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RESEARCH

Adjuvant cytokine-induced killer cell immunotherapy in hepatocellular carcinoma: real-world data and 9-year extended follow-up of a randomized controlled trial

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Abstract

Background Most adjuvant therapies for hepatocellular carcinoma (HCC) have failed except for autologous cytokine-induced killer (CIK) therapy, which prolonged recurrence-free survival (RFS) in a previously reported randomized controlled trial (RCT) and real-world data (RWD).

Methods This study aimed to assess the long-term outcomes of adjuvant CIK therapy using both an extended follow-up of the original RCT and a retrospective cohort study. An extended follow-up analysis of the RCT included 226 patients (114 in the CIK group and 112 in the control group). The follow-up duration was extended from 2 to 9 years after the enrollment of the last patient. In parallel, a retrospective RWD study was performed involving 577 patients from two tertiary centers in Korea, including 251 who received adjuvant CIK therapy and 326 controls. Propensity score matching (PSM) was applied to adjust for baseline imbalances. The primary endpoint was RFS in both studies.

Results In the RWD study (median follow-up = 57.2 months), the CIK group demonstrated significantly prolonged RFS than controls both before PSM (median = 101.2 versus 64.7 months; HR = 0.69, 95% CI 0.53–0.90, $P = 0.006$) and after PSM (median = 101.2 vs. 65.7 months; HR = 0.64, 95% CI 0.45–0.91, $P = 0.01$). In the extended follow-up of the RCT (median follow-up = 116.1 months), the CIK group exhibited significantly prolonged RFS (median = 44.0 vs. 30.0 months; hazard ratio [HR] = 0.72, 95% confidence interval [CI] 0.54–0.97, $P = 0.033$) compared to the control group.

Conclusions Adjuvant CIK cell therapy significantly improved RFS in both a RWD study and a 9-year extended RCT follow-up, supporting its reproducible benefit in reducing recurrence after curative treatment of HCC. These consistent findings provide strong evidence for the clinical utility of CIK therapy as a durable adjuvant immunotherapeutic strategy for HCC.

Keywords Hepatocellular carcinoma · Adjuvant immunotherapy · Cytokine-induced killer cells · Recurrence-free survival · Real-world evidence

Abbreviations

AFP	α-Fetoprotein
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
CI	Confidence interval
CIK	Cytokine-induced killer

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CSS	Cancer-specific survival
CT	Computed tomography
ECOG	Eastern cooperative oncology group
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HR	Hazard ratio
IQR	Interquartile range
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
NK	Natural killer
NKG2D	Natural killer group 2D
NLR	Neutrophil-to-lymphocyte ratio
OS	Overall survival
PEI	Percutaneous ethanol injection
RCT	Randomized controlled trial
RFA	Radiofrequency ablation
RFS	Recurrence-free survival
RWD	Real-world data
TACE	Transarterial chemoembolization
TCR	T-cell receptor
Treg	Regulatory T cell
TTR	Time to recurrence

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers and a leading cause of cancer-related death worldwide [1]. It accounts for the majority of primary liver cancers, particularly in Asia where chronic hepatitis B virus (HBV) infection is highly prevalent [2]. With nationwide surveillance programs, more patients are now diagnosed at an early stage and receive potentially curative treatments such as surgical resection, radiofrequency ablation (RFA), or percutaneous ethanol injection (PEI) [3]. Nonetheless, prognosis remains poor because recurrence occurs in up to 70% of patients within 5 years, and the median time to recurrence is less than 3 years. [4] This underscores the urgent need for effective adjuvant therapies to reduce recurrence and improve long-term outcomes.

However, no adjuvant therapy has been successfully established to date [5, 6]. The STORM trial showed no benefit of sorafenib compared with placebo [7], and the more recent IMbrave050 trial while initially encouraging during treatment, ultimately failed to sustain efficacy after discontinuation, resulting in an overall negative outcome with a high incidence of adverse events [8, 9]. Accordingly, current global guidelines do not recommend adjuvant treatment outside clinical trials [10–12]. This lack of durable benefit highlights the need for alternative adjuvant approaches.

By contrast, cytokine-induced killer (CIK) cells are ex vivo–expanded lymphocytes that include $CD3^+CD56^+$ NK/T-like cells with potent antitumor activity [13, 14]. Our multicenter randomized controlled trial (RCT), which followed patients for up to 2 years after enrollment of the last patient, previously demonstrated that adjuvant CIK immunotherapy significantly improved recurrence-free survival (RFS) and overall survival (OS) after curative treatment for HCC [15]. Whether such effects persist in the long term, when late recurrences and de novo tumors become more frequent, remains uncertain.

