size**;** Child–Pugh A	Single lesion, with vascular or bile duct invasion, or 2–3 nodules of any size; Child–Pugh A/B	69%	≤3 nodules ≤3 cm; Child–Pugh A/B	Single lesion ≤5 cm Child–Pugh A/B	65%
China	Single lesion or 2–3 nodules of any size**;** Child–Pugh A/B	≥ 4 nodules or portal vein invasion; Child–Pugh A/B	~50%	Single lesion ≤5 cm or 2–3 nodules ≤3 cm; Child–Pugh A/B	Single lesion >5 cm or 2–3 nodules >3 cm; Child–Pugh A/B	45%

[a]Based on a nationwide registry [47]. BCLC, Barcelona Clinic Liver Cancer classification; HCC, hepatocellular carcinoma; MWA, microwave ablation; NA, not applicable; OS, overall survival; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; Vp1/2, segmented, right anterior or posterior portal vein invasion; Vp3/4, right, left, or main portal vein invasion; Vv1/2, peripheral or major hepatic vein invasion; Vv3, hepatic vein invasion extending into inferior vena cava.

TABLE 2 | Phase III trials of adjuvant therapies for patients with HCC

Trial	Treatment groups and patients (*n*)	Median follow-up (months)	RFS	OS
IMbrave050[b] (Ref. [12])	Atezolizumab + bevacizumab (334) versus active surveillance (334)	17.4	NR (HR 0.72, 95% CI 0.56–0.93)[a]	NA
Mazzaferro et al.[88]	IFNα (76) versus no treatment (74)	45	24.3% versus 5.8% at 5 years (HR NA)	58.5% at 5 years (HR NA)
Yoshida et al.[87]	Vitamin K2 45 mg (182), 90 mg (185) and placebo (181)	NA	17.8 months in all groups (HR 1.15, 95% CI 0.84–1.57)	99.2%, 98.7% and 97.2%, respectively, at 1 year (HR NA)
Chen et al.[89]	IFNα-2b (133) versus no treatment (135)	63.8	42.8 months versus 48.6 months (HR NA)	75.4% versus 72.5% at 5 years (HR NA)
Lee et al.[95]	CIK cells (115) versus placebo (115)	40 and 36.5, respectively	44 months versus 30 (HR 0.63, 95% CI 0.43–0.94)[a]	NR (HR 0.21, 95% CI 0.06–0.75)[a]
NIK-333[86]	Peretinoin 600 mg (134), 300 mg (134) and placebo (129)	30	43.7%, 24.9% and 29.3% at 3 years (HR NA)	NA
STORM[91]	Sorafenib (556) versus placebo (558)	23 and 22, respectively	33.3 months versus 33.7 months (HR 0.94, 95% CI 0.78–1.13)	NR (HR 1.00, 95% CI 0.76–1.3)

SiLVER[92]	Sirolimus-based (261) versus sirolimus-free (264) immunosuppressive regimen	96	70.2% versus 64.5% at 5 years (HR 0.84, 95% CI 0.62–1.15)	74.6% versus 68.4% (HR 0.81, 95% CI 0.58–1.13)
Li et al.[93]	FOLFOX-HAIC (157) versus no treatment (158)	23.7 and 21.5, respectively	20.3 months versus 10.0 months (HR 0.59, 95% CI 0.43–0.81)[a]	80.4% versus 74.9% at 3 years (HR 0.64, 95% CI 0.36–1.14)

[a]Reported statistically significant differences. [b]Recommended by guidelines as first-line preferred treatment[7]. CIK, cytokine-induced killer; FOLFOX-HAIC, hepatic arterial infusion of oxaliplatin, fluorouracil and leucovorin; HCC, hepatocellular carcinoma; NA, not available; NR, not reached OS, overall survival; RFS, recurrence-free survival.

