Adjuvant treatment of hepatocellular carcinoma after resection

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Abstract

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer, and surgical resection offers an opportunity for cure in patients fortunate enough to have tumors amenable to resection. Unfortunately, recurrence rates are as high as 70% five years after resection, and recurrent disease proves to be a major obstacle to improving prognosis. Many adjuvant treatments have been utilized after resection in hopes of improving survival and have failed. This review outlines previous adjuvant strategies for patients with resected HCC and discusses potential steps forward to finding a successful adjuvant therapy.

Keywords: Hepatocellular carcinoma, adjuvant therapy, immunotherapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer and is the third leading cause of cancer death worldwide\(^1\). Surgical resection offers an opportunity to cure those patients fortunate enough to have tumors amenable to resection with well-preserved liver function\(^2\). However, patients who undergo “curative” resection have high recurrence rates where up to 70% of patients experience recurrence 5 years after resection\(^2\). The high rate of HCC recurrence is a major obstacle to improving patient prognosis, where early recurrence, within 2 years, is mainly related to metastasis and tumor dissemination of the primary HCC. In contrast, after 2 years, late recurrence is mostly the result of new tumors arising in the diseased
liver\cite{2}. Therefore, adjuvant therapies are in dire need to reduce intrahepatic recurrence rates in patients with resected or ablated HCC. However, many adjuvant strategies have failed to reduce recurrence-free or overall survival (OS).

The past several years have seen advances in the systemic treatment of HCC. For the past decade, sorafenib has not only been the mainstay of systemic treatment but the only drug approved for first-line systemic treatment since 2017. Recently, lenvatinib has been approved in the first-line setting, while regorafenib, cabozantinib, ramucirumab, and the immune checkpoint inhibitor nivolumab are approved as second-line therapy\cite{6}. Despite these recent advances, no therapy has consistently proven effective in the adjuvant setting. Instead, systemic therapy and intra-arterial therapy, as well as several immune-based strategies, have been utilized. Here we will review previous strategies that have been tried and largely failed in the adjuvant treatment of HCC. We will then discuss a potential pathway forward toward achieving success in the adjuvant setting.

### SYSTEMIC THERAPIES

The current standard of care for patients with advanced HCC for almost the past decade has been the multi-kinase inhibitor sorafenib which in a clinical trial showed a meager survival advantage of approximately three months over placebo\cite{3}. Adjuvant treatment is often derived from studies based on results found to be efficacious in the advanced setting. For example, capecitabine was established as an alternative to the standard bolus fluorouracil as first-line treatment for metastatic colorectal cancer and therefore was evaluated in the adjuvant setting and subsequently found to be an effective alternative to intravenous fluorouracil\cite{4}. However, the same results were not observed with sorafenib in the adjuvant setting. In phase III double-blind study, HCC patients with a complete radiologic response after surgical resection or local ablation were randomized to receive sorafenib or placebo with a primary endpoint of recurrence-free survival (RFS) and a secondary endpoint of OS. Unfortunately, there was no significant difference in median RFS or OS between the groups\cite{5}. Other randomized clinical trials of adjuvant systemic therapies have found broadly similar disappointing results. Based on pre-clinical studies, capecitabine was found to inhibit postoperative recurrence and lung metastasis in mice\cite{6}. In a randomized control trial, capecitabine was found to inhibit post-operative recurrence of HCC but failed to improve OS\cite{7}. Similarly, others have utilized the chemotherapy tegafur/uracil (UFT) in patients who underwent curative hepatic resection and found no difference in RFS or OS compared to surgery alone\cite{8} [Table 1].

In addition to traditional chemotherapy, Vitamin K\textsubscript{2} has been utilized in the adjuvant setting for patients with HCC. Pre-clinical data demonstrated that Vitamin K\textsubscript{2} suppressed the growth of three HCC cell lines through the regulation and suppression of the hepatoma-derived growth factor (HDGF) gene\cite{9}. HDGF is highly expressed in HCC cells stimulating their proliferation with oncogenic and angiogenic activity\cite{10,11}. However, adjuvant trials with Vitamin K\textsubscript{2} in HCC have largely disappointing results. Vitamin K\textsubscript{2} failed to improve the recurrence of HCC\cite{12,13} or significantly improved the recurrence without an improvement in OS\cite{14,15} [Table 1]. Vitamin K\textsubscript{2} has been tested in combination with an angiotensin-converting enzyme (ACE) inhibitor as the combination demonstrated more potent anti-angiogenic and anti-tumor effects than single-agent treatment in rats\cite{16}. However, the combination of vitamin K\textsubscript{2} and an ACE inhibitor reduced HCC recurrence without a survival advantage\cite{17}.

