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Radiotherapy combined with atezolizumab plus bevacizumab for hepatocellular carcinoma with portal vein tumor thrombus

Short running title: Radiotherapy plus T+A for HCC with PVTT

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Abstract

Introduction: Vascular invasion and metastasis are poor prognostic factors in patients with hepatocellular carcinoma (HCC). The efficacy of available therapeutic regimens for unresectable HCC is not satisfactory in HCC with portal vein tumor thrombosis (PVTT). Therefore, this open-label, single-arm phase II clinical trial aims to investigate the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating HCC patients with PVTT.

Methods and analysis: We plan to enroll patients diagnosed with unresectable hepatocellular carcinoma complicated by PVTT. Radiotherapy combined with atezolizumab plus bevacizumab will be administered for treatment. Patients will initially receive radiotherapy, with each radiotherapy cycle lasting for 28 days and the total dose of tumor (DT) of 40 Gy/20 f/26 d. CT scan will be performed again, and the treatment plan will be reformulated after field constriction. The treatment will continue until the total DT is up to 54-56 Gy/27-28 f. The treatment with atezolizumab plus bevacizumab will be started at 3±1 d after the initiation of radiotherapy and will continue until unacceptable toxicity or disease progression. The primary endpoint is objective response rate (ORR), while the secondary endpoints include overall survival, disease control rate, progression-free survival, time to progression, duration of response, and the rate of surgical conversions. Assuming an ORR of 47%, with a two-sided alpha error of 0.1, 90% power and 10% dropout rate, the required number of evaluable patients is 42.

Ethics and dissemination: This study will be conducted according to the standards of Good Clinical Practice and in compliance with the principles of the Declaration of Helsinki. The Ethics Committee of our Hospital has approved the protocol (EHBHKY2021-K-017). All participants are required to provide written informed consent. The results of the trial will be published in peer-reviewed journals and presented at international conferences.

Registration details: ChiCTR.org, registration number: ChiCTR2100049831.

Keywords: *Hepatocellular carcinoma, portal vein tumor thrombus, radiotherapy, atezolizumab, bevacizumab*

Strengths and limitations of this study

- While optimal treatment are yet to be determined, this study might help explore effective treatment strategies for patients with HCC complicated by PVTT.
- The combination of radiotherapy and immunotherapy plus targeted therapy in this study might improve the efficacy in patients with HCC complicated by PVTT.
- This study was a single-arm trial without a control group with small sample size.

Introduction

Liver cancer is the seventh most common cancer and the second most deadly cancer, with more than 900,000 new cases being diagnosed worldwide every year.[1] In China, the prevalence of liver cancer is about 26.67/100000,[2] while the 5-year survival rate is only around 12%.[3] Hepatocellular carcinoma (HCC) is the most common histological subtype of primary liver cancer,[4] and surgery is the preferred and most efficacious approach for the treatment of HCC. However, some patients lose the chance for surgery at the time of diagnosis due to their intermediate to advanced tumors, liver dysfunction, etc.[5] Local therapies (including radiotherapy) and systemic therapies are important options for prolonging the survival of HCC patients who cannot undergo surgical resection. Due to the promising antitumor activity and favorable safety profile in the IMbrave150 trial with the mortality risk being decreased by 42% comparing with sorafenib,[6] atezolizumab plus bevacizumab have become the standard first-line therapy for unresectable HCC. Yet, the objective response rate (ORR) with atezolizumab plus bevacizumab in unresectable HCC patients was reported to be only 27.3%.[6] Hence, improving the therapeutic effect in patients is still a great clinical challenge.

Vascular invasion and metastasis are commonly seen in patients with intermediate to advanced-stage HC as 44%-62% of patients with advanced HCC have been reported to develop portal vein tumor

thrombosis (PVTT).[7] In addition, the range and severity of PVTT have been significantly associated with the prognosis of unresectable HCC patients, with the median overall survival was just 2.7 to 4 months in naive HCC patients with PVTT.[8] However, there are significant differences between eastern and western countries in the treatment strategies used for HCC patients with PVTT.[9] Guidelines from western countries argue that HCC complicated with PVTT represents the advanced stage of HCC, advocating sorafenib as the only treatment option.[10-12] On the contrary, eastern countries adopt positive strategies, such as surgical resection, radiotherapy, transcatheter arterial chemoembolization (TACE), and surgery combined with multidisciplinary therapies, which are expected to improve the long-term survival of these patients.[13]