Supplementary Table 1 | Ongoing phase II/III adjuvant clinical trials and selected neoadjuvant phase II trials testing immune therapies alone or in combination in HCC

Setting	Agent(s) and targets	Primary end point(s)	Phase	Cohort size	NCT/identifier
Single-agent immunotherapies					
Adj	Pembrolizumab (PD-1)	RFS, OS	III	950	KEYNOTE-937 (NCT03867084)
Adj	Toripalimab (PD-1)	BICR RFS	II/III	402	JUPITER 04 (NCT03859128)
Adj	Nivolumab (PD-1)	RFS	III	545	CheckMate 9DX (NCT03383458)
Adj	Highly purified CTLs	RFS	III	210	NCT02709070
Adj	Durvalumab (PD-L1)	RFS	III	908	EMERALD-2 (NCT03847428)[1]
Neo	Nivolumab (PD-1)	PTRR	II	20	NCT05471674
Neo + adj	Cemiplimab (PD-1)	STN	II	73	NCT03916627
ICIs in combination with targeted therapies					
Adj	Tislelizumab (PD-1), lenvatinib (VEGFR1–3, PDGFR, FGFR1–4, RET)	RFS	III	300	PREVENT-2 (NCT05910970)
Adj	Camrelizumab (PD-1), apatinib (VEGFR2)	BICR RFS	III	687	NCT04639180
Adj	Sintilimab (PD-1), bevacizumab (VEGFA)	RFS	III	246	DaDaLi (NCT04682210)
Adj	Durvalumab (PD-L1), bevacizumab (VEGFA)	RFS	III	908	EMERALD-2 (NCT03847428)
Adj	Penpulimab (PD-1), anlotinib (VEGFR1–3, PDGFR, FGFR1–3, c-Kit)	RFS	III	480	NCT05862337
Neo	Pembrolizumab (PD-1), lenvatinib (VEGFR1–3, PDGFR, FGFR1–4, RET)	MPR	II	60	PRIMER-1 (NCT05185739)
Neo	Atezolizumab (PD-L1), bevacizumab (VEGFA)	pCR, AEs	II	30	NCT04721132
Neo + Adj	Camrelizumab (PD-1), apatinib (VEGFR2)	Tumour recurrence-free rate at 1 year	II	78	CAPT (NCT04930315)
Neo + Adj	Atezolizumab (PD-L1), bevacizumab (VEGFA)	RFS	II	202	AB-LATE02 (NCT04727307)

ICIs in combination with other ICIs					
Adj	Cadonilimab (PD-1/CTLA4 bispecific)	BICR RFS	III	405	NCT05489289
Neo	Nivolumab (PD-1), ipilimumab (CTLA4)	Percentage of patients with tumour shrinkage after treatment	II	40	NCT03510871
Triplet combinations of ICIs					
Neo + Adj	Camrelizumab (PD-1), apatinib (VEGFR2), TACE	RFS	III	130	NCT05613478
Other combination therapies involving ICIs					
Neo	Nivolumab (PD-1), BMS-813160 (CCR2/5), BMS-986253 (IL-8)	MPR, STN	II	50	NCT04123379

Trials in bold indicate trials involving neoadjuvancy. Adj, adjuvant; AE, adverse event; BICR, blinded independent central review; CTL, cytotoxic T lymphocyte; Neo, neoadjuvant; MPR, major pathological response; OS, overall survival; pCR, pathological complete response; PTRR, pathological tumour response rate; RFS, recurrence-free survival; STN, significant tumour necrosis; TACE, transarterial chemoembolization. [1] This trial tested two approaches: single agent durvalumab, and the combination of Durvalumab (PD-L1), with bevacizumab (VEGFA). Thus, it appears twice in the table.