In addition to vitamin K\textsubscript{2}, vitamin A derivatives have been utilized in the chemoprevention of HCC as studies have suggested that loss of retinoid activity may be linked to carcinogenesis in HCC\cite{18}. In a prospective randomized control trial, the acyclic retinoid, polyprenoid acid, was found to prevent recurrent HCC after surgical resection or percutaneous injection of ethanol compared to placebo\cite{19}. However, this
study has been criticized as the patients in the study had low plasma retinol levels below the level of severe vitamin A deficiency. This has been shown to predispose patients to alterations in differentiation and increased cellular proliferation, and therefore treatment may have restored vitamin A levels, and the broader implication for cancer prevention in patients with normal vitamin A remains unclear[22].

Heparanase has been shown to be elevated in a wide variety of tumors and is linked to the development of pathological processes such as tumor invasion and metastasis[23]. Therefore, heparanase inhibitor PI-88 was studied in patients with HCC in the adjuvant setting [Table 1]. PI-88 demonstrated a benefit of RFS at the 160 mg dose but not 250 mg dose, with no benefit in OS[24,25]. Additionally, in the phase II trial, more than 95% of patients who received PI-88 experienced mild or moderately severe adverse events[25,26].

### LOCOREGIONAL THERAPY

Locoregional therapies such as transcatheter arterial chemoembolization (TACE) are a cornerstone of treatment for patients with HCC[44]. These modalities have also been utilized in the adjuvant setting with largely mixed results.

### Intra-arterial therapies

Hepatic arterial-based therapies such as TACE, which combines the arterial delivery of beads to restrict tumor blood flow with local administration of chemotherapy, are recommended for patients with the intermediate stage (BCLC stage B) HCC[45]. Chemoembolization with doxorubicin provided a significant survival benefit in stringently selected patients with unresectable HCC[27]. Additionally, chemoembolization

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Rationale for adjuvant</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruix et al. (2015) [1]</td>
<td>Sorafenib</td>
<td>Sorafenib approved for advanced HCC</td>
<td>No difference in median RFS or OS</td>
</tr>
<tr>
<td>Xia et al. (2010)</td>
<td>Adjuvant capecitabine</td>
<td>Capcitabine significantly inhibited postoperative recurrence and lung metastasis in mice[9]</td>
<td>Improved DFS and probability of recurrence with no difference in OS</td>
</tr>
<tr>
<td>Ishizuka et al. (2016)</td>
<td>Adjuvant UFT vs. surgery alone</td>
<td>UFT has shown efficacy in some HCC patients with advanced disease[26]</td>
<td>No difference in RFS and OS</td>
</tr>
<tr>
<td>Hotta et al. (2007)</td>
<td>Vitamin K2</td>
<td>Vitamin K2 has been shown to inhibit the expression of heptoma-derived growth factors by suppressing the promoter activity of the HDGF protein[15]</td>
<td>No difference in HCC recurrence</td>
</tr>
<tr>
<td>Yoshida et al. (2011)</td>
<td>Vitamin K2</td>
<td>No improvement of DFS</td>
<td></td>
</tr>
<tr>
<td>Ishizuka et al. (2012)</td>
<td>Vitamin K2</td>
<td>No improvement of DFS or OS</td>
<td></td>
</tr>
<tr>
<td>Kakizaki et al. (2007)</td>
<td>Vitamin K2</td>
<td>Disease recurrence significantly lower in vitamin K2 group (P = 0.045) but no difference in OS</td>
<td></td>
</tr>
<tr>
<td>Mizuta et al. (2006)</td>
<td>Vitamin K2</td>
<td>Disease recurrence lower in vitamin K2 group but no significant difference in OS</td>
<td></td>
</tr>
<tr>
<td>Yoshiji et al. (2009)</td>
<td>Vitamin K2 and ACE inhibitor</td>
<td>Combination of vitamin K2 and ACE inhibitor significantly suppressed recurrence of HCC but no significant difference in cumulative survival</td>
<td></td>
</tr>
<tr>
<td>Muto et al. (1996)</td>
<td>Acyclic retinoid, polyrenoid acid</td>
<td>Polyrenoid acid prevented recurrent hepatoma after surgical resection or percutaneous injection of ethanol</td>
<td></td>
</tr>
<tr>
<td>Liu et al. (2009)</td>
<td>Heparanase inhibitor PI-88</td>
<td>Heparanase is elevated in a wide variety of tumors and is linked to the development of pathological processes such as tumor invasion and metastasis[23]</td>
<td>160 mg dosage of PI-88 but not 250 mg dose, improved RFS at 1 year</td>
</tr>
</tbody>
</table>