Radiotherapy is recommended for unresectable HCC patients with types I, II, and III PVTT, whose liver function is graded as Child-Pugh A and B. In addition, previous studies have shown that radiotherapy can synergistically enhance the anti-tumor effect of immunotherapy and targeted therapy by changing the tumor microenvironment.[14, 15] At present, radiotherapy combined with immunotherapy and targeted therapy is currently one of the dominant research topics within the tumor area. A number of studies have also suggested that radiotherapy combined with sorafenib alone improves OS in patients with HCC complicated by PVTT.[16] In addition, the results of a previous retrospective study showed that radiotherapy combined with atezolizumab plus bevacizumab was feasible in unresectable HCC.[17] However, whether the combination of radiotherapy and immunotherapy plus targeted therapy can improve the efficacy in patients with HCC complicated by PVTT needs to be further verified.

Therefore, we proposed an open-label, multi-center, single-arm phase II clinical trial. Patients diagnosed with locally advanced or metastatic and/or unresectable HCC complicated with PVTT will be preliminarily enrolled to explore the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab, which is expected to provide an efficacious therapeutic regimen for

HCC patients with PVTT and further improve the treatment response and prognosis. Here, we present the protocol in accordance with the SPIRIT reporting checklist.

Methods and analysis

Study design and objective

This open-label, multi-center, single-arm clinical trial will enroll patients diagnosed with locally advanced or metastatic and/or unresectable HCC complicated with PVTT from the departments of hepatobiliary surgery of our Hospitals between August 2021 and December 2021 so as to initially explore the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in naive patients with unresectable HCC complicated by PVTT.

Eligibility criteria

The key inclusion criteria are as follows: 1) Males or non-pregnant females aged between 18 and 70 years old; 2) Signed informed consent form; 3) Able to comply with the study protocol according to the investigator's judgment; 4) Histologically, cytologically or clinically diagnosed with hepatocellular carcinoma (HCC); 5) Confirmed portal vein tumor thrombus by imaging findings; 6) Not suitable for radical surgical treatment; 7) Have not received any anti-tumor treatment; 8) At least one measurable untreated lesion (per RECIST 1.1); 9) Pre-treatment tumor tissue sample (if available); 10) ECOG Performance Status of 0 or 1 within 7 days prior to initiation of study treatment; 11) Child-Pugh class A within 7 days prior to initiation of study treatment; 12) Adequate hematologic and organ functions, based on the following laboratory test results obtained within 7 days prior to initiation of study treatment; 13) Resolution of any acute, clinically significant treatment-related toxicity from prior therapy to grade < 1 prior to study entry, with the exception of alopecia; 14) Negative HIV test at screening.

The key exclusion criteria are as follows: 1) History of the leptomeningeal disease; 2) Active or history of autoimmune disease or immune deficiency; 3) History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), drug-induced pneumonitis, or idiopathic pneumonitis, or evidence of active pneumonitis on screening chest computed tomography (CT) scan. History of radiation pneumonitis in the radiation ield (fibrosis) is permitted; 4) Active tuberculosis; 5) Significant cardiovascular disease (such as New York Heart Association Class II or greater cardiac disease, myocardial infarction, or cerebrovascular accident) within 3 months prior to initiation of study treatment, unstable arrhythmia, or unstable angina; 6) History of congenital long QT syndrome or corrected QT interval > 500 ms (calculated with the use of the Fridericia method) at screening; 7) History of uncorrectable electrolyte disorder affecting serum levels of potassium, calcium, or magnesium; 8) The major surgical procedure, other than for diagnosis, within 4 weeks prior to initiation of study treatment, or anticipation of the need for a major surgical procedure during the study; 9) History of malignancy other than HCC within 5 years prior to screening; 10) Severe infection within 4 weeks prior to initiation of study treatment,; 11) Treatment with therapeutic oral or intravenous antibiotics within 2 weeks prior to initiation of study treatment; 12) Prior allogeneic stem cell or solid organ transplantation; 13) Untreated or incompletely treated esophageal and/or gastric varices that are bleeding or have a high risk for bleeding; 15) Co-infection of HBV and HCV; 16) Symptomatic, untreated, or actively progressing central nervous system (CNS) metastases.

