Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Prognosis of Interventional Therapy Combined with Targeted Therapy and Immune Checkpoint Inhibitors in Patients with Recurrent Dual-Phenotype versus Conventional Hepatocellular Carcinoma: A Retrospective Cohort Study

ShenPing Hu
Women and Children's Hospital, School of Medicine, Xiamen University
PeiPei Li
Xiamen Hospital of Traditional Chinese Medicine
XiWang Lin
Xiamen Hospital of Traditional Chinese Medicine
Zhenyu Yin
Zhongshan Hospital of Xiamen University
Heping Zhu
chinaxm.happy@163.com

Xiamen Hospital of Traditional Chinese Medicine

Research Article

Keywords: dual-phenotype hepatocellular carcinoma, hepatocellular carcinoma, Local Treatment, Systemic Therapy, Recurrent

Posted Date: November 18th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11527037/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Dual-phenotype hepatocellular carcinoma (DPHCC) is a rare subtype of HCC that co-expresses hepatocellular and cholangiocellular immunohistochemical markers and exhibits high aggressiveness. Evidence regarding the prognosis of patients with postoperative recurrent DPHCC receiving interventional therapy combined with targeted therapy and immune checkpoint inhibitors (ICIs) remains scarce.

Methods

In this retrospective cohort study, 35 patients with postoperative recurrent HCC treated at Xiamen Hospital of Traditional Chinese Medicine between January 2019 and March 2026 were analyzed; all received interventional therapy (transarterial chemoembolization [TACE] or hepatic arterial infusion chemotherapy [HAIC]) combined with targeted therapy and ICIs. Patients were classified into a DPHCC group (n = 17) and a conventional HCC group (n = 18) according to pathological immunohistochemical findings. The primary endpoints were post-recurrence overall survival (OS) and progression-free survival (PFS). Survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using Cox proportional hazards models. Restricted mean survival time (RMST) was calculated to complement median survival estimates.

Results

Post-recurrence OS was significantly shorter in the DPHCC group than in the conventional group (median, 18.2 vs. 36.8 months; HR, 2.75; 95% CI, 1.15–6.59; P = 0.019), and the RMST for OS at 12 months was correspondingly shorter in the DPHCC group. Post-recurrence PFS also tended to be shorter in the DPHCC group (median, 4.6 vs. 9.8 months; HR, 1.78; 95% CI, 0.86–3.68; P = 0.120).

Conclusions

Among patients with postoperative recurrent HCC treated with interventional therapy combined with targeted therapy and ICIs, the dual-phenotype subtype was associated with poorer post-recurrence survival. DPHCC may represent a high-risk subtype requiring closer surveillance and more aggressive systemic treatment strategies after postoperative recurrence.

Introduction

- Dual-phenotype hepatocellular carcinoma (DPHCC) is a rare subtype of HCC that co-expresses hepatocellular and cholangiocellular immunohistochemical markers, accounting for approximately 10% of all HCC cases. Unlike classic combined hepatocellular–cholangiocarcinoma (cHCC-CCA), DPHCC retains a predominantly hepatocellular morphology on histological examination, yet immunohistochemical staining reveals aberrant expression of cholangiocellular markers such as CK19, CK7, and EpCAM [1]. Previous studies have shown that DPHCC is associated with higher rates of microvascular invasion, a greater tendency toward satellite lesion formation, and more aggressive biological behavior, with significantly inferior postoperative overall survival and recurrence-free survival compared with conventional HCC [2].
- At present, evidence on systemic therapy for DPHCC is extremely limited. Previous studies have focused mainly on the preoperative imaging diagnosis and postoperative prognosis of DPHCC, whereas the efficacy and survival outcomes of interventional therapy combined with targeted and immunotherapy in patients with postoperative recurrence of this subtype have not been reported. Given the unique biological features of DPHCC, its response to interventional therapy combined with targeted and immunotherapy may differ from that of conventional HCC: on the one hand, the cholangiocytic phenotype in DPHCC may confer distinct immune response characteristics to ICIs; on the other hand, its heightened aggressiveness may lead to earlier treatment resistance and progression under combination therapy. Therefore, elucidating the prognosis of DPHCC under the interventional plus targeted and immunotherapy paradigm is of considerable clinical importance for individualized treatment decisions in this high-risk subtype.
- Against this background, we retrospectively analyzed 35 patients with postoperative recurrent HCC who received interventional therapy combined with targeted agents and ICIs at our center. Patients were assigned to a dual-phenotype group or a conventional group according to pathological immunohistochemical findings, with the aim of comparing post-recurrence OS and PFS between the two groups and preliminarily exploring the impact of the dual-phenotype subtype on prognosis in patients with postoperative recurrence in the era of combination therapy.

