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Adjuvant treatment of hepatocellular carcinoma after resection

Zachary J. Brown, Allan Tsung

Division of Surgical Oncology, Department of Surgery, The Ohio State University Wexner Medical Center, Columbus, OH 43210, USA.

Correspondence to: Prof. Allan Tsung, Division of Surgical Oncology, Department of Surgery, The Ohio State University Wexner Medical Center, 410 W 10th Ave, N-924 Doan Hall, Columbus, OH 43210, USA. E-mail: Allan.Tsung@osumc.edu

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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[3]. 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[4]. 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[2,5]. 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[6]. 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[7]. 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[8]. In a randomized control trial, capecitabine was found to inhibit post-operative recurrence of HCC but failed to improve OS[9]. 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[10] [Table 1].

In addition to traditional chemotherapy, Vitamin K_2 has been utilized in the adjuvant setting for patients with HCC. Pre-clinical data demonstrated that Vitamin K_2 suppressed the growth of three HCC cell lines through the regulation and suppression of the hepatoma-derived growth factor (HDGF) gene[11]. HDGF is highly expressed in HCC cells stimulating their proliferation with oncogenic and angiogenic activity[11,12]. However, adjuvant trials with Vitamin K_2 in HCC have largely disappointing results. Vitamin K_2 failed to improve the recurrence of HCC[13-15] or significantly improved the recurrence without an improvement in OS[16,17] [Table 1]. Vitamin K_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[18]. However, the combination of vitamin K_2 and an ACE inhibitor reduced HCC recurrence without a survival advantage[19].

In addition to vitamin K_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[20]. In a prospective randomized control trial, the acyclic retinoid, polyprenoic acid, was found to prevent recurrent HCC after surgical resection or percutaneous injection of ethanol compared to placebo[21]. However, this

Table 1. Systemic (non-arterial) adjuvant therapies for HCC

Study	Treatment	Rationale for adjuvant	Outcome
Bruix *et al.*[7] 2015 (STORM)	Sorafenib	Sorafenib approved for advanced HCC	No difference in median RFS or OS
Xia *et al.*[9] 2010	Adjuvant capecitabine	Capecitabine significantly inhibited postoperative recurrence and lung metastasis in mice[8]	Improved DFS and probability of recurrence with no difference in OS
Ishizuka *et al.*[10] 2016	Adjuvant UFT *vs.* surgery alone	UFT has shown efficacy in some HCC patients with advanced disease[10]	No difference in RFS and OS
Hotta *et al.*[13] 2007	Vitamin K_2	Vitamin K_2 has been shown to inhibit the expression of hepatoma-derived growth factors by suppressing the promotor activity of the HDGF protein[11]	No difference in HCC recurrence
Yoshida *et al.*[15] 2011	Vitamin K_2		No improvement of DFS
Ishizuka *et al.*[14] 2012	Vitamin K_2		No improvement of DFS or OS
Kakizaki *et al.*[16] 2007	Vitamin K_2		Disease recurrence significantly lower in vitamin K_2 group ($P = 0.045$) but no difference in OS
Mizuta *et al.*[17] 2006	Vitamin K_2		Disease recurrence lower in vitamin K_2 group but no significant difference in OS
Yoshiji *et al.*[19] 2009	Vitamin K_2 and ACE inhibitor	ACE inhibitor and vitamin K_2 exert strong anti-angiogenic activities, and the combination showed suppressive effects against the development of HCC in rats[18]	Combination of vitamin K_2 and ACE inhibitor significantly suppressed recurrence of HCC but no significant difference in cumulative survival
Muto *et al.*[21] 1996	Acyclic retinoid, polyprenoic acid	Pre-clinical models show polyprenoic acid inhibits hepatocarcinogenesis and induces apoptotis in human hepatoma cell lines[111]	Polyprenoic acid prevented recurrent hepatoma after surgical resection or percutaneous injection of ethanol
Liu *et al.*[25] 2009	Heparanase inhibitor PI-88	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]	160 mg dosage of PI-88 but not 250 mg dose, improved RFS at 1 year

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.

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 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[1]. 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[2]. Chemoembolization with doxorubicin provided a significant survival benefit in stringently selected patients with unresectable HCC[27]. Additionally, chemoembolization

with the emulsion of cisplatin in lipiodol displayed improved survival in Asian patients with unresectable HCC[28]. However, results are mixed in the adjuvant setting. Izumi *et al.*[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.*[30] 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[31] and patients who required removal of a portal vein tumor thrombus[32]. In addition, the use of TACE with portal vein chemotherapy (PVC) was evaluated. Li *et al.*[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[34-36] [Table 2].

In addition to TACE, several studies have evaluated the use of other arterial-based regiments such as intra-arterial I^{131} lipiodol or hepatic artery infusion chemotherapy. Trans-arterial I^{131} lipiodol has largely demonstrated a lack of efficacy[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 improvement in RFS over UFT alone[40]. HAI of 5-fluoruracil (5-FU) and cisplatin failed to produce any benefit on recurrence after curative resection of HCC[41]. 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[42]. However, several studies with trans-arterial therapies did not improve DFS or OS and may have been associated with worse outcomes[43-46].