Treatment plan

Patients will initially receive radiotherapy with each cycle for 28 days. A treatment plan will be established after CT location. The clinical target volume (CTV) includes tumors and tumor thrombi. The planning target volume (PTV) will be expanded by 0.5-1.0 cm, with conventional fractionation at a single dose of 180-220 cGy. Total DT will be 40 Gy/20 f/26 d. CT scan will be performed at the end of every cycle, and the treatment plan will be reformulated after field constriction. The treatment will continue until the total DT is up to 54-56 Gy/27-28 f.

The treatment with atezolizumab plus bevacizumab will be started at 3±1 d after the initiation of radiotherapy. Each treatment cycle will last for 21 days. On the first day of each cycle, atezolizumab injection will be intravenously administered at a fixed dose of 1,200 mg (initial intravenous injection for 60 min, and subsequent intravenous injections for 30 min if well-tolerated). Bevacizumab at a dose of 15 mg/kg will be intravenously administered at least 5 min apart (initial intravenous injection for 90 min, and subsequent intravenous injections reduced to 60 min and 30 min if well-tolerated). The treatment will continue until any unacceptable toxicity is found or disease progression occurs.

In case of severe toxicity, the administration will be delayed and/or the dose will be reduced. If any serious adverse event (SAE) occurs during the radiotherapy, or the radiotherapy is delayed for more than 4 weeks for any reason, radiotherapy sessions will be terminated. The doses of atezolizumab and bevacizumab will be adjusted according to the drug's instructions.

Endpoints

The primary endpoint is ORR, which is defined as the proportion of patients with Complete Response (CR) and Partial Response (PR). CR or PR is assessed in accordance with Response Evaluation Criteria In Solid Tumors (RECIST) 1.1.

Secondary endpoints include: (1) OS, defined as the time between first study treatment and death (for any reason). (2) Disease control rate (DCR), defined as the percentage of subjects with the best response as CR, PR, or stable disease (SD). (3) Progression free survival (PFS), defined as the time between first treatment and first tumor progression or appearance of a new lesion or death due to any cause, whichever occurs first. (4) Time to progression (TTP), defined as the time between first treatment to first tumor progression. (5) Duration of response (DOR), defined as the time from first tumor remission to first tumor progression or death due to any cause. The DCRs, PFSs, TTPs and DORs will be calculated in accordance with the RECIST version 1.1, modified RECIST (mRECIST), and immune-modified RECIST (imRECIST), respectively. (6) The rate of surgical conversions is

defined as the proportion of subjects who receive the study treatment and are assessed to be viable for surgical resection. The resectability criteria include successful down-staging of the tumor, sufficient future liver remnant (FLR), and technically resectable assessed by the investigator.

Adverse events (AEs) are assessed in accordance with the NCI Common Terminology Criteria for Adverse Event (CTCAE V5.0). Monitoring will be performed from the date on which the patient signs the informed consent to 90 days after the last treatment.

Biomarkers for exploratory endpoints include metagenomic sequencing of fecal organisms, 5 gene indexes of hepatocellular carcinoma (HN1, RAN, RAMP3, KRT19, TAF9), immune correlation detection (programmed death-ligand 1 [PD-L1] expression, CD8 expression; microsatellite instability [MSI], and tumor mutational burden [TMB], immune-related gene expression, RNA-seq) and other checkpoint proteins and cell surface markers.

Participant timeline

Table 1 lists the time points for assessing efficacy, adverse events, laboratory safety assessments (hematology, coagulation, and chemistry), physical examination, electrocardiogram (ECG), and tumor measurements.

Table 1. Schema of Single-arm Clinical Trial

Item	Screening Period		Treatment Period				End of Trial	Survival Follow-up
	-28 - 0d	-7 - 0d	4 weeks after the first treatment session	7 weeks after the first treatment session	n cycles	Perioperative Period	30 days after checkout	
Window period (days)	——	——					±7	
Informed consent	×							
Demographics								
Past medical history	×							
History of tumor treatment								
Physical examination		×	×	Every 3 weeks		×	×	
Vital signs		×	×	Every 3 weeks		×	×	
ECOG		×	×	Every 3 weeks ×			×	
Pregnancy test		×					×	
Infection screening	×						×	
Imaging	×		×	Every 6 weeks			×	×
Tumor markers		×	×	Every 6 weeks			×	
Echocardiography	×						×	
Tumor tissue	×							
12-lead electrocardiogram		×	When clinically indicated				×	
Blood biochemistry		×	×	Every 3 weeks			×	