HCC: Hepatocellular carcinoma; RFS: recurrence-free survival; OS: overall survival; DFS: disease-free survival; UFT: tegafur/uracil; HDGF: hepatoma-derived growth factor; ACE: angiotensin-converting enzyme.
with the emulsion of cisplatin in lipiodol displayed improved survival in Asian patients with unresectable HCC\cite{26}. However, results are mixed in the adjuvant setting. Izumi et al.\cite{29} studied the use of TACE with lipiodol containing doxorubicin and mitomycin, finding an improvement in disease-free survival (DFS) but no effect on OS. On the other hand, Li et al.\cite{36} found a significant improvement in intrahepatic recurrence and OS in patients who underwent TACE with lipiodol containing doxorubicin and mitomycin. Additional studies showed improved survival with TACE in patients with resected stage IIIA HCC\cite{31} and patients who required removal of a portal vein tumor thrombus\cite{32}. In addition, the use of TACE with portal vein chemotherapy (PVC) was evaluated. Li et al.\cite{33} noted a significant increase in DFS with TACE + PVC but no difference in DFS with TACE alone. More recent studies have also evaluated the role of TACE in the adjuvant setting for HCC with again largely mixed results\cite{28-36} [Table 2].

In addition to TACE, several studies have evaluated the use of other arterial-based regimens such as intra-arterial I\textsuperscript{131} lipiodol or hepatic artery infusion chemotherapy. Trans-arterial I\textsuperscript{131} lipiodol has largely demonstrated a lack of efficacy\cite{37-39} [Table 3]. Hepatic arterial infusion (HAI) chemotherapy has gained a foothold in the treatment of advanced colorectal liver metastasis, but its role in the treatment in patients with HCC remains limited. Bolus arterial infusion of epirubicin in combination with UFT produced no benefit on recurrence after curative resection of HCC\cite{40}. However, in a retrospective study of 85 patients who underwent radical hepatectomy where 42 received HAI of 5-FU, oxaliplatin, and mitomycin-C, the HAI group demonstrated significantly higher 5-year intrahepatic RFS, DFS, and OS than the control group\cite{41}. However, several studies with trans-arterial therapies did not improve DFS or OS and may have been associated with worse outcomes\cite{42-46}.

**Brachytherapy**

Adjuvant iodine\textsuperscript{125} brachytherapy has been utilized in patients with resected HCC. In a randomized control trial, 68 HCC patients undergoing curative resection were assigned to receive either iodine\textsuperscript{125} brachytherapy at the raw surface or resection or best supportive care. Iodine\textsuperscript{125} brachytherapy was found to improve time to recurrence and OS after curative resection\cite{47}. However, these results have yet to been validated in a larger patient cohort in the adjuvant setting.

**Radiation therapy**

Although radiation therapy (RT) has traditionally been avoided in the liver due to the risk of radiation-induced liver disease and limited response, recent advances in RT may allow its application as an adjunct to other therapies\cite{48}. In a retrospective study, stereotactic body radiation therapy demonstrated superior efficacy in HCC than sorafenib\cite{49}. A narrow-margin resection (< 1 cm) of HCC has been found to be associated with a significantly higher recurrence rate than patients who underwent wide-margin excision (> 1 cm)\cite{50}. Postoperative RT has been found to be associated with improved recurrence rates in patients who underwent narrow margin hepatectomy\cite{51,52}. In a study comparing conservative therapy, TACE, or radiotherapy in HCC patients with microvascular invasion, adjuvant RT revealed significantly improved RFS and OS compared to TACE and conservative therapy\cite{53}. Additionally, in an analysis of the SEER database, 244 were identified who received preoperative (93 patients) or postoperative (151 patients) RT. Patients who received preoperative RT had improved OS and cancer-specific survival compared to patients who received postoperative RT\cite{54}. The use of RT after hepatectomy remains understudied, and there remains interested in its utilization in both the adjuvant setting and as a bridge to liver transplant\cite{46}.