Brachytherapy

Adjuvant iodine^{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^{125} brachytherapy at the raw surface or resection or best supportive care. Iodine^{125} brachytherapy was found to improve time to recurrence and OS after curative resection[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[48]. In a retrospective study, stereotactic body radiation therapy demonstrated superior efficacy in HCC than sorafenib[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)[50]. Postoperative RT has been found to be associated with improved recurrence rates in patients who underwent narrow margin hepatectomy[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[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[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[48].

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

Study	Groups	Rationale for adjuvant	Outcome
Izumi *et al.*[29] 1994	TACE: lipiodol + doxorubicin + mitomycin + gelatin sponge	Previous evidence within the advanced setting[112]	Significant improvement of DFS but no effect on OS
Li *et al.*[30] 1995	TACE: lipiodol + doxorubicin + mitomycin		Significant improvement of DFS and OS
Li *et al.*[33] 2006	TACE (lipiodol + Adriamycin + mitomycin + cisplatin or carboplatinum +/- portal vein chemotherapy	Microscopic venous invasion is common and related to post-resection outcome[113]	Post-operative TACE + PVC increased DFS. No difference in DFS with TACE alone
Peng *et al.*[32] 2009	TACE: lipiodol + Adriamycin + 5-fluorouracil + gelatin sponge	The study population involved patients with hepatectomy with portal vein tumor thrombus removal	TACE improved OS
Zhong *et al.*[31] 2009	TACE: lipiodol + mitomycin + carboplatin + epirubicin		Significantly improved DFS and OS in Stage IIIA HCC
Lai *et al.*[43] 1998	TACE: lipiodol + cisplatin IV: epirubicin	Regiment and route of administration for adjuvant therapy varied and sought to study in RCT	Adjuvant therapy associated with significantly worse DFS and no significance in OS
Li *et al.*[36] 2017	TACE: lipiodol, epirubicin, oxaliplatin, 5-FU	Unresectable HCC with benefits of TACE[114]	TACE improved RFS and OS
Liu *et al.*[35] 2016	TACE: epirubicin, oxaliplatin, 5-FU	Unresectable HCC with benefits of TACE[114]	TACE improved 1-year, no difference in 2- or 3-year DFS
Ye *et al.*[34] 2017	TACE: lipiodol, raltitrexed, lobaplatin	Unresectable HCC with benefits of TACE[114]	TACE improved OS and DFS in patients with HCC with microvascular invasion
Qi *et al.*[115] 2019	TACE: lipiodol, oxaliplatin or lobaplatin, pirarubicin or pharmorubicin	Unresectable HCC with benefits of TACE[114]	No difference in 1-, 2-, 3-year DFS Tumor size > 5 cm had improved DFS and OS with TACE

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; 5-FU: 5-fluoruracil.

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

Study	Groups	Rationale for adjuvant	Outcome
Kim *et al.*[41] 2011	Adjuvant hepatic artery infusion 5-FU and cisplatin	Repetitive short course 5-FU and cisplatin showed antitumor effects in advanced HCC[116]	No benefit in recurrence rate or median recurrence-free survival at 2 years
Kohno *et al.*[40] 1996	Bolus arterial injection of epirubicin post-op day 28 with UFT *vs.* UFT alone		Bolus injection of epirubicin did not change long-term results
Ono *et al.*[44] 2001	Analysis of three trials of three different post-operative adjuvant therapies: arterial epirubicin with oral tegafur, arterial epirubicin+ IV epirubicin + carmofur, IV epirubicin	Pre-op transarterial chemoembolization displayed no significant benefit and tried therapy in the adjuvant setting[117]	No improvement in DFS or OS with possible worse outcomes for patient receiving adjuvant therapy
Lau *et al.*[38] 2008	Intra-arterial I^{131} lipiodol	I^{131} lipiodol has active uptake and prolonged retention in hepatoma cells	No difference in DFS or OS at 8 years
Lau *et al.*[37] 1999	Intra-arterial I^{131} lipiodol		Decrease RFS and increase OS at 3 years
Chung *et al.*[39] 2013	Intra-arterial I^{131} lipiodol		No difference in RFS or OS
Hirokawa *et al.*[45] 2020	Transarterial catheter infusion of cisplatin three months after surgery then three months later TACE with lipiodol and cisplatin	Unresectable HCC with benefits of TACE[114]	No difference in relapse-free survival or overall survival
Hamada *et al.*[46] 2020	Hepatic artery infusion: cisplatin	Hepatic artery infusion chemotherapy may be associated with survival benefits in advanced disease[118]	No significant difference in tumor-free or overall survival in HCC patients with portal vein infiltration
Feng *et al.*[42] 2017	Hepatic artery infusion: cisplatin		HAI group demonstrated significantly higher 5-year intrahepatic RFS, DFS, and OS
Li *et al.*[109] 2020	Transarterial injection of ^{131}I-metuximab 4-6 weeks after hepatectomy	Previous studies have shown survival benefits of ^{131}I-metuximab in advanced HCC[108]	^{131}I-metuximab was associated with improved 5-year RFS in HCC tumors expressing CD147