To address this, we conducted an extended 9-year follow-up of the original RCT to evaluate the durability of CIK cell immunotherapy. In parallel, we performed a large two-center real-world data (RWD) study to validate the clinical effectiveness of adjuvant CIK therapy in routine practice. By combining long-term randomized trial evidence with RWD, this study provides comprehensive insights into the durability and generalizability of CIK therapy as an adjuvant strategy for HCC.

Methods

Study populations

We conducted a two-center, retrospective RWD study at Seoul National University Hospital (Seoul, Korea) and Samsung Medical Center (Seoul, Korea). Consecutive adults with stage I–II HCC who underwent potentially curative therapy (surgical resection or RFA) were identified from institutional electronic medical records. Patients were excluded for inadequate baseline data, recurrent or extrahepatic disease at index, other active malignancies, prior liver transplantation, autoimmune or immunodeficiency disorders precluding immunotherapy, or loss to follow-up immediately after the index treatment. In total, 577 eligible patients were included and categorized into a CIK group ($n = 251$) or a control group ($n = 326$) according to receipt of adjuvant autologous CIK in routine practice (Figure S1). The study was approved by the IRB at each center.

The design and primary results of the original multicenter RCT have been described previously [15]. In the extended RCT follow-up, a total of 230 patients with stage I or II HCC who had undergone potentially curative treatment (surgical resection, RFA, or PEI) at five university-affiliated hospitals in Korea were enrolled between July 2008 and December 2010. Eligible patients had Child–Pugh class A liver function and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Key exclusion criteria included autoimmune disease, immunodeficiency, other active malignancies, or severe comorbid conditions. After screening, 226 patients were randomized in a 1:1 ratio to receive either adjuvant immunotherapy with autologous CIK cells or no adjuvant therapy (control group). The trial protocol was

approved by the institutional review board (IRB) at each participating center, and all patients provided written informed consent.

Follow-up procedures

In the RWD study, follow-up began at the date of curative treatment (index date) and continued until recurrence, death, or last visit. For the extended RCT follow-up, patients were followed until January 2020, approximately 9 years after enrollment of the last patient. The data cutoff date was set to January 2020 because of the practical challenges of maintaining long-term follow-up during the COVID-19 pandemic. Survival status and recurrence events were determined from medical records and linkage to national death registries.

Interventions

In the RWD study, exposure was defined as receipt of adjuvant autologous CIK during the planned post-curative treatment period by institutional protocols. CIK manufacturing and infusion procedures followed each center's standards. Patients who did not receive CIK constituted the control group. In the extended RCT follow-up, the immunotherapy group received 16 intravenous infusions of CIK cells (mean, 6.4×10^9 cells per infusion) over 60 weeks, consisting of four weekly doses, followed by four doses every two weeks, four doses every four weeks, and four doses every eight weeks.

Endpoints

In the RWD study, the primary endpoint was RFS, defined as the time from index date to the first documented intrahepatic or extrahepatic recurrence or death from any cause, whichever occurred first. Secondary endpoints were OS and CSS. Patients without an event were censored at the date of last follow-up. Recurrence was ascertained on dynamic contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) interpreted according to institutional standards. Causes of death were obtained from medical records.

In the extended RCT follow-up, the primary endpoint was RFS, defined as the time from randomization to the first documented recurrence or death from any cause. Secondary endpoints included OS, CSS, and time to recurrence (TTR). OS as the time from randomization to death from any cause, and CSS was defined as the time from randomization to death from HCC. TTR was defined as the time to recurrence after excluding patients who died without recurrence. Tumor recurrence was assessed by dynamic contrast-enhanced CT or MRI at 3-month intervals for the first 2 years and every 3–6 months thereafter. All imaging studies were reviewed by experienced radiologists who were blinded to treatment allocation, and any discrepancies were resolved by additional independent review.

Covariates

For the RWD study, baseline variables at the index date included demographics (age and sex), ECOG performance status, etiology of liver disease (HBV, hepatitis C virus, and the others), cirrhosis, tumor burden (number and maximum diameter), tumor stage, treatment modality, α-fetoprotein, protein induced by vitamin K absence or antagonist-II (PIVKA-II), platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, bilirubin, prothrombin time, and creatinine. The propensity score (PS) for receiving CIK was estimated using these covariates in a logistic regression model, and patients were matched 1:1 by nearest neighbor methods. Covariate balance after matching was assessed using standardized mean differences (SMDs).