**ANTI-VIRAL THERAPY**

Chronic viral hepatitis is the greatest risk factor for the development of HCC, where the worldwide
### Table 2. Adjuvant transcatheter arterial chemoembolization studies for hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Groups</th>
<th>Rationale for adjuvant</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Izumi et al.</td>
<td>TACE: lipiodol + doxorubicin + mitomycin + gelatin sponge</td>
<td>Previous evidence within the advanced setting</td>
<td>Significant improvement of DFS but no effect on OS</td>
</tr>
<tr>
<td>Li et al.</td>
<td>TACE: lipiodol + doxorubicin + mitomycin</td>
<td>Microscopic venous invasion is common and related to post-resection outcome</td>
<td>Post-operative TACE + PVC increased DFS. No difference in DFS with TACE alone</td>
</tr>
<tr>
<td>Li et al.</td>
<td>TACE (lipiodol + Adriamycin + mitomycin + cisplatin or carboplatinum +/- portal vein chemotherapy</td>
<td>The study population involved patients with hepatectomy with portal vein tumor thrombus removal</td>
<td>TACE improved OS</td>
</tr>
<tr>
<td>Peng et al.</td>
<td>TACE: lipiodol + Adriamycin + 5-fluorouracil + gelatin sponge</td>
<td>Microscopic venous invasion is common and related to post-resection outcome</td>
<td>Post-operative TACE + PVC increased DFS. No difference in DFS with TACE alone</td>
</tr>
<tr>
<td>Zhong et al.</td>
<td>TACE: lipiodol + mitomycin + carboplatin + epirubicin</td>
<td>The study population involved patients with hepatectomy with portal vein tumor thrombus removal</td>
<td>TACE improved OS</td>
</tr>
<tr>
<td>Lai et al.</td>
<td>TACE: lipiodol + cisplatin IV: epirubicin</td>
<td>Regimen and route of administration for adjuvant therapy varied and sought to study in RCT</td>
<td>Adjuvant therapy associated with significantly worse DFS and no significance in OS</td>
</tr>
<tr>
<td>Li et al.</td>
<td>TACE: lipiodol, epirubicin, oxaliplatin, S-FU</td>
<td>Unresectable HCC with benefits of TACE</td>
<td>TACE improved RFS and OS</td>
</tr>
<tr>
<td>Liu et al.</td>
<td>TACE: epirubicin, oxaliplatin, S-FU</td>
<td>Unresectable HCC with benefits of TACE</td>
<td>TACE improved 1-year, no difference in 2- or 3-year DFS</td>
</tr>
<tr>
<td>Ye et al.</td>
<td>TACE: lipiodol, raltitrexed, lobaplatin</td>
<td>Unresectable HCC with benefits of TACE</td>
<td>TACE improved OS and DFS in patients with HCC with microvascular invasion</td>
</tr>
<tr>
<td>Qi et al.</td>
<td>TACE: lipiodol, oxaliplatin or lobaplatin, pirarubicin or pharorubicin</td>
<td>Unresectable HCC with benefits of TACE</td>
<td>No difference in 1-, 2-, 3-year DFS</td>
</tr>
</tbody>
</table>

TACE: Transcatheter arterial chemoembolization; DFS: disease-free survival; OS: overall survival; PVC: portal vein chemotherapy; HCC: hepatocellular carcinoma; RCT: randomized controlled trial; RFS: recurrence free survival; S-FU: 5-fluorouracil.

Incidence of HCC follows that of chronic viral hepatitis\(^2\). Therefore, anti-viral therapy before and after curative treatment may be crucial in preventing late HCC recurrence\(^3\). Several groups have evaluated the use of nucleoside analogues in the adjuvant treatment of Hepatitis B virus-related HCC with mixed results [Table 4]. Several randomized control trials found lamivudine not to affect post-operative DFS or OS\(^{55-58}\). Other studies demonstrated lamivudine or other nucleoside analogues to improve tumor-free survival in patients with high serum HBV DNA\(^{59,60}\) or reducing recurrence with prolonged post-operative survival with improved liver function reserve\(^{61}\). However, the above studies indicate nucleoside analogues may improve liver function and clearance of HBV in patients with HCC.

**STUDIES WITH ADJUVANT IMMUNE-BASED THERAPIES IN HCC**

Several randomized clinical trials have been performed utilizing immune-based therapies in the adjuvant setting. This section will discuss the application of immunotherapies in the adjuvant setting focusing on cytokine-based therapy, cell-based therapies, and vaccine-based therapies.