Material and methods

1.1 Study Subjects

The inclusion criteria were as follows: (1) initial curative-intent hepatectomy performed at our center with tumor recurrence within 2 years after surgery; (2) histopathological confirmation of HCC on postoperative examination; (3) age 18–80 years, good performance status, and no major organ dysfunction; and (4) Child–Pugh class A or B liver function at recurrence.

The exclusion criteria were as follows: (1) receipt of any anticancer treatment other than interventional therapy and targeted/immunotherapy after recurrence; (2) a history of other malignancies; (3) incomplete clinical data; and (4) an Eastern Cooperative Oncology Group performance status (ECOG PS) score > 2 at recurrence.

The diagnosis of DPHCC was based on histopathological criteria. Briefly, the tumor had to fulfill both of the following conditions: (1) morphological features consistent with HCC; and (2) immunohistochemical evidence of dual-phenotype differentiation—that is, within the same lesion, more than 15% of tumor cells co-expressing at least one cholangiocellular marker (e.g., MUC1, CK7, or CK19) and at least one hepatocellular marker (e.g., arginase-1, HepPar-1, glypican-3.

1.2 Treatment

All patients with postoperative recurrence received TACE/HAIC combined with targeted and immunotherapy. All TACE/HAIC procedures were performed under sterile conditions via a common femoral artery approach using the Seldinger technique. Under digital subtraction angiography (DSA) fluoroscopic guidance, a microcatheter was advanced into the suspected feeding arteries for superselective catheterization. For TACE, a chemotherapeutic emulsion containing lobaplatin, epirubicin, and raltitrexed together with lipiodol (3–5 mL) was injected into the target artery, followed by embolization. For HAIC, fluorouracil and oxaliplatin were continuously infused into the target artery.

Targeted anti-angiogenic agents included lenvatinib, apatinib, regorafenib, and bevacizumab; ICIs included tislelizumab, camrelizumab, and sintilimab. All agents were dosed according to body weight and administered in repeated cycles (treatment schedule, Fig. 2).Contrast-enhanced abdominal CT/MRI was performed approximately 3–4 weeks after TACE to evaluate tumor viability and determine whether repeat TACE/HAIC was required.

1.3 Outcomes and follow-up

The primary endpoints were post-recurrence OS and PFS. Post-recurrence OS was defined as the time from the initiation of treatment to death from any cause or the last follow-up; post-recurrence PFS was defined as the time from the start of treatment to the date of confirmed tumor progression, death, or the last follow-up.

After the initiation of treatment, patients were followed up monthly through outpatient visits or telephone contact to document disease progression or death. Each follow-up visit included a physical examination, laboratory tests (complete blood count; liver and renal function tests; tumor markers), and imaging examinations (chest X-ray or CT; contrast-enhanced abdominal CT or MRI). When tumor markers were stable or declining, contrast-enhanced abdominal CT/MRI was performed every 3 months. Suspected tumor progression was confirmed by appropriate imaging examinations, with biopsy when necessary. The follow-up cutoff date was September 1, 2026; patients who died before this date were followed until death.

1.4 Statistical analysis

Continuous variables are presented as medians with interquartile ranges (IQRs) and were compared using the Mann–Whitney U test. Categorical variables are presented as counts and percentages and were compared using Fisher's exact test or the χ^2 test. Survival curves were estimated using the Kaplan–

Meier method, and between-group differences were compared using the log-rank test. Cox proportional hazards regression was used to estimate HRs and their 95% CIs to assess the effect of the dual-phenotype subtype on post-recurrence survival. In addition, the RMST was calculated to complement the median survival estimates and to quantify the between-group difference in average survival over a fixed time window. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using Python (Lifelines, Matplotlib, and Pandas libraries).

Results

As of September 1, 2026, a total of 35 patients with postoperative recurrent HCC were included: 17 with DPHCC and 18 with conventional HCC. Baseline clinical characteristics were generally comparable between the two groups; however, the DPHCC group had a younger mean age, a lower proportion of patients with intermediate/advanced-stage disease before surgery, and lower mean des-γ-carboxy prothrombin (PIVKA-II) levels at recurrence (Table 1).

Table 1
Baseline characteristics of patients with recurrent dual-phenotype HCC and conventional HCC

Characteristic	Dual-phenotype HCC (n = 17)	Conventional HCC (n = 18)	P value
Age, years	49.0 (38.0–63.0)	55.0 (47.8–68.2)	0.030
Largest tumour diameter before resection, cm	7.5 (4.6–9.5)	7.5 (5.2–12.0)	0.509
Serum AFP, ng/mL	192.8 (21.6–1522.0)	18.7 (3.6–115.0)	0.077
Serum PIVKA-II, mAU/mL	22.0 (16.0–63.0)	91.0 (46.2–1247.0)	0.003
Serum CA19-9, U/mL	12.0 (8.5–29.2)	9.1 (6.3–14.2)	0.216
Serum CEA, ng/mL	2.5 (1.7–3.3)	2.2 (1.6–3.2)	1.000
Recurrence tumour diameter, cm	2.2 (1.8–4.0)	3.2 (2.1–4.5)	0.498
Sex			0.088
Male	12 (70.6)	17 (94.4)	
Female	5 (29.4)	1 (5.6)	
Hepatitis B virus infection			1.000
Yes	15 (88.2)	16 (88.9)	
No	2 (11.8)	2 (11.1)	
Preoperative conversion / neoadjuvant therapy			0.489
Yes	7 (41.2)	5 (27.8)	
No	10 (58.8)	13 (72.2)	
Macrovascular invasion			0.443
Yes	5 (29.4)	3 (16.7)	
No	12 (70.6)	15 (83.3)	
Microvascular invasion (pathological)			0.305
Yes	12 (70.6)	9 (50.0)	