Statistical analysis

Kaplan–Meier methods were used to estimate survival curves, and group comparisons were performed using log-rank tests. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox proportional hazards regression models. Prespecified subgroup analyses were performed according to baseline demographic and clinical characteristics. Statistical significance was defined as $P < 0.05$. Analyses were conducted with SAS version 9.4 (SAS Institute, Cary, NC, USA) and R version 4.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

Results

RWD of CIK therapy

Patient characteristics

In the two-center RWD study, 577 patients met eligibility criteria, including 251 in the CIK group and 323 control group. The median follow-up was 57.2 months (IQR, 35.9–82.5). Before matching, the CIK group had a higher proportion of multiple tumors (26.3% vs. 6.1%) and larger tumor size (median, 2.5 versus 2.0 cm; both $P < 0.001$). PS matching yielded 154 balanced pairs ($n = 308$) with all SMDs < 0.20 (Table 1).

Table 1 Baseline demographic and clinical characteristics of unmatched and propensity score matched groups in the real-world population

Variables	Before PS matching			After PS matching			
	Immunotherapy ($n=251$)	Control ($n=323$)	*P*	Immunotherapy ($n=154$)	Control ($n=154$)	*P*	*SMD*
Male sex, *n* (%)	190 (75.7%)	261 (80.1%)	0.25[c]	114 (74.0%)	123 (79.9%)	0.28[c]	0.09
Age, years	57.1 (50.3–63.3)	61.0 (54.0–67.7)	< 0.001[f]	58.9 (50.9–65.0)	59.2 (53.0–66.0)	0.34[d]	0.08
Treatment modality, *n* (%)			< 0.001[c]			0.09[c]	0.16
RFA	33 (13.1%)	128 (39.3%)		33 (21.4%)	47 (30.5%)		
Surgical resection	218 (86.9%)	198 (60.7%)		121 (78.6%)	107 (69.5%)		
HCC stage, *n* (%)[a]			< 0.001[c]			0.57[c]	0.08
Stage I	93 (37.1%)	228 (69.9%)		84 (54.5%)	90 (58.4%)		
Stage II	158 (62.9%)	98 (30.1%)		70 (45.5%)	64 (41.6%)		
Number of HCC, *n* (%)			< 0.001[c]			0.62[c]	0.08
1	185 (73.7%)	306 (93.9%)		131 (85.1%)	135 (87.7%)		
≥ 2	66 (26.3%)	20 (6.1%)		23 (14.9%)	19 (12.3%)		
Size of HCC, cm	2.5 (2.0–4.0)	2.0 (1.5–3.0)	< 0.001[f]	2.4 (1.6–3.7)	2.2 (1.5–3.4)	0.20[f]	0.04
Underlying liver disease, *n* (%)			0.02[e]			0.09[e]	0.19
HBV infection only	217 (86.5%)	266 (81.6%)		134 (87.0%)	123 (79.9%)		
HCV infection only	8 (3.2%)	20 (6.1%)		5 (3.2%)	9 (5.8%)		
HBV + HCV co-infection	0 (0.0%)	4 (1.2%)		0 (0.0%)	3 (1.9%)		
Others	26 (10.4%)	36 (11.0%)		15 (9.7%)	19 (12.3%)		
Cirrhosis, *n* (%)[b]	137 (54.6%)	263 (80.7%)	< 0.001[c]	99 (64.3%)	113 (73.4%)	0.11[c]	0.15
α-fetoprotein, ng/mL	3.9 (2.6–6.7)	3.8 (2.6–7.1)	0.68[f]	3.8 (2.5–6.8)	3.8 (2.6–7.3)	0.67[f]	0.14
PIVKA-II, mAU/mL	23.0 (18.0–29.5)	22.0 (17.0–28.0)	0.10[f]	22.0 (18.0–26.5)	23.0 (18.0–29.0)	0.83[f]	0.15
AST, IU/L	28.0 (22.0–36.0)	27.0 (22.0–35.0)	0.53[f]	27.0 (22.0–37.0)	26.5 (21.0–36.0)	0.41[f]	0.09
ALT, IU/L	26.0 (18.0–37.0)	24.0 (18.0–36.0)	0.46[f]	26.5 (18.0–37.0)	24.0 (16.0–42.0)	0.32[f]	0.09
Albumin, g/dL	4.3 (4.1–4.6)	4.1 (3.8–4.3)	< 0.001[f]	4.2 (3.9–4.5)	4.2 (3.8–4.4)	0.33[f]	0.13
Total bilirubin, mg/dL	0.9 (0.7–1.0)	0.9 (0.7–1.0)	0.65[f]	0.6 (0.4–1.0)	0.7 (0.5–0.8)	0.17[f]	0.15
Prothrombin time, INR	1.0 (1.0–1.1)	1.1 (1.0–1.1)	< 0.002[f]	1.0 (1.0–1.1)	1.0 (1.0–1.1)	0.43[f]	0.09
Creatinine, mg/dL	0.8 (0.8–0.9)	0.8 (0.7–0.9)	0.46[f]	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.70[f]	0.02
Platelet, $\times 10^3/mm^3$	169.0 (129.0–207.0)	177.5 (134.0–220.5)	0.21[f]	167.0 (129.0–207.0)	161.5 (130.0–214.0)	0.76[f]	0.06