**Cytokine based therapy: interferon**

The use of interferon (IFN) appeared as a logical first choice for the treatment of HCC as it may show both anti-viral and anti-tumor functions. However, the tumor response rates to IFN therapy were poor in patients with advanced disease, with a partial response rate of 6% (2 of 30 patients) and no benefit in OS. Additionally, IFN therapy was not well tolerated in patients with cirrhosis and HCC, where nearly half of the patients discontinued treatment due to intolerance or adverse events\(^{62}\).
Table 3. Intra-arterial adjuvant studies for hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Study</th>
<th>Groups</th>
<th>Rationale for adjuvant</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim et al.[41] 2011</td>
<td>Adjuvant hepatic artery infusion 5-FU and cisplatin</td>
<td>Repetitive short course 5-FU and cisplatin showed antitumor effects in advanced HCC[116]</td>
<td>No benefit in recurrence rate or median recurrence-free survival at 2 years</td>
</tr>
<tr>
<td>Kohno et al.[40] 1996</td>
<td>Bolus arterial injection of epirubicin post-op day 28 with UFT vs. UFT alone</td>
<td>Bolus injection of epirubicin did not change long-term results</td>
<td></td>
</tr>
<tr>
<td>Ohno et al.[44] 2001</td>
<td>Analysis of three trials of three different post-operative adjuvant therapies: arterial epirubicin with oral tegafur, arterial epirubicin + carmustine, IV epirubicin</td>
<td>Pre-op transarterial chemoembolization displayed no significant benefit and tried therapy in the adjuvant setting[117]</td>
<td>No improvement in DFS or OS with possible worse outcomes for patient receiving adjuvant therapy</td>
</tr>
<tr>
<td>Lau et al.[38] 2008</td>
<td>Intra-arterial [131] lipiodol</td>
<td>[131] lipiodol has active uptake and prolonged retention in hepatoma cells</td>
<td>No difference in DFS or OS at 8 years</td>
</tr>
<tr>
<td>Lau et al.[37] 1999</td>
<td>Intra-arterial [131] lipiodol</td>
<td>Decrease RFS and increase OS at 3 years</td>
<td></td>
</tr>
<tr>
<td>Chung et al.[39] 2013</td>
<td>Intra-arterial [131] lipiodol</td>
<td>No difference in RFS or OS</td>
<td></td>
</tr>
<tr>
<td>Hirokawa et al.[45] 2020</td>
<td>Transarterial catheter infusion of cisplatin three months after surgery then three months later TACE with lipiodol and cisplatin</td>
<td>Unresectable HCC with benefits of TACE[114]</td>
<td>No difference in relapse-free survival or overall survival</td>
</tr>
<tr>
<td>Hamada et al.[46] 2020</td>
<td>Hepatic artery infusion: cisplatin</td>
<td>Hepatic artery infusion chemotherapy may be associated with survival benefits in advanced disease[103]</td>
<td>No significant difference in tumor-free or overall survival in HCC patients with portal vein infiltration</td>
</tr>
<tr>
<td>Feng et al.[42] 2017</td>
<td>Hepatic artery infusion: cisplatin</td>
<td></td>
<td>HAI group demonstrated significantly higher 5-year intrahepatic RFS, DFS, and OS[106]</td>
</tr>
<tr>
<td>Li et al.[109] 2020</td>
<td>Transarterial injection of [131] metuximab 4-6 weeks after hepatectomy</td>
<td>Previous studies have shown survival benefits of [131] metuximab in advanced HCC[108]</td>
<td>[131] metuximab was associated with improved 5-year RFS in HCC tumors expressing CD147</td>
</tr>
</tbody>
</table>

5-FU: 5-fluorouracil; HCC: hepatocellular carcinoma; UFT: tegafur/uracil; DFS: disease-free survival; OS: overall survival; RFS: recurrence-free survival; TACE: transcatheter arterial chemoembolization; HAI: hepatic arterial infusion.

As with many of the treatment strategies mentioned above in the adjuvant setting for HCC, IFN has been met with mixed results [Table 5]. Several groups found no improvement of RFS or OS in HBV/HCV-HCC patients[63] and HBV-HCC patients[64] after curative resection. Meanwhile, other studies found IFN-improved post-ablation HCC recurrence[65] and preventing late recurrence after resection in HCV-HCC patients but found no improvement in disease-specific survival[66]. Furthermore, several studies found IFN- to not improve HCC recurrence but improved survival after procedures performed for curative intent for HCC[64-70]. Additionally, IFN was shown to improve RFS at 25 months after curative resection or ablation[71]. Pegylated-interferon (PEG-IFN) was associated with a decrease in 1- and 2-year recurrence with a higher survival[72], and the addition of ribavirin was associated with a decreased recurrence and mortality in HCV associated HCC[73].
### Table 4. Anti-viral therapies

<table>
<thead>
<tr>
<th>Study</th>
<th>Groups</th>
<th>Rationale for adjuvant</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yin et al.</td>
<td>Anti-viral medication: lamivudine, adefovir dipivoxil, or entecavir</td>
<td>Retrospective studies reported that post-operative treatment conferred postoperative survival[69-72]</td>
<td>Improve liver function reserve and reduces HBV-HCC recurrence and prolonged post-operative survival</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>Adefovir</td>
<td></td>
<td>Adefovir reduced late HBV-HCC recurrence and improved OS</td>
</tr>
<tr>
<td>Kuzuya et al.</td>
<td>Lamivudine</td>
<td>Lamivudine treatment may reduce HBV replication and improve remnant liver function, prevent liver failure and prolong survival[73]</td>
<td>No significant difference in cumulative recurrence of survival rates in HBV-HCC</td>
</tr>
<tr>
<td>Kubo et al.</td>
<td>Lamivudine</td>
<td></td>
<td>Lamivudine improved tumor-free survival rate after curative resection in patients with high serum concentrations of HBV DNA</td>
</tr>
<tr>
<td>Li et al.</td>
<td>Lamivudine</td>
<td></td>
<td>No difference in disease-free survival but promoted post-operative HBV clearance and increased residual liver volume</td>
</tr>
<tr>
<td>Piao et al.</td>
<td>Lamivudine</td>
<td></td>
<td>No difference in HBV-HCC recurrence or survival, but the treatment group was associated with a low death rate due to liver failure</td>
</tr>
<tr>
<td>Yoshida et al.</td>
<td>Lamivudine</td>
<td></td>
<td>No difference in overall survival or recurrence-free survival but did improve liver function for HBV-HCC</td>
</tr>
<tr>
<td>Wu et al.</td>
<td>Nucleoside analogues: lamivudine, entecavir, and telbivudine</td>
<td></td>
<td>Antiviral therapy was associated with a lower risk of HCC recurrence among patients with HBV-related HCC</td>
</tr>
<tr>
<td>Yang et al.</td>
<td>Nucleoside analogues: lamivudine, entecavir, or adefovir</td>
<td></td>
<td>Antiviral therapy was associated with improved RFS for the patient with high HBV viral load</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>Telbivudine</td>
<td></td>
<td>Telbivudine reduced HCC recurrence in patients with low preoperative HBV-DNA levels</td>
</tr>
</tbody>
</table>

HBV-HCC: Hepatitis B virus-hepatocellular carcinoma; OS: overall survival; RFS: recurrence-free survival.