Note: Continuous variables: median (IQR), Mann-Whitney U test;. Categorical variables: n (%), Fisher exact (2x2) or chi-square (> 2x2); shown stratified by category. P < 0.05 significant (bold).

HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; CA19-9, carbohydrate antigen 19 – 9; CEA, carcinoembryonic antigen; MVI, microvascular invasion; PS, performance status; IQR, interquartile range.

Characteristic	Dual-phenotype HCC (n = 17)	Conventional HCC (n = 18)	P value
No	5 (29.4)	9 (50.0)	
Distant metastasis at recurrence			0.711
Yes	5 (29.4)	4 (22.2)	
No	12 (70.6)	14 (77.8)	
Number of tumours at baseline			0.658
Solitary	15 (88.2)	14 (77.8)	
Multiple	2 (11.8)	4 (22.2)	
Number of intrahepatic recurrence			0.733
Solitary	6 (35.3)	8 (44.4)	
Multiple	11 (64.7)	10 (55.6)	
Preoperative BCLC stage			0.031
Stage A	9 (52.9)	4 (22.2)	
Stage B	3 (17.6)	11 (61.1)	
Stage C	5 (29.4)	3 (16.7)	
BCLC stage at recurrence			0.876
Stage A	4 (23.5)	3 (16.7)	
Stage B	8 (47.1)	9 (50.0)	
Stage C	5 (29.4)	6 (33.3)	
Child-Pugh class at recurrence			1.000
Class A	13 (76.5)	14 (77.8)	
Class B	4 (23.5)	4 (22.2)	
ECOG PS at recurrence			1.000
PS 1	15 (88.2)	16 (88.9)	

Note: Continuous variables: median (IQR), Mann-Whitney U test;. Categorical variables: n (%), Fisher exact (2x2) or chi-square (> 2x2); shown stratified by category. P < 0.05 significant (bold).

HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; CA19-9, carbohydrate antigen 19 – 9; CEA, carcinoembryonic antigen; MVI, microvascular invasion; PS, performance status; IQR, interquartile range.

Characteristic	Dual-phenotype HCC (n = 17)	Conventional HCC (n = 18)	P value
PS 2	2 (11.8)	2 (11.1)	
Note: Continuous variables: median (IQR), Mann-Whitney U test;. Categorical variables: n (%), Fisher exact (2x2) or chi-square (> 2x2); shown stratified by category. P < 0.05 significant (bold).			
HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; CA19-9, carbohydrate antigen 19 – 9; CEA, carcinoembryonic antigen; MVI, microvascular invasion; PS, performance status; IQR, interquartile range.			

The median post-recurrence OS was 18.2 months in the DPHCC group and 36.8 months in the conventional group(Fig. 3), and the difference was statistically significant (HR, 2.75; 95% CI, 1.15–6.59; P = 0.019) (Table 2). The RMST for OS was shorter in the DPHCC group, indicating that the average survival benefit during the first year after recurrence was substantially lower in patients with DPHCC than in those with conventional HCC.

Table 2
Univariate Cox Regression Analysis.

OS median	Conventional HCC(n = 18)	Dual-phenotypeHCC(n = 17)	P value	HR(95%CI)
	36.8 months	**18.2 months**	**0.019**	**2.75 (1.15–6.59),P = 0.023**
PFS median	9.8 months	4.6 months	0.120	1.78 (0.86–3.68),P = 0.117
Univariate Cox regression analysis showed that DPHCC was significantly associated with worse OS (HR = 2.75, 95% CI: 1.15–6.59, p = 0.023). For PFS, DPHCC showed a trend toward worse outcomes (HR = 1.78, 95% CI: 0.86–3.68, p = 0.117), which did not reach statistical significance, likely due to the small sample size.				

The median post-recurrence PFS was 4.6 months in the DPHCC group and 9.8 months in the conventional group(Fig. 4); the difference did not reach statistical significance (HR, 1.78; 95% CI, 0.86–3.68; P = 0.120). Nevertheless, in the RMST analysis, the DPHCC group still showed a shorter progression-free time than the conventional group, suggesting a clinically meaningful trend toward a difference in PFS between the two groups(Fig. 5).