Data are expressed as n (%), mean ± SE, or median (interquartile range [Q1–Q3])

RFA Radiofrequency ablation, *PEI* Percutaneous ethanol injection, *HCC* Hepatocellular carcinoma, *IQR* Interquartile range, *ECOG* Eastern cooperative oncology group, *HBV* Hepatitis B virus, *HCV* Hepatitis C virus, *PIVKA*-II Protein induced by vitamin K absence-II, *AST* Aspartate aminotransferase, *ALT* alanine aminotransferase

[a]The HCC staging was done according to AJCC staging system (8th edition)

[b]Liver cirrhosis was diagnosed by the presence of histological and/or radiological evidence

[c]By Chi-square test

[d]By two sample t test

[e]By Fisher's exact test

[f]By Wilcoxon rank sum test

Recurrence-free survival

In the unmatched cohort, the CIK group showed significantly longer RFS than control group (median, 101.2 vs. 64.7 months; HR = 0.69, 95% CI 0.53–0.90, $P = 0.006$; Fig. 1A). The RFS benefit persisted after PS matching (median, 101.2 vs. 65.7 months; HR = 0.64, 95% CI 0.45–0.91, $P = 0.01$; Fig. 1B).

Overall and cancer-specific survivals

OS did not differ significantly between groups in either the unmatched (HR = 0.96, 95% CI 0.55–1.68, $P = 0.88$) or PS-matched cohort (HR = 1.00, 95% CI = 0.48–2.08, $P = 0.99$; Figure S2). CSS likewise showed no significant between-group difference after PS matching (HR = 1.30, 95% CI = 0.51–3.31, $P = 0.58$; Figure S3).

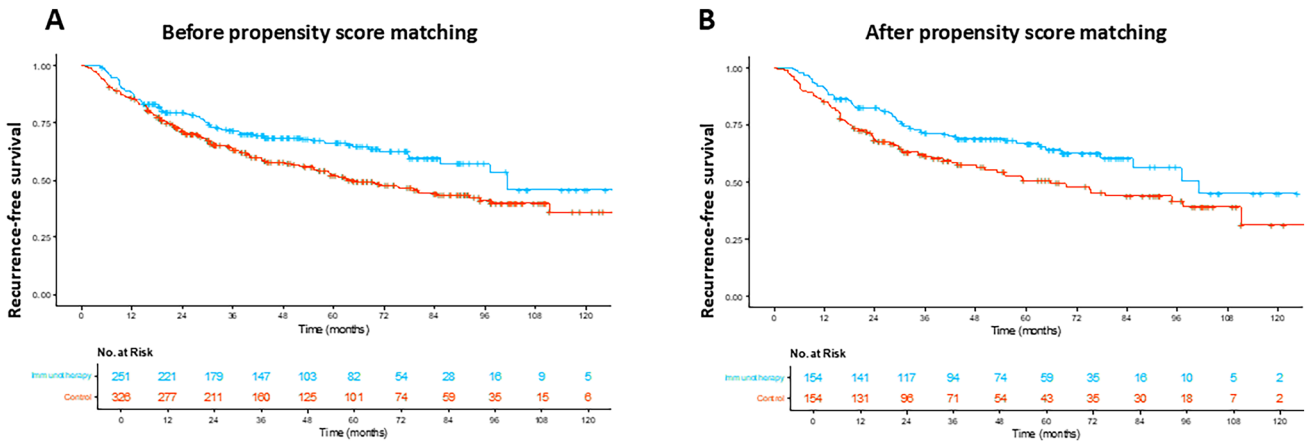

Fig. 1 Recurrence-free survival before (**A**) and after (**B**) propensity score matching in real-world population

Subgroup analyses

Subgroup analyses according to baseline clinical, tumor-related, and laboratory variables demonstrated a consistent benefit of adjuvant CIK immunotherapy over the control group for recurrence-free survival across most subgroups (Figure S4). Notably, patients with baseline liver cirrhosis showed a significant reduction in recurrence risk with adjuvant CIK therapy (HR = 0.66, 95% CI 0.48–0.91, $P = 0.01$). Similarly, a favorable effect was observed among patients with low platelet count ($< 150 \times 10^3$/μL; HR = 0.64, 95% CI 0.43–0.96, $P = 0.03$).

Adverse events Treatment-related adverse events were infrequent and mild, occurring in 9 patients (5.8%) in the immunotherapy group, while no adverse events were reported in the control group. All observed adverse events were grade 1 and included chills, fatigue, pyrexia, productive cough, vomiting, or other minor symptoms. No grade ≥ 2 adverse events, serious adverse events, or treatment discontinuations related to immunotherapy were observed (Table S1).