### Cell and vaccine-based therapy

Adoptive cell transfer (ACT) is a highly personalized form of cancer immunotherapy that involves the transfer of host-derived expanded immune cells[74]. Adoptive transfer of autologous tumor-infiltrating lymphocytes (TIL) has been shown to produce complete and durable tumor regression in patients with metastatic melanoma, and a particular case of metastatic cholangiocarcinoma[75,76]. ACT has been studied in the adjuvant setting for HCC. Wang et al.[77] found that TIL can be expanded and activated in vitro and reduce recurrence rates in patients with HCC compared to patients who did not receive TIL infusion. Meanwhile, adjuvant activated autologous lymphocyte infusions were found to increase RFS but had no impact on OS[79].

Activated T cell transfer has also been applied with adjunctive treatments such as an autologous tumor lysate-pulsed dendritic cell vaccine[79]. Patients who underwent a curative HCC liver resection received an adjuvant dendritic cell vaccine made from a patient’s dendritic cells pulsed with a tumor lysate created from the resected tumor along with activated CD3+ T cells. There was a significant difference in RFS and OS in favor of the combination DC vaccine and ACT vs. no adjuvant therapy[79]. However, other studies have shown mixed results with the use of lymphocyte-activated killer cells along with intra-arterial therapies[80,81]. Tumor associated-antigen pulsed DCs without ACT has also been utilized in the adjuvant setting with no difference in RFS and upon subgroup analysis may have increased the risk of recurrence in patients who were treated with RFA while reducing recurrence in patients who received an operation[82] [Table 6].
Table 5. Adjuvant interferon therapy for HCC

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Rationale for adjuvant</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al. (^{(63)}) 2012</td>
<td>IFN-α</td>
<td>IFN may show both anti-viral and anti-tumor function</td>
<td>No improvement in RFS or OS in the total patient population of HBV/HCV patients</td>
</tr>
<tr>
<td>Sun et al. (^{(70)}) 2006</td>
<td>IFN-α</td>
<td></td>
<td>No improvement in DFS in HBV-related HCC but improved OS in HBV related disease</td>
</tr>
<tr>
<td>Lo et al. (^{(64)}) 2007</td>
<td>IFN-α</td>
<td></td>
<td>No improvement in DFS or OS in HBV-related HCC</td>
</tr>
<tr>
<td>Shiratori et al. (^{(69)}) 2003</td>
<td>IFN-α</td>
<td></td>
<td>No improvement in RFS but may improve OS in HCV-related disease</td>
</tr>
<tr>
<td>Kubo et al. (^{(67)}) 2002</td>
<td>IFN-α</td>
<td>Post-operative IFN did not statistically decrease intrahepatic recurrence but improved cumulative survival in HCV-related HCC</td>
<td></td>
</tr>
<tr>
<td>Lin et al. (^{(65)}) 2004</td>
<td>IFN-α</td>
<td>Post-ablation IFN reduced the recurrence of HCC recurrence No survival data were reported</td>
<td></td>
</tr>
<tr>
<td>Nishiguchi et al. (^{(68)}) 2005</td>
<td>IFN-α</td>
<td>Post-operative IFN group improved cumulative survival but failed to improve intrahepatic recurrence</td>
<td></td>
</tr>
<tr>
<td>Mazzaferro et al. (^{(66)}) 2006</td>
<td>IFN-α</td>
<td>No difference in disease-specific survival, but IFN may prevent late recurrence in HCV-HCC</td>
<td></td>
</tr>
<tr>
<td>Ikeda et al. (^{(71)}) 2000</td>
<td>IFN-β</td>
<td>Improved RFS at 25 months. OS not reported</td>
<td></td>
</tr>
<tr>
<td>Hsu et al. (^{(73)}) 2013</td>
<td>PEG-IFN with ribavirin</td>
<td>The antiviral regimen of IFN-β with ribavirin used to treat HCV-related infection(^{(126)})</td>
<td>IFN-β with ribavirin was associated with decreased recurrence rate and mortality in patients with HCV-HCC</td>
</tr>
<tr>
<td>Lee et al. (^{(72)}) 2013</td>
<td>PEG-IFN</td>
<td>PEG-IFN associated with a decrease in 1- and 2-year recurrence and higher survival</td>
<td></td>
</tr>
</tbody>
</table>

HCC: Hepatocellular carcinoma; IFN: interferon; OS: overall survival; RFS: recurrence-free survival; HBV: hepatitis B virus; DFS: disease-free survival; HCV: hepatitis C virus; PEG-IFN: pegylated-interferon.