Discussion

In this study, we retrospectively analyzed the survival outcomes of 35 patients with postoperative recurrent HCC who received interventional therapy combined with targeted agents and ICIs. Post-recurrence OS in the dual-phenotype group was significantly shorter than that in the conventional group (median, 18.2 vs. 36.8 months; HR, 2.75; P = 0.019), and PFS also showed a trend toward shortening (median, 4.6 vs. 9.8 months; HR, 1.78; P = 0.120).

For recurrent conventional HCC, the triplet regimen of interventional therapy combined with targeted and immunotherapy has accumulated substantial evidence. A retrospective study of 88 patients with recurrent HCC demonstrated that a triplet regimen of TACE combined with molecular targeted agents and camrelizumab achieved a median PFS of 14.0 months and a median OS of 31.1 months [3]. In our study, the median PFS (9.8 months) and median OS (36.8 months) in the conventional group were broadly consistent with these previously reported data, indirectly validating the efficacy of the interventional plus targeted and immunotherapy regimen in the post-recurrence setting. In contrast, the median PFS (4.6 months) and median OS (18.2 months) in the dual-phenotype group were markedly inferior to these historical benchmarks, suggesting that even under a potent combination regimen, this subtype fails to achieve survival benefits comparable to those of conventional HCC.

In our cohort, DPHCC was less sensitive to interventional therapy and combined targeted and immunotherapy than conventional HCC. This may be because the dual-phenotype features endow the tumor with greater proliferative and metastatic potential, higher chemoresistance, and a higher propensity for vascular invasion [4]; these properties are likely related to the high expression of CK19 in DPHCC. Lai et al. [5] examined surgical specimens from 57 patients with HCC treated with TACE and found that tumor cells within TACE-treated areas overexpressed the hypoxia marker carbonic anhydrase IX (CAIX) and strongly expressed CK19, whereas tumor cells in untreated areas expressed neither CAIX nor CK19, suggesting that CK19-positive DPHCC cells may better tolerate the hypoxic environment induced by TACE. Similarly, Zhuo et al. [6], investigating subsequent treatment in patients with recurrent HCC after liver transplantation, found that only regorafenib suppressed the growth and induced the apoptosis of CK19 + cells, whereas sorafenib, apatinib, and 5-fluorouracil showed no such effect. Further studies have demonstrated that CK19-positive HCC cells highly express ATP-binding cassette (ABC) transporters, which actively pump a variety of drugs out of cells and constitute an important mechanism of resistance to multiple chemotherapeutic and targeted agents [7]. Moreover, CK19-positive HCC cells highly express immunosuppressive molecules such as PD-L1 and TGFB1 and display an exhausted phenotype, thereby hindering the infiltration of CD8 + T cells and other immune cells and substantially attenuating the efficacy of ICIs [8].

The findings of this study have several implications for clinical practice. First, for patients with pathologically confirmed dual-phenotype HCC, clinicians should be fully aware of the high-risk nature of this subtype. Even when postoperative recurrence is treated actively with the triplet regimen of interventional therapy plus targeted and immunotherapy, survival may still be significantly inferior to that of conventional HCC; hence, closer surveillance and earlier, more frequent treatment evaluation are warranted. Second, when formulating postoperative adjuvant treatment strategies, the dual-phenotype subtype can serve as an important basis for risk stratification. For patients with DPHCC, adjuvant therapy may need to go beyond the current standard regimens and incorporate more targeted treatment combinations.

This study has several limitations. First, as a single-center retrospective study with a small sample size (n = 35), the statistical power was limited, and some differences (e.g., PFS) may have failed to reach

statistical significance because of the limited sample size. Second, selection bias inherent in the retrospective design cannot be entirely excluded; although baseline characteristics were generally comparable between the groups, unknown confounders may still have influenced the results. Third, molecular pathological analyses of tumor tissues (e.g., mutational profiles, tumor mutational burden, and features of the immune microenvironment) were not performed; therefore, the underlying mechanisms of the differential response of the dual-phenotype subtype to combination therapy could not be elucidated at the molecular level. Fourth, the specific modality of interventional therapy (TACE, HAIC, or both) and the types of targeted agents and ICIs varied among patients, and this heterogeneity in treatment regimens may have affected the precision of the results to some extent.

Conclusion

In conclusion, to the best of our knowledge, this is the first study to report that among patients with postoperative recurrent HCC treated with interventional therapy combined with targeted agents and ICIs, the dual-phenotype subtype is associated with significantly worse post-recurrence OS and a trend toward shorter PFS. These findings suggest that DPHCC may represent a high-risk subtype with a poor prognosis, facing formidable survival challenges even in the era of combination therapy. In clinical practice, patients with postoperative recurrence of DPHCC should undergo closer surveillance, and more aggressive systemic treatment strategies should be considered for post-recurrence therapy.