Long-term follow up of RCT

Patient characteristics

Of the 230 patients initially enrolled, 226 were randomized and included in the intention-to-treat population, with 114 assigned to the immunotherapy group and 112 to the control group (Figure S1B). Baseline demographic and disease characteristics were well balanced between the two groups (Table 2). The median age was 55 years, and 183 patients (81.0%) had underlying HBV infection. Tumor size was comparable between groups (median, 2.1 cm vs. 2.0 cm; $P = 0.79$), and the distribution of treatment modality did not differ significantly (surgical resection 30.2% versus 32.1%, RFA 59.6% versus 57.1%, PEI 10.2% versus 10.8%; $P = 0.64$). The median follow-up duration for the overall cohort was 116.1 months (IQR, 77.5–130.5), including 118.2 months in the immunotherapy group and 114.0 months in the control group.

Recurrence-free survival

During the 9-year follow-up, tumor recurrence or death occurred in 67 of 114 patients (58.8%) in the immunotherapy group and in 78 of 112 patients (69.6%) in the control group. The median RFS was significantly longer in the immunotherapy group (44.0 months) than in the control group (30.0 months) (HR = 0.72, 95% CI 0.54–0.97, $P = 0.033$; Fig. 2A). The RFS rates at 9 years was 25.4% in the immunotherapy group, compared with 16.1% in the control group.

Time-to-recurrence

After excluding patients who died without recurrence, TTR was significantly longer in the immunotherapy group than in the control group (median, 45.7 vs. 30.6 months; HR = 0.71, 95% CI 0.52–0.96; $P = 0.028$; Fig. 2B).

Cancer-specific and overall survival

At the time of data cutoff, 28 patients in the immunotherapy group and 39 in the control group had died. CSS was significantly improved in the immunotherapy group (HR = 0.49, 95% CI 0.25–0.95, $P = 0.036$; Fig. 3A). The 9-year CSS rates were 64.9% in the immunotherapy group and 59.8% in the control group. OS also favored the immunotherapy group, although the difference did not reach statistical significance

Table 2 Baseline demographic and clinical characteristics in the RCT population

Variables	Immunotherapy ($n=114$)	Control ($n=112$)	P
Male sex, *n* (%)	95 (83.3%)	91 (81.3%)	0.68[e]
Age, years	55.4 ± 8.2	56.4 ± 10.6	0.41[f]
Treatment modality, *n* (%)			0.06[g]
PEI	13 (11.4%)	4 (3.6%)	
RFA	69 (60.5%)	70 (62.5%)	
Surgical resection	32 (28.1%)	38d (33.9%)	
HCC stage, *n* (%)[a]			0.67[e]
Stage I	98 (86.0%)	94 (83.9%)	
Stage II	16 (14.0%)	18 (16.1%)	
Number of HCC, *n* (%)			0.98[e]
< 3	112 (98.2%)	110 (98.2%)	
≥ 3	2 (1.8%)	2 (1.8%)	
Size of HCC, cm	1.8 (1.4–2.3)	2.3 (1.5–3.1)	0.03[h]
ECOG status, *n* (%)[b]			0.83[e]
0	81 (71.1%)	81 (72.3%)	
1	33 (28.9%)	31 (27.7%)	
Underlying liver disease, *n* (%)			0.87[g]
HBV infection only	96 (84.2%)	90 (80.4%)	
HCV infection only	9 (7.9%)	10 (8.9%)	
HBV + HCV co-infection	2 (1.8%)	2 (1.8%)	
Others	7 (6.1%)	10 (8.9%)	
Cirrhosis, *n* (%)[c]	76 (66.7%)	70 (62.5%)	0.51[e]
α-fetoprotein, ng/mL	5.2 (3.1–9.9)	5.4 (3.0–13.0)	0.56[h]
PIVKA-II, mAU/mL	19.0 (14.0–24.8)	18.0 (14.0–24.0)	0.96[h]
AST, IU/L	33.0 (27.0–43.5)	34.0 (26.8–44.0)	0.87[h]
ALT, IU/L	33.0 (25.0–45.8)	33.0 (23.0–47.5)	0.55[h]
ALP, IU/L	82.5 (70.0–101.5)	82.0 (65.0–100.0)	0.45[h]
Albumin, g/dL	4.1 (3.9–4.3)	4.1 (3.9–4.3)	0.99[h]
Total bilirubin, mg/dL	0.8 (0.6–1.0)	0.8 (0.6–1.0)	0.71[h]
Prothrombin time, s	13.7 (13.1–14.7)	13.9 (13.2–14.4)	0.74[h]
Creatinine, mg/dL	0.9 (0.8–1.0)	0.9 (0.7–1.0)	0.86[h]
Platelet, $\times 10^3/mm^3$	116.5 (92.3–158.0)	141.0 (117.5–166.3)	0.01[h]