Other vaccine trials have been conducted utilizing a peptide vaccine against the carcinoembryonic antigen glypican-3 (GPC3). GPC3 makes an appealing target for HCC vaccines as it is specifically overexpressed in HCC and often is associated with a poor prognosis. Early studies utilizing a GPC3 peptide vaccine found the treatment to be safe and induce tumor infiltration of CD8⁺ T cells. The GPC3 vaccine in the adjuvant setting demonstrating a significantly improved recurrence rate in patients treated with surgery plus vaccine compared to surgery alone at 1 year but was found to be no longer statistically significant at 2 years\(^{(83)}\). However, recently, Taniguchi \(^{(84)}\) found the GPC3 peptide vaccine decreased 1-year recurrence rate after surgery by 15%, and the 5-year and 8-year survival rates were improved by 10% and 30%, respectively, compared to an unvaccinated group. Additionally, Kuang et al. \(^{(85)}\) utilized an autologous formalin-fixed tumor vaccine to improve RFS and OS after curative resection [Table 6].

Another strategy of ACT that has been tried in the adjuvant setting for HCC is through cytokine-induced killer (CIK) cells. CIK cells are autologous cells expanded \textit{ex vivo} from a patient’s peripheral blood mononuclear cells and cultured with a cytokine cocktail and anti-CD3 antibodies. The resulting cell population has potent antitumor effects with the dual-functional capability of T cells and NK cells\(^{(86,87)}\). CIK cell treatment was found to be an independent prognostic factor for improved OS in patients after HCC resection\(^{(89)}\). In a non-randomized study, Chen et al. \(^{(89)}\) found CIK cells improved DFS and OS for patients without microvascular invasion. Furthermore, a randomized controlled trial found CIK cells decreased HCC recurrence after radical resection\(^{(89)}\). Lee et al. \(^{(87)}\) studied CIK cells in the adjuvant setting in patients with resected HCC. The primary endpoint of this study was RFS and secondary endpoints, including OS and cancer-specific survival. They found that CIK cell immunotherapy was associated with improved recurrence-free, overall, and cancer-specific survival\(^{(87)}\).
Table 6. Immune approaches for adjuvant HCC

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Rationale for adjuvant</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takayama et al. [78] 2000</td>
<td>Adoptive cell transfer</td>
<td>Activated autologous lymphocyte infusions</td>
<td>Increased RFS and disease-specific survival but had no impact on OS</td>
</tr>
<tr>
<td>Wang et al. [77] 1997</td>
<td>ACT</td>
<td></td>
<td>Reduced recurrence</td>
</tr>
<tr>
<td>Lee et al. [87] 2015</td>
<td>CIK cells</td>
<td>CIK cells have potent antitumor effects with the dual-functional capability of T cells and NK cells</td>
<td>CIK cell immunotherapy was associated with improved recurrence-free, overall, and cancer-specific survival</td>
</tr>
<tr>
<td>Hui et al. [90] 2009</td>
<td>CIK cells</td>
<td>CIK cells demonstrated decreased recurrence after radical resection</td>
<td>CIK cell immunotherapy was associated with improved recurrence-free, overall, and cancer-specific survival</td>
</tr>
<tr>
<td>Kuang et al. [85] 2004</td>
<td>Autologous formalin-fixed tumor vaccine</td>
<td>HCC vaccine consisted of autologous formalin-fixed tumor tissue fragments, biodegradable microparticles containing GM-CSF, IL2, and tuberculin[127]</td>
<td>Vaccine improved RFS and OS</td>
</tr>
<tr>
<td>Xie et al. [81] 2000</td>
<td>Hepatic artery chemoembolization with lymphocyte-activated killer cells/IL2 after radical surgery</td>
<td></td>
<td>Reduced intrahepatic recurrence and improved survival</td>
</tr>
<tr>
<td>Shimizu et al. [79] 2014</td>
<td>Dendritic cell vaccine with activated T-cell transfer</td>
<td>Dendritic cell vaccine was made from a patient’s isolated dendritic cells pulsing with tumor lysate created from the resected tumor and CD3+ activated T cells</td>
<td>Significant difference in both RFS and OS</td>
</tr>
<tr>
<td>Lee et al. [82] 2017</td>
<td>TAA-pulsed DC vaccine</td>
<td>A previous study demonstrated that IV vaccination with ex vivo DCs showed evidence of antitumor efficacy in advanced HCC[128]</td>
<td>No overall difference in RFS. Subgroup analysis with improved RFS in non-RFA patients and increased risk of recurrence in RFA patients</td>
</tr>
<tr>
<td>Sawada et al. [83] 2016</td>
<td>GPC3 vaccine</td>
<td>Early studies utilizing a GPC3 peptide vaccine found the treatment to be safe and able to induce tumor infiltration of CD8+ T cells</td>
<td>No statistically significant reduction in recurrence at 2 years</td>
</tr>
<tr>
<td>Kawata et al. [80] 1995</td>
<td>Intra-arterial Adriamycin, IL-2, lymphocyte-activated killer cells</td>
<td></td>
<td>No difference in DFS or OS compared to intra-arterial Adriamycin alone</td>
</tr>
</tbody>
</table>