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Funding

This research was funded by Fujian Provincial Natural Science Foundation Project (2023D001) and Xiamen Health Guidance Project (3502Z20244D1191).

Acknowledgments

Not applicable

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Approval

The present retrospective study was approved by the Ethics Committee of Xiamen Hospital of Traditional Chinese Medicine (approval number: 2024-K034-01). The study was conducted in accordance with the principles of the Declaration of Helsinki and local legislative and institutional requirements. The Ethics Committee formally waived the requirement for written informed consent for this retrospective study, given its retrospective design based on anonymized clinical data that poses minimal risk to participants.

References

1. Lu X, Xi T, Lau WY, et al. Hepatocellular carcinoma expressing cholangiocyte phenotype is a novel subtype with highly aggressive behavior. Ann Surg Oncol. 2011;18(8):2210–7. 10.1245/s10434-011-1585-7.
2. Yu ZC, Fang ZK, Yu Y, et al. The clinical characteristics, patterns of recurrence, and long-term survival outcomes of dual-phenotype hepatocellular carcinoma after curative liver resection. J Hepatocell Carcinoma. 2025;12:183–92. 10.2147/JHC.S493094.
3. Hou C, Xiong B, Zhou L, et al. Transarterial chemoembolization with molecular targeted therapies plus camrelizumab for recurrent hepatocellular carcinoma. BMC Cancer. 2024;24:387. 10.1186/s12885-024-12136-2.
4. Lee TK, Guan XY, Ma S. Cancer stem cells in hepatocellular carcinoma—from origin to clinical implications. Nat Rev Gastroenterol Hepatol. 2022;19(1):26–44. 10.1038/s41575-021-00508-3.
5. Lai J, Conley A, Knudsen B, et al. Hypoxia after transarterial chemoembolization may trigger a progenitor cell phenotype in hepatocellular carcinoma. Histopathology. 2015;67(4):442–50. 10.1111/his.12623.
6. Zhuo J, Lu D, Lin Z, et al. The distinct responsiveness of cytokeratin 19-positive hepatocellular carcinoma to regorafenib. Cell Death Dis. 2021;12(12):1084. 10.1038/s41419-021-04320-4.
7. Govaere O, Komuta M, Berkers J, et al. Keratin 19: a key role player in the invasion of human hepatocellular carcinomas. Gut. 2014;63(4):674–85. 10.1136/gutjnl-2012-304351.
8. Zhang XF, Zuo XY, Zhu Y, et al. Targeting cell-state plasticity driven by FOXM1/CEBPB axis disrupts developmental heterogeneity and therapeutic resistance in hepatocellular carcinoma. J Hepatol. 2026;84(2):370–84. 10.1016/j.jhep.2025.09.022.