BOX 1 | Summary of IMbrave 050 trial

Patients

- Eligible patients had undergone complete resection (R0, or negative gross and microscopic margins) or local ablation (microwave or radiofrequency ablation with a radiological complete response) for newly diagnosed HCC 4–12 weeks before randomization.
- Patients were deemed to have a high risk of HCC recurrence after resection or local ablation (described below).
- Patients had Child–Pugh Class A liver function, adequate haematological and organ function, and an Eastern Cooperative Oncology Group Performance Status score of 0 or 1.
- Most patients were Asian (82%). In both study groups, the major underlying aetiology for HCC was hepatitis B HCC (62%), followed by hepatitis C (11%) and non-viral aetiologies (12%).
- Most patients (84%) had disease defined as Barcelona Clinical Liver Classification (BCLC) A.
- The majority of patients (88%) had undergone resection. Of these patients, 90% had a single tumour with a median tumour size (longer diameter) of 5.5 cm, 61% had microvascular invasion and 7% had segmented, right anterior or posterior portal vein invasion
- Most patients who underwent ablation had a single tumour with a median size of 2.5 cm.

Definition of high risk of recurrence

- In patients who had undergone resection, the risk of recurrence was defined as either one of the following conditions: 1) ≤3 tumours, with the largest having a size of >5 cm regardless of vascular invasion, or poor tumour differentiation; 2) ≥4 tumours, with the largest having a size of ≤5 cm regardless of vascular invasion, or poor tumour differentiation; or 3) ≤3 tumours, with the largest having a size of ≤5 cm with vascular invasion, and/or poor tumour differentiation.
- In patients who had received local ablation, the risk of recurrence was defined as either one of following conditions: a) 1 tumour sized >2– ≤5 cm; or 2) ≤4 tumours, all sized ≤5 cm.

Crossover

- Crossover was allowed in the active surveillance group after the detection of recurrence.

BOX 2 | Considerations for trials testing immunotherapies for HCC in the neoadjuvant and adjuvant settings

End points

- In the adjuvant setting, the most commonly used primary end point is recurrence-free survival (RFS)[120].

- Overall survival (OS) would be an important secondary end point in this setting, but given the number of available treatments after recurrence (including potential unintended crossover to the study treatment) OS data can be difficult to interpret.
- In the true neoadjuvant setting, the primary end point is pathological complete response (pCR), assuming that a pCR will translate into improved OS.
- 'Window-of-opportunity' studies involving patients with resectable liver cancer are increasingly performed. These approaches are designed to evaluate biomarker changes and potentially immune priming. In these pharmacodynamic studies, baseline biopsy samples should be obtained followed by a short course of systemic therapy with additional tumour tissue obtained at the time of resection. Molecular studies can be performed on these samples[153] .

Target population

- In the adjuvant setting, in order to demonstrate a decreased in RFS patients with a higher risk should be selected. Risk assessment should be based on histopathological assessment of resected tumour tissue.
- In the neoadjuvant setting, the target population needs to be clearly defined and studies should involve patients with resectable disease at the time of enrolment. If patients are beyond resectable criteria at that time, the question becomes whether the intervention results in downstaging, as opposed to the intent of neoadjuvant studies, which is the assessment of pathological response.
- In window-of-opportunity studies, patients should have resectable HCC accessible to biopsy at baseline.

Response assessments

- In adjuvant studies, RFS is assessed with imaging to detect both intrahepatic and distant recurrences. The interval for surveillance imaging depends on the study size and statistical assumptions, but is typically 2–3 months.
- In the neoadjuvant setting, the primary end point is based on histopathological assessments. Interval imaging during treatment can be considered to rule out progression that would compromise resectability and to assess the secondary end point of RECIST 1.1 objective response rate.
- In window-of-opportunity studies, molecular end points are typically descriptive without formal statistical testing. If the study cohort is large, RFS can be included as a secondary end point. Alternatively, a brief clinical exposure could be included in large-cohort studies aimed at registrational purposes, serving as a co-primary end point alongside pathological response and/or specific biomarkers to validate and correlate laboratory findings with clinically meaningful end points.

Biomarkers

- Studies should have a prospective plan for tumour collection and patients' informed consent processes that allows for broad assessments as new technologies become available. Peripheral blood samples should also be collected for correlative studies, such as analyses of circulating tumour DNA, immune cell subtyping and/or inflammatory biomarkers[154].