Checkpoint inhibition with nivolumab is now a mainstream treatment for patients with sorafenib refractory HCC[91]. Several clinical trials are currently in progress investigating immune checkpoint inhibition for patients with HCC in the adjuvant setting (CheckMate 9DX, nivolumab vs. placebo after resection or ablation; EMERALD-2, adjuvant durvalumab +/- bevacizumab after curative treatment)[92].

FUTURE DIRECTIONS

As the recurrence for HCC after attempted curative ablation or resection remains high, additional studies are required to reduce post-operative recurrence. Many of the treatment strategies mentioned above have been met with largely mixed results. The key to success may be found in selecting patients and pairing them with the appropriate therapy properly[93]. For example, early and late recurrences are linked to different predictive risk factors[94]. Early recurrence is often
seen as an intrahepatic metastasis associated with risk factors such as large tumors, incomplete tumor capsules, and venous or microvascular invasion\textsuperscript{[95-97]}. On the other hand, late recurrence is often thought of as the development of a new tumor in a persistently diseased liver with risk factors centered on the extent of the patient’s underlying liver disease and alpha-fetoprotein (AFP) level\textsuperscript{[95]}. Additionally, gene signatures and circulating micro-RNA have been used to predict survival and recurrence\textsuperscript{[98-101]}. Ng et al.\textsuperscript{[102]} investigated a prediction model for early (< 18 months) recurrence following resection. They developed a Recurrent Liver Cancer Score based on four risk factors: AFP, tumor size, multiple tumors or satellite nodules, and microvascular invasion. Low and high-risk groups were at statistically significant risk of recurrence and 5-year survival\textsuperscript{[102]}. The ability to properly select patients may prove vital in achieving success with HCC in the adjuvant setting.

The question remains of what therapy to administer, and we can predict patients who will get a response with potential biomarkers. Pan et al.\textsuperscript{[103]} looked at 48 patients with HCC treated with postoperative adjuvant CIK cell immunotherapy to identify a predictive biomarker for adjuvant CIK cell treatment. They found that there was no association between prognosis and CIK cell phenotype. However, there was a statistically significant improvement in OS and RFS in patients with the high cytotoxic activity of CIK cells compared to the low cytotoxic activity of CIK cells\textsuperscript{[103]}. Additionally, low expression of microRNA miR-26 was found to be a predictor of the worse OS and a better response to IFN therapy\textsuperscript{[104]}. Antiviral-based therapies have not been found to reduce HBV or HCC related recurrence after resection but seem to improve overall liver function which then in turn may improve post-operative survival\textsuperscript{[61]}. Furthermore, with the introduction and application of Harvoni (ledipasvir/sofosbuvir) allowing for the potential cure of HCV, it would be interesting to witness potential changes in HCC recurrence over the next decade. Treating HCV-infected patients before the development of cirrhosis may reduce the risk of HCC\textsuperscript{[105]}. However, data is emerging that treatment of HCV in patients with successfully treated HCC may result in high rates of recurrence, especially within the first six months of treatment\textsuperscript{[106]}.

Medicine is becoming more increasingly personalized with the development of targeted therapies aimed at specific molecular alterations. For example, up to 60% of patients with HCC have positive CD147 expression in tumor tissues\textsuperscript{[107]}. CD147 is associated with increased metastatic potential and worse disease outcomes than tumors that are negative for CD147 expression\textsuperscript{[107]}. Blocking CD147 with metuximab, a monoclonal antibody specifically against CD147, has been shown to inhibit HCC cell growth and metastasis\textsuperscript{[108]}. \textsuperscript{[109]}\textsuperscript{131}I-metuximab was found to be associated with improved 5-year RFS in patients with HCC tumors expressing CD147\textsuperscript{[109]}.

CONCLUSION

The past several years have seen advancement in systemic therapies for HCC in the advanced setting. However, patients fortunate enough to undergo resection with intent for cure are plagued by high recurrence rates, and there is currently no approved therapy in the adjuvant setting to help this complex problem. Nevertheless, future success may be found as we continue to learn more about the molecular drivers of tumorigenesis and improved selection of patients, proper therapy, and the development of more targeted therapies.

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Authors’ contributions

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Not applicable.

Consent for publication
Not applicable.

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