Figures

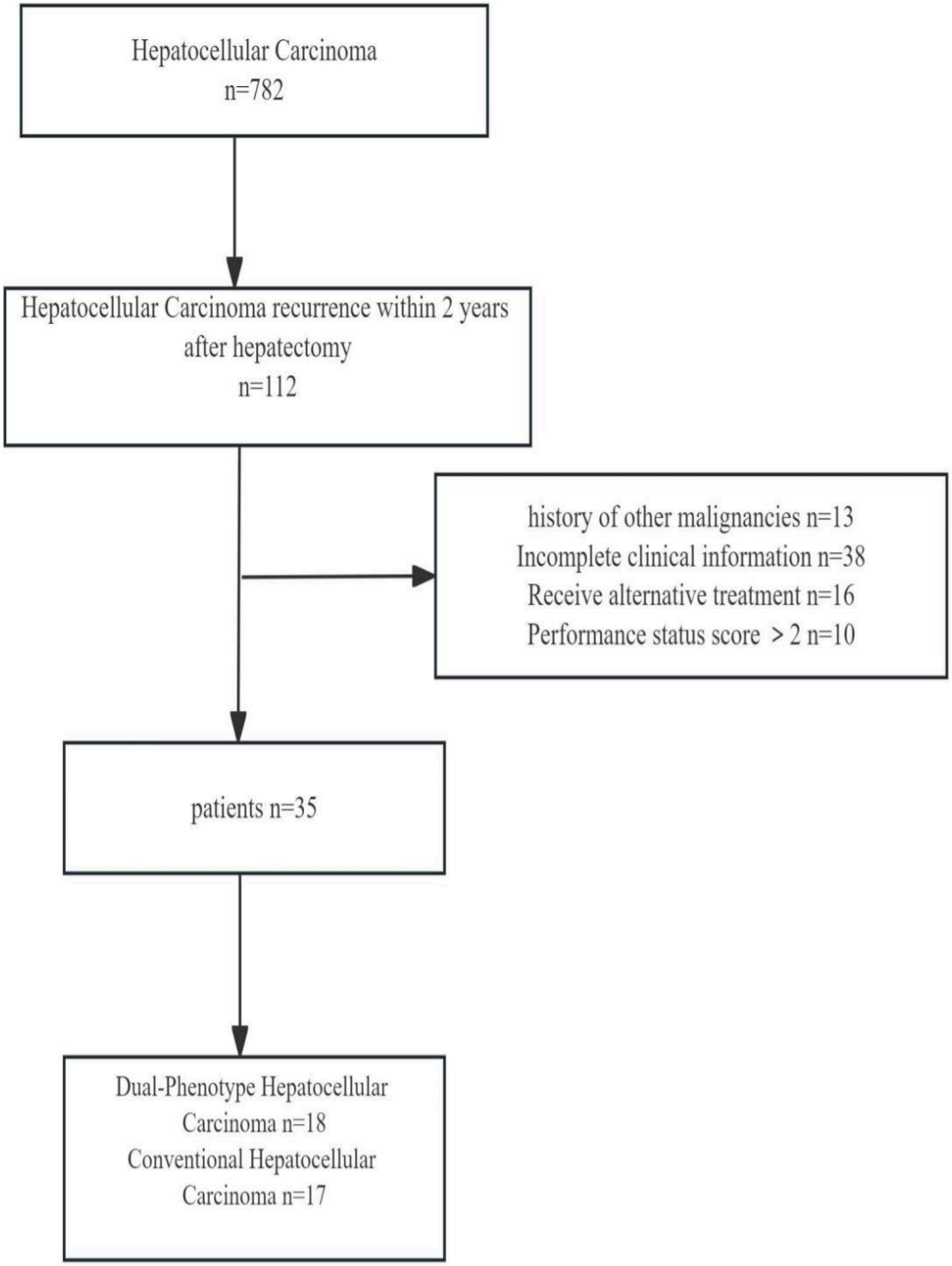


Figure 1

Patient Selection Flowchart.



Figure 2

Treatment trajectories of 35 patients.

The swimlane plot illustrates the treatment timelines of 19 patients, with the horizontal axis representing months from treatment start and each patient assigned a horizontal lane. The upper colored bar in each lane denotes targeted therapy, with Lenvatinib shown in red, Regorafenib in green, Apatinib in yellow, and

Bevacizumab in blue. The lower colored bar represents immunotherapy, with Camrelizumab shown in crimson, Tislelizumab in orange, and Sintilimab in purple. Intervention timepoints are marked by black solid circles (●) placed within the lower bar. Progression timepoints are indicated by black downward arrows (▼) of moderate size positioned above the upper bar.