Routine blood test		×	×	Every 3 weeks	×	
Routine urine test		×	×	Every 3 weeks	×	
Routine stool test		×	×	Every 3 weeks	×	
Thyroid function, pituitary function, coagulation		×	×	Every 3 weeks	×	
Gut microbiome testing for the stool sample		×		×	×	
Biomarker testing for the blood sample		×		×	×	
Surgical resection					×	
Surgical complications					×	
Concomitant medication	×	×	×	Every 3 weeks	×	
Adverse events	×	×	×	Every 3 weeks	×	
Compliance evaluation	×	×	×	Every 3 weeks		
Dispensing of drugs			×	Every 3 weeks		
Recovery of drugs			×	Every 3 weeks	×	
Follow-up on survival status and anti-tumor treatment						×

Furthermore, for the testing of biomarkers as exploratory endpoints, stool and blood samples will be collected from patients prior to the first treatment, at 7 weeks after the first treatment, and after treatment discontinuation, respectively.

Data collection and management

The completed CRFs will be submitted to the respective sites for archiving. All data will be recorded in an electronic case report form (eCRF) by investigators or clinical research coordinators. All study documents will be confidential. All study data, including confirmation of all patients (effective check on recorded information, such as study cases), all original copies of signed ICFs, as well as detailed original records of drug distribution, shall be kept uniformly by the study institution until 5 years after the end of the trial.

Statistical methods

According to the results of IMbrave150,[6] the ORR of atezolizumab plus bevacizumab in unresectable HCC patients was 27.3%. Assuming an ORR of 47%, with a two-sided alpha error of 0.1, 90% power, and 10% dropout rate, the required number of evaluable patients is 42.

The efficacy assessment is based on the full analysis set (FAS). The intention-to-treat (ITT) efficacy analysis will be performed for subjects who received at least one session of the study treatment based on the FAS. No supplementation will be made for missing values. The safety analysis set (SAS) includes all enrolled subjects who have received at least one session of study treatment and have a post-treatment safety record. This dataset will be used for safety analysis.

Monitoring

An independent Data and Safety Monitoring Committee has been established to assess the safety data if serious adverse events occur. Any adverse events will be registered. A qualified and independent

auditor is appointed to audit the trial systems, and the audit will be conducted before and during the study following a written procedure.

Patient and public involvement

The patients and general public were not involved in the trial design.

Discussion

Atezolizumab plus bevacizumab is the standard first-line therapy for unresectable HCC patients. Although the combination can significantly improve the prognosis in patients, the ORR is still not satisfactory.[6] Improving the therapeutic effect in patients is still a great clinical challenge. For patients with HCC, vascular invasion and metastasis are important reasons these patients cannot undergo surgical resection as well as poor prognostic factors. Radiotherapy has an important role in treating patients with PVTT; however, the therapeutic effects are still limited. Previous clinical studies have shown that radiotherapy combined with targeted therapy, such as sorafenib, tends to prolong the overall survival of patients with HCC complicated by PVTT. In the meanwhile, a retrospective study reported the feasibility of radiotherapy combined with atezolizumab plus bevacizumab in unresectable HCC.[17] However, evidence on whether the combination of radiotherapy with atezolizumab plus bevacizumab can improve the therapeutic effects in unresectable HCC patients with PVTT are still warranted. Accordingly, this open-label, multi-center, single-arm clinical trial was designed to preliminarily explore the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in naive unresectable HCC patients with PVTT.

There are several limitations in the present study that should be considered. First, this is a single-arm clinical trial without a control group that could be used to compare the efficacy and safety between treatment regimens. Second, since this study is an exploratory trial with small sample size, no confirmatory efficacy conclusions can be drawn. The conclusions of this study may serve as preliminary evidence for subsequent large-scale, randomized, controlled clinical trials.

As the prognosis in unresectable HCC patients with PVTT remains poor, there is an urgent need to find an efficacious and safe therapeutic regimen. In this study, the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in native unresectable HCC patients with PVTT will be investigated so as to explore a new therapeutic regimen and further improve the efficacy and prognosis in these patients.

Ethics and dissemination

This study will be conducted according to the standards of Good Clinical Practice and in compliance with the principles of the Declaration of Helsinki. The Ethics Committee of our Hospital has approved the protocol (EHBHKY2021-K-017). All participants are required to provide written informed consent. The results of the trial will be published in peer-reviewed journals and presented at international conferences.