Data are expressed as n (%), mean ± SE, or median (interquartile range [Q1–Q3])

RCT Randomized controlled trial, *RFA* Radiofrequency ablation, *PEI* Percutaneous ethanol injection, *HCC* Hepatocellular carcinoma, *IQR* Interquartile range, *ECOG* Eastern cooperative oncology group, *HBV* Hepatitis B virus, *HCV* Hepatitis C virus, *PIVKA*-II Protein induced by vitamin K absence-II, *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase, *ALP* Alkaline phosphatase

[a]The HCC staging was done according to AJCC staging system (6th edition)

[b]The ECOG performance status assesses the daily living abilities of the patient, on a scale ranging from 0 (fully active) to 5 (dead)

[c]Liver cirrhosis was diagnosed by the presence of histological and/or radiological evidence

[d]Two of them underwent intrahepatic RFA in addition to surgical resection

[e]By Chi-square test

[f]By two sample t test

[g]By Fisher's exact test

[h]By Wilcoxon rank sum test

(HR = 0.70, 95% CI 0.44–1.11, $P = 0.13$; Fig. 3B). The 9-year OS rates were 64.0% in the immunotherapy group

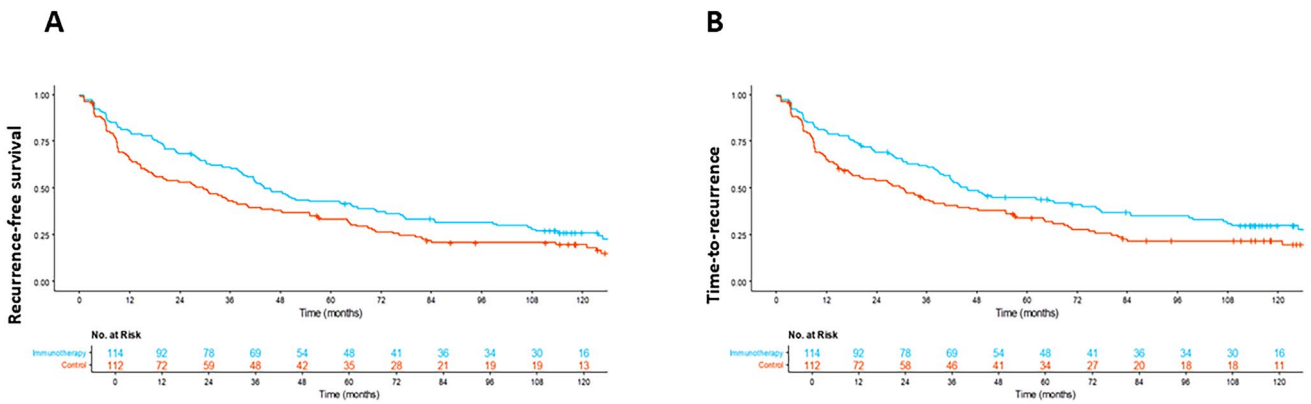

Fig. 2 Recurrence-free survival (**A**) and time to recurrence (**B**) in the RCT population

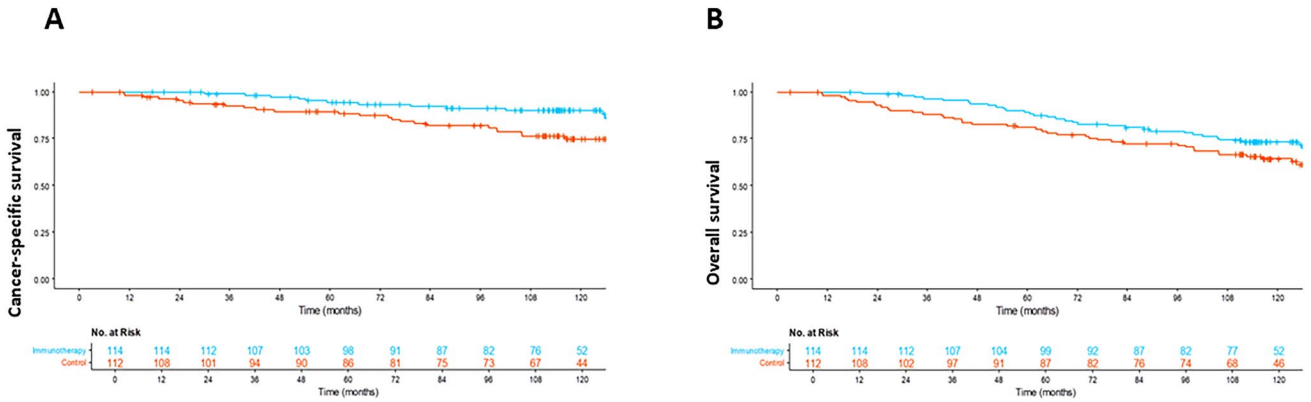

Fig. 3 Cancer-specific survival (**A**) and overall survival (**B**) in the RCT population

and 56.3% in the control group. Of all deaths, 51.0% were unrelated to HCC.