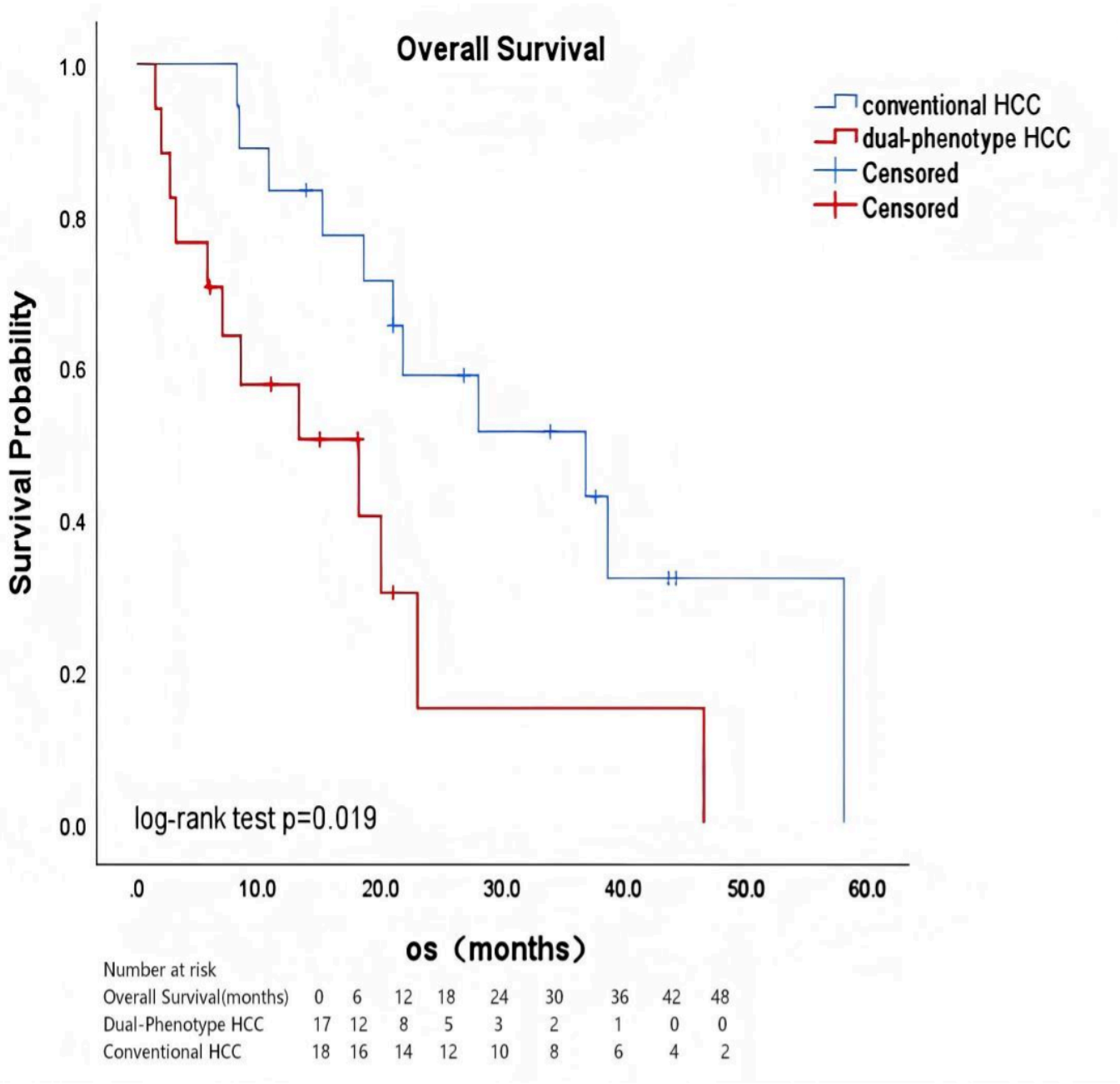


Figure 3

Kaplan-Meier estimate of overall survival.

Patients with dual-phenotype hepatocellular carcinoma (HCC) (n=17) exhibited significantly shorter median OS compared to those with conventional HCC (n=18) (18.2 vs. 36.8 months; log-rank chi-squared = 5.50, P = 0.019)