Trial status

The trial has been registered in ChiCTR.org (Registration number: ChiCTR2100049831; registration date: 2021-08-10; http://www.chictr.org.cn/showproj.aspx?proj=126593). Recruitment is ongoing. The protocol version number is 1.0. The study protocol has been reported in accordance with the Standard Protocol Items: Recommendations for Clinical Interventional Trials (SPIRIT) guidelines.

Acknowledgements

The authors would like to thank the patients, their families, and all the investigators who will participate in the present study.

Data Sharing Statement

We will widely disseminate the results of this clinical trial through conference presentations and publications in relevant journals. All evaluation forms, reports, and other records will be identified in

a manner designed to maintain participants' confidentiality. All records will be kept in a secure storage area with limited access. Data during the study are available from the study leader upon reasonable request.

Authors' contributions

Kang Wang and Yan-Jun Xiang drafted the manuscript. Kang Wang, Yan-Jun Xiang, and Hong-Ming Yu contributed equally to this work. Shu-Qun Cheng, Kang Wang, Yan-Jun Xiang, and Hong-Ming Yu designed the trial. Yu-Qiang Cheng, Qian-Zhi Ni, Wei-Xing Guo, Jie Shi, Shuang Feng, and Jian Zhai are responsible for planning the data analysis and analyzing the data resulting from the trial. All named authors adhere to the authorship guidelines of Trials. All authors have agreed to publication. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no conflicts of interest to declare.

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SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P2/10
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	P10
Funding	4	Sources and types of financial, material, and other support	P14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P14
	5b	Name and contact information for the trial sponsor	P14
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P14
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P3-4

	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	P4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P5/16-21
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P5-6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P6-7
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P7

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P7
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P7
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P8
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P8
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P8
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	P8
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P8
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P8
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P9
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	P10
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P14
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P8
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating hepatocellular carcinoma with portal vein tumor thrombus: A study protocol

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Efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating hepatocellular carcinoma with portal vein tumor thrombus: A study protocol

Short running title: Radiotherapy plus T+A for HCC with PVTT

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Abstract

Introduction: Vascular invasion and metastasis are poor prognostic factors in patients with hepatocellular carcinoma (HCC). The efficacy of available therapeutic regimens for unresectable HCC is not satisfactory in HCC with portal vein tumor thrombosis (PVTT). Therefore, this open-label, single-arm phase II clinical trial aims to investigate the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating HCC patients with PVTT.

Methods and analysis: We plan to enroll patients diagnosed with unresectable hepatocellular carcinoma complicated by PVTT. Intensity-modulated radiotherapy (IMRT) combined with atezolizumab plus bevacizumab will be administered for treatment. Patients will initially receive radiotherapy, with each IMRT cycle lasting for 28 days and the total dose of tumor (DT) of 40 Gy/20 f/26 d. CT scan will be performed again, and the treatment plan will be reformulated after field constriction. The treatment will continue until the total DT is up to 54-56 Gy/27-28 f. The treatment with atezolizumab plus bevacizumab will be started at 3±1 d after the initiation of radiotherapy and will continue until unacceptable toxicity or disease progression. The primary endpoint is objective response rate (ORR), while the secondary endpoints include overall survival, disease control rate, progression-free survival, time to progression, duration of response, and the rate of surgical conversions. Assuming an ORR of 47%, with a two-sided alpha error of 0.1, 90% power, and a 10% dropout rate, the required number of evaluable patients is 42.

Ethics and dissemination: This study will be conducted according to the standards of Good Clinical Practice and in compliance with the principles of the Declaration of Helsinki. The Ethics Committee of our Hospital has approved the protocol (EHBHKY2021-K-017). All participants are required to provide written informed consent. The results of the trial will be published in peer-reviewed journals and presented at international conferences.

Registration details: ChiCTR.org, registration number: ChiCTR2100049831.

Keywords: *Hepatocellular carcinoma, portal vein tumor thrombus, radiotherapy, atezolizumab, bevacizumab*

Strengths and limitations of this study

- This study was a single-arm trial without a control group.
- This study is an exploratory trial with a small sample size.
- The conclusions of this study may serve as preliminary evidence for subsequent large-scale, randomized, controlled clinical trials.