Post-recurrence treatment

After recurrence, patients received either curative or non-curative therapies. Curative treatments were administered in 41 of 83 patients (49.4%) in the immunotherapy group and 41 of 87 patients (47.1%) in the control group. Non-curative treatments were given in 41 patients (49.4%) and 43 patients (49.4%), respectively. The distribution of post-recurrence therapies did not differ significantly between the two groups ($P > 0.05$, Table 3).

Discussion

In this 9-year extended RCT follow-up, adjuvant immunotherapy with autologous CIK cells after curative treatment for HCC continued to demonstrate significant improvements in RFS and CSS, findings that were further supported by

Table 3 Post-recurrence treatment modalities in the RCT population

	Immunotherapy ($n=114$)	Control ($n=112$)
Recurrence	83 (72.8%)	87 (77.7%)
Non-recurrence	31 (27.2%)	25 (22.3%)
Curative treatment	41 (49.4%)	41 (47.1%)
Surgical resection	6 (7.2%)	12 (13.8%)
Liver transplantation	1 (1.2%)	1 (1.1%)
RFA	25 (30.1%)	22 (25.3%)
PEI	9 (10.8%)	6 (6.9%)
Non-curative treatment	41 (49.4%)	43 (49.4%)
RT	1 (1.2%)	1 (1.1%)
TACE	34 (41.0%)	37 (42.5%)
TACE + RT	1 (1.2%)	0 (0.0%)
TACE + RFA	3 (3.6%)	3 (3.4%)
Systemic treatment[a]	2 (2.4%)	2 (2.3%)
Best supportive care	1 (1.2%)	3 (3.4%)

RFA Radiofrequency ablation, *PEI* Percutaneous ethanol injection, *TACE* Transarterial chemoembolization, *RT* radiotherapy

[a]sorafenib, atezolizumab plus bevacizumab

real-world evidence, thereby demonstrating long-term efficacy. In contrast, several clinical trials investigating adjuvant treatments for HCC have failed to improve RFS. Specifically, although atezolizumab plus bevacizumab in the IMbrave050 trial appeared effective in the interim analysis, it ultimately failed to sustain its benefit after completion of the 12–month treatment period as per the study protocol [7, 9]. Moreover, CIK cell therapy was associated with a favorable safety profile with low rates of serious adverse events and no treatment discontinuations, in contrast to the higher incidence of treatment-related toxicities and even mortality observed with atezolizumab plus bevacizumab [8]. While caution is required in making cross-trial comparisons, our results suggest that adoptive immunotherapy using CIK cells may offer a unique advantage in this clinical setting [15, 16].

The RWD study further provides external validation of these findings in routine practice settings, including cases with suspected unfavorable features before or after curative treatment. Despite less favorable baseline characteristics (e.g., a higher proportion of multiple tumors [26.3% vs. 6.1%] and larger median tumor size [2.5 vs. 2.0 cm], both $P < 0.001$) in patients who received CIK, the RFS advantage remained significant both before and after PS matching, with effect sizes comparable to those observed in the extended RCT follow-up. By demonstrating consistent RFS benefit across methodologically distinct settings, the combined evidence supports the conclusion that CIK meaningfully reduces the risk of post-curative recurrence. Importantly, subgroup analyses demonstrated a sustained RFS benefit in patients with baseline liver cirrhosis, a clinical condition closely associated with late recurrence, indirectly supporting a long-term antitumor effect of adjuvant CIK therapy beyond early postoperative tumor control.

The sustained efficacy may reflect CIK cells' immunologic properties, which enable durable immune surveillance beyond treatment, unlike other adjuvant treatment candidates whose effects wane after treatment discontinuation. In our preliminary immune-profiling study, patients who received repeated CIK transfers exhibited a significant expansion of $CD8^+$ classical memory T cells. These long-lived memory populations can rapidly activate upon antigen re-encounter and are known to mediate durable tumor-specific immune responses [17–21]. The enrichment of memory T cells thus provides a plausible mechanistic explanation for the continued reduction in recurrence and cancer-related death beyond the treatment period.