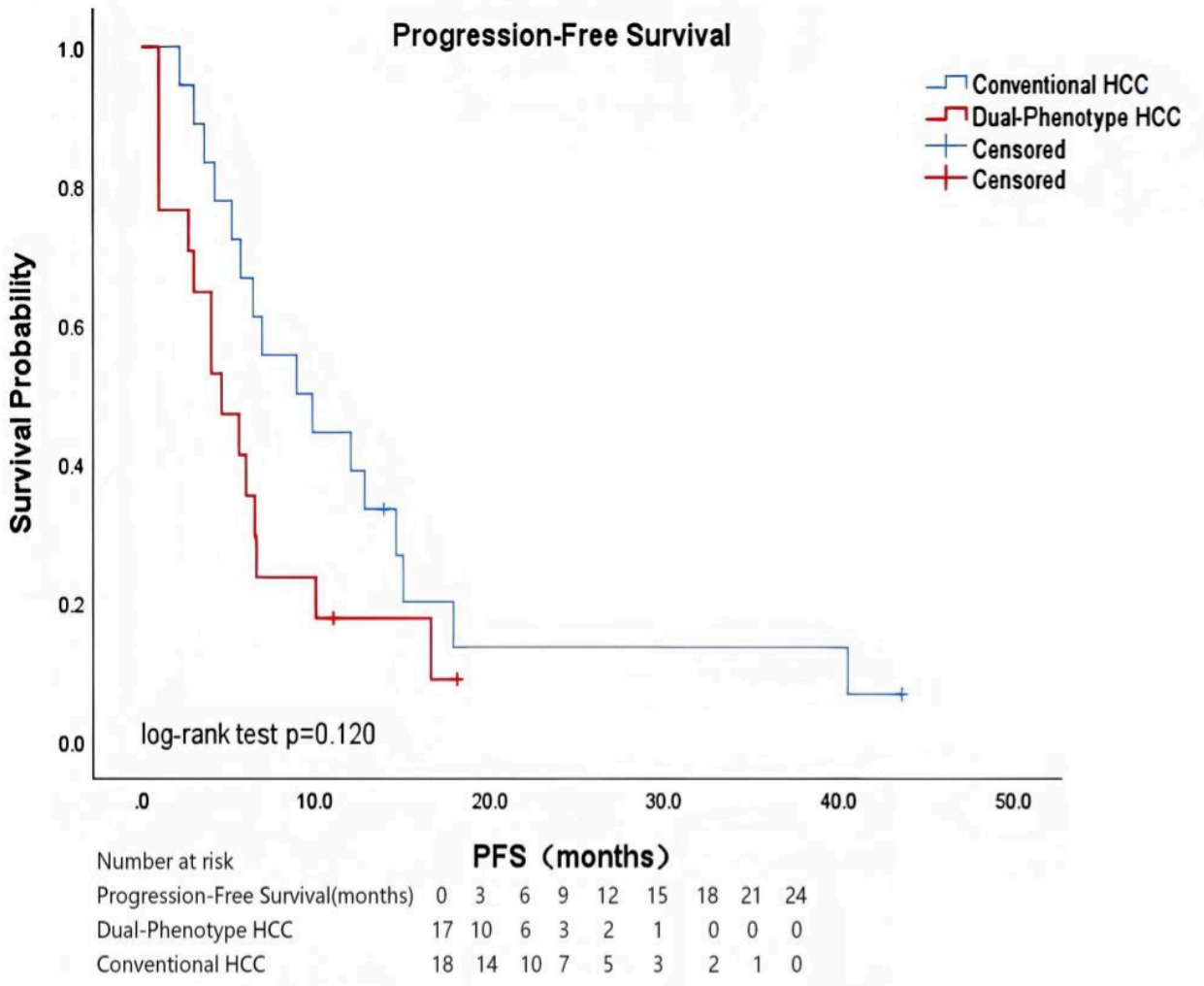


Figure 4

Kaplan-Meier estimate of progression-free survival.

Dual-phenotype HCC showed shorter median PFS compared to those with conventional HCC (n=18) (4.6 vs. 9.8 months), the difference did not reach statistical significance (log-rank chi-squared = 2.41, P = 0.120)

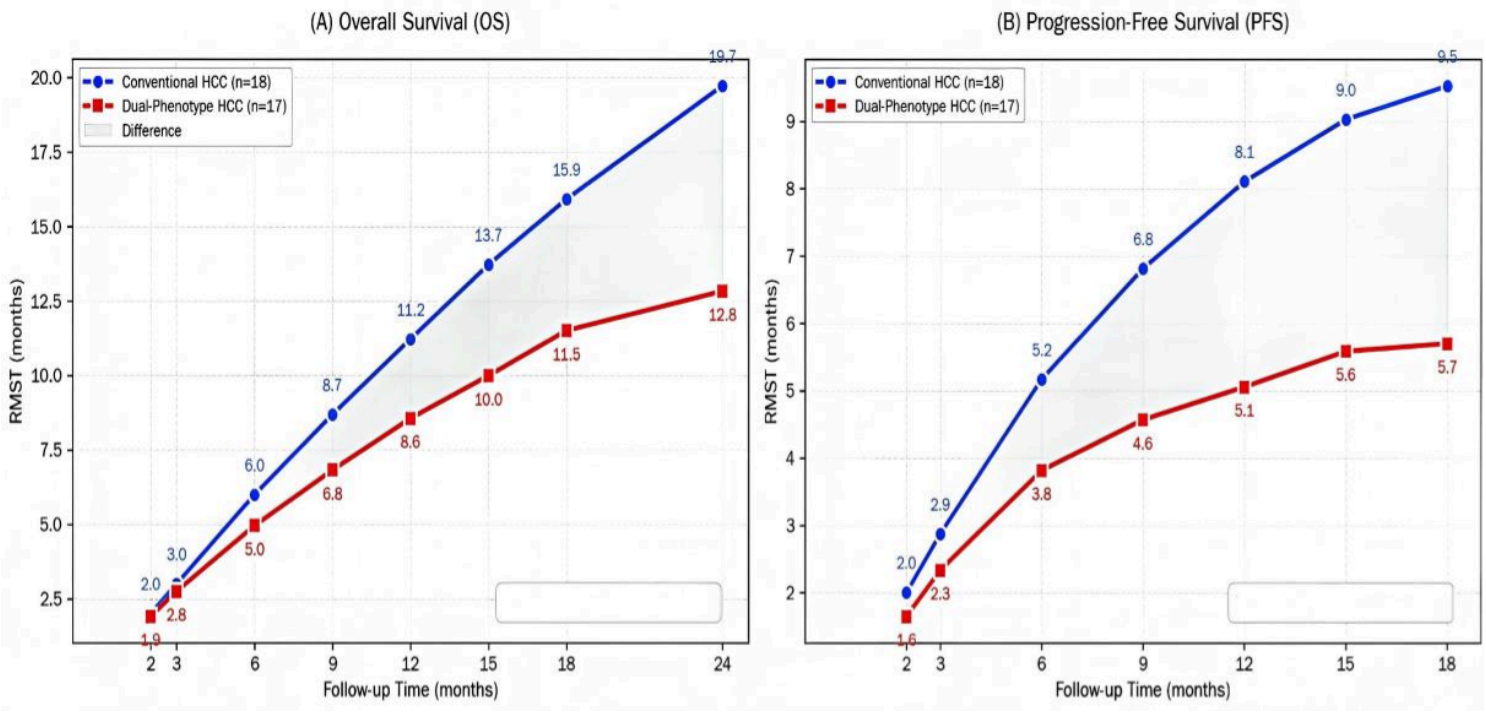


Figure 5

Restricted Mean Survival Time (RMST) Comparison Dual-Phenotype vs. Conventional Hepatocellular Carcinoma