5-FU: 5-fluoruracil; 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[67-70]. 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

Study	Groups	Rationale for adjuvant	Outcome
Yin *et al.*[61] 2013	Anti-viral medication: lamivudine, adefovir dipivoxil, or entecavir	Retrospective studies reported that post-operative treatment conferred postoperative survival[119-122]	Improve liver function reserve and reduces HBV-HCC recurrence and prolonged post-operative survival
Huang *et al.*[123] 2015	Adefovir		Adefovir reduced late HBV-HCC recurrence and improved OS
Kuzuya *et al.*[55] 2007	Lamivudine	Lamivudine treatment may reduce HBV replication and improve remnant liver function, prevent liver failure and prolong survival[124]	No significant difference in cumulative recurrence of survival rates in HBV-HCC
Kubo *et al.*[59] 2007	Lamivudine		Lamivudine improved tumor-free survival rate after curative resection in patients with high serum concentrations of HBV DNA
Li *et al.*[56] 2010	Lamivudine		No difference in disease-free survival but promoted post-operative HBV clearance and increased residual liver volume
Piao *et al.*[57] 2005	Lamivudine		No difference in HBV-HCC recurrence or survival, but the treatment group was associated with a low death rate due to liver failure
Yoshida *et al.*[58] 2008	Lamivudine		No difference in overall survival or recurrence-free survival but did improve liver function for HBV-HCC
Wu *et al.*[122] 2012	Nucleoside analogues: lamivudine, entecavir, and telbivudine		Antiviral therapy was associated with a lower risk of HCC recurrence among patients with HBV-related HCC
Yang *et al.*[60] 2012	Nucleoside analogues: lamivudine, entecavir, or adefovir		Antiviral therapy was associated with improved RFS for the patient with high HBV viral load
Huang *et al.*[125] 2018	Telbivudine		Telbivudine reduced HCC recurrence in patients with low preoperative HBV-DNA levels

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[78].

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

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

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 *et al.*[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 *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[88]. 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[90]. 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

Study	Treatment	Rationale for adjuvant	Outcome
Takayama *et al.*[78] 2000	Adoptive cell transfer	Activated autologous lymphocyte infusions	Increased RFS and disease-specific survival but had no impact on OS
Wang *et al.*[77] 1997	ACT		Reduced recurrence
Lee *et al.*[87] 2015	CIK cells	CIK cells have potent antitumor effects with the dual-functional capability of T cells and NK cells	CIK cell immunotherapy was associated with improved recurrence-free, overall, and cancer-specific survival
Hui *et al.*[90] 2009	CIK cells		CIK cells demonstrated decreased recurrence after radical resection
Kuang *et al.*[85] 2004	Autologous formalin-fixed tumor vaccine	HCC vaccine consisted of autologous formalin-fixed tumor tissue fragments, biodegradable microparticles containing GM-CSF, IL2, and tuberculin[127]	Vaccine improved RFS and OS
Xie *et al.*[81] 2000	Hepatic artery chemoembolization with lymphocyte-activated killer cells/IL2 after radical surgery		Reduced intrahepatic recurrence and improved survival
Shimizu *et al.*[79] 2014	Dendritic call vaccine with activated T-cell transfer	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	Significant difference in both RFS and OS
Lee *et al.*[82] 2017	TAA-pulsed DC vaccine	A previous study demonstrated that IV vaccination with *ex vivo* DCs showed evidence of antitumor efficacy in advanced HCC[128]	No overall difference in RFS. Subgroup analysis with improved RFS in non-RFA patients and increased risk of recurrence in RFA patients
Sawada *et al.*[83] 2016	GPC3 vaccine	Early studies utilizing a GPC3 peptide vaccine found the treatment to be safe and able to induce tumor infiltration of $CD8^+$ T cells	No statistically significant reduction in recurrence at 2 years
Kawata *et al.*[80] 1995	Intra-arterial Adriamycin, IL-2, lymphocyte-activated killer cells		No difference in DFS or OS compared to intra-arterial Adriamycin alone

HCC: Hepatocellular carcinoma; OS: overall survival; RFS: recurrence-free survival; ACT: adoptive cell transfer; CIK: cytokine induced killer; GM-CSF: granulocyte-macrophage colony-stimulating factor; TAA: tumor associated antigen; RFA: radiofrequency ablation; GPC3: glypican-3.

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[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[95]. Additionally, gene signatures and circulating micro-RNA have been used to predict survival and recurrence[98-101]. Ng *et al.*[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[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.*[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[103]. Additionally, low expression of microRNA miR-26 was found to be a predictor of the worse OS but a better response to IFN therapy[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[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[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[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[107]. CD147 is associated with increased metastatic potential and worse disease outcomes than tumors that are negative for CD147 expression[107]. Blocking CD147 with metuximab, a monoclonal antibody specifically against CD147, has been shown to inhibit HCC cell growth and metastasis[108]. ^{131}I-metuximab was found to be associated with improved 5-year RFS in patients with HCC tumors expressing CD147[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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