CIK cells, particularly the $CD3^+CD56^+$ NK/T-like subset, contribute to antitumor immunity through both direct tumor eradication and immune orchestration [22–24]. These heterogeneous effector populations exert MHC-unrestricted cytotoxicity by releasing perforin and granzymes through poly-perforin channels and by engaging NK-like receptors such as NKG2D, which recognize stress ligands on tumor cells, thereby inducing apoptosis even when classical antigen presentation is impaired [25]. This activity is mediated by cytotoxic pathways involving perforin, granzymes, and Fas–FasL interactions, thereby bypassing immune evasion caused by tumor downregulation of MHC-I [26]. In addition, CIK cells have been shown in preclinical studies to effectively target and eliminate cancer stem-like cells, which represent highly resistant subpopulations implicated in recurrence and therapeutic failure [27, 28]. By eliminating these reservoirs of disease and overcoming established mechanisms of immune escape, CIK therapy provides a strong biological rationale for its long-term antitumor efficacy. Beyond direct cytotoxicity, CIK therapy also enhances adaptive immunity by expanding $CD8^+$ classical memory T cells which are theoretically capable of mounting durable antitumor responses [29]. In the clinical setting of adjuvant therapy, previous evidence has consistently demonstrated that CIK therapy exerts its most pronounced effects in the early post-treatment period, effectively reducing recurrence driven by residual disease or micrometastases [30–32]. Similar patterns have been observed in other cancer types currently under investigation with adjuvant CIK therapy [33–35], as well as in trials employing CIK cells manufactured under alternative protocols, reinforcing the robustness of this short-term benefit [36, 37]. However, whether CIK therapy could also prevent late recurrence arising de novo has long been a matter of debate, with limited data to support such an effect. In this context, the present long-term follow-up and RWD provides compelling evidence that CIK therapy not only mitigates early recurrence but may also confer protection against late recurrence, thereby highlighting its potential to achieve durable long-term disease control beyond the immediate treatment window. Together, these dual mechanisms, namely the elimination of minimal residual disease and the induction of durable immune surveillance, likely account for the long-term improvement in RFS and CSS observed in our study. Future immunologic studies, including single-cell transcriptomics and T-cell receptor profiling, will be essential to clarify the mechanisms of CIK-induced immune remodeling and its role in sustaining long-term tumor control. [38, 39]

Our study has limitations. First, the RWD study is retrospective and, despite PS matching, remains susceptible to unmeasured confounding and treatment-selection bias. Second, heterogeneity in recurrence patterns and subsequent treatments after recurrence may also influence long-term survival outcomes, necessitating more granular analyses beyond survival comparisons and incorporating immunologic evaluations stratified by recurrence patterns.

In summary, adjuvant CIK therapy after curative treatment for early-stage HCC provided sustained improvements in RFS over 9 years of RCT follow-up and showed consistent RFS benefits in real-world practice. These converging data

support the clinical utility of CIK as an adjuvant strategy to reduce recurrence. Future pragmatic trials and translational immunology studies are warranted to elucidate the link between clinical benefit and underlying mechanisms, refine patient selection, and define optimal integration with contemporary post-recurrence treatments, thereby advancing an immunologically grounded adjuvant approach for HCC.

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Author contributions J.-H.L. had full access to all of the data of this study and took responsibility for the integrity of the data and the accuracy of the data analysis. H.S. collected the data and performed the statistical analysis. Y.P., B.G.S., W.M.C., H.J.H., Y.L., T.J.S., J.E.Y., Y.S.L., J.H.L., M.H.H., Y.B.L., E.J.C., S.J.Y., Y.J.K., and J.H.Y collected and reviewed the data. H.S. and J.-H.L. wrote the manuscript with comments from all authors.

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Data availability The data that support the findings of this study are not publicly available but are available from the corresponding author upon request.

Declarations

Conflict of interest Yun Bin Lee: Receives research grants from Samjin Pharmaceuticals and Yuhan Pharmaceuticals; Su Jong Yu: Receives research grants from Yuhan Pharmaceuticals and Daewoong Pharmaceuticals; Yoon Jun Kim: Receives research grants from BTG, Boston Scientific, AstraZeneca, Gilead Sciences, Samjin, BL&H, and Bayer, and lecture fees from Roche, Abbvie, Eisai, Boston Scientific, BMS, BTG, Bayer, MSD, Novo Nordisk, GC Cell, Boehringer Ingelheim, and Gilead Sciences; Jung-Hwan Yoon: Receives research grants from Bayer, Daewoong Pharmaceuticals, and Bukwang Pharmaceutical; Jeong-Hoon Lee: Receives research grants from Yuhan Pharmaceuticals and GC Cell, and lecture fees from GC Cell, Daewoong Pharmaceuticals, and Gilead Korea; All other authors: Nothing to declare.

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