Introduction

Liver cancer is the seventh most common cancer and the second most deadly cancer, with more than 900,000 new cases being diagnosed worldwide every year.[1] In China, the age-standardized annual incidence of liver cancer is about 26.67/100,000,[2] while the 5-year survival rate is only around 12%.[3] Hepatocellular carcinoma (HCC) is the most common histological subtype of primary liver cancer,[4] and surgery is the preferred and most efficacious approach for the treatment of HCC. However, some patients lose the chance for surgery at the time of diagnosis due to their intermediate to advanced tumors, liver dysfunction, etc. [5] Local therapies (including radiotherapy) and systemic therapies are important options for prolonging the survival of HCC patients who cannot undergo surgical resection. Due to the promising antitumor activity and favorable safety profile in the IMbrave150 trial, with the mortality risk being decreased by 42% compared with sorafenib,[6] atezolizumab plus bevacizumab have become the standard first-line therapy for unresectable HCC. Yet, the objective response rate (ORR) with atezolizumab plus bevacizumab in unresectable HCC patients was reported to be only 27.3%.[6] Hence, improving the therapeutic effect in patients is still a great clinical challenge.

Vascular invasion and metastasis are commonly seen in patients with intermediate to advanced-stage HC, as 44%-62% of patients with advanced HCC have been reported to develop portal vein tumor thrombosis (PVTT).[7] In addition, the range and severity of PVTT have been significantly associated with the prognosis of unresectable HCC patients, with the median overall survival being just 2.7 to 4 months in naive HCC patients with PVTT.[8] However, there are significant differences between eastern and western countries in the treatment strategies used for HCC patients with PVTT.[9] Guidelines from western countries argue that HCC complicated with PVTT represents the advanced stage of HCC, advocating sorafenib as the only treatment option.[10-12] On the contrary, eastern countries adopt positive strategies, such as surgical resection, radiotherapy, transcatheter arterial chemoembolization (TACE), and surgery combined with multidisciplinary therapies, which are expected to improve the long-term survival of these patients.[13-16]

Radiotherapy is recommended for unresectable HCC patients with types I, II, and III PVTT, whose liver function is graded as Child-Pugh A and B. In addition, previous studies have shown that radiotherapy can synergistically enhance the antitumor effect of immunotherapy and targeted therapy by changing the tumor microenvironment.[17] [18] At present, radiotherapy combined with immunotherapy and targeted therapy is currently one of the dominant research topics within the tumor area. A number of studies have also suggested that radiotherapy combined with sorafenib alone improves OS in patients with HCC complicated by PVTT,[19] and the feasibility of concurrent radiotherapy and sorafenib.[20] [21] In addition, the results of a previous retrospective study showed that radiotherapy combined with atezolizumab plus bevacizumab was feasible in unresectable HCC.[22] However, whether the combination of radiotherapy and immunotherapy plus targeted therapy can improve the efficacy in patients with HCC complicated by PVTT needs to be further verified.

Therefore, we proposed an open-label, multicenter, single-arm phase II clinical trial. Patients diagnosed with locally advanced or metastatic and/or unresectable HCC complicated with PVTT will be preliminarily enrolled to explore the efficacy and safety of radiotherapy combined with

atezolizumab plus bevacizumab, which is expected to provide an efficacious therapeutic regimen for HCC patients with PVTT and further improve the treatment response and prognosis. Here, we present the protocol in accordance with the SPIRIT reporting checklist.

Methods and analysis

Study design and objective

This open-label, multicenter, single-arm clinical trial will enroll patients diagnosed with locally advanced or metastatic and/or unresectable HCC complicated with PVTT from the departments of hepatobiliary surgery of our Hospitals between August 2021 and December 2021 so as to initially exploring the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in naive patients with unresectable HCC complicated by PVTT.

Eligibility criteria

The key inclusion criteria are as follows: 1) Males or non-pregnant females aged between 18 and 70 years old; 2) Signed informed consent form; 3) Able to comply with the study protocol according to the investigator's judgment; 4) Histologically, cytologically or clinically diagnosed with hepatocellular carcinoma (HCC); 5) Confirmed portal vein tumor thrombus by imaging findings; 6) Not suitable for radical surgical treatment; 7) Have not received any antitumor treatment; 8) At least one measurable untreated lesion (per RECIST 1.1); 9) Pre-treatment tumor tissue sample (if available); 10) ECOG Performance Status of 0 or 1 within 7 days prior to initiation of study treatment; 11) Child-Pugh class A within 7 days prior to initiation of study treatment; 12) Adequate hematologic and organ functions, based on the following laboratory test results obtained within 7 days prior to initiation of study treatment; 13) Resolution of any acute, clinically significant treatment-related toxicity from prior therapy to grade < 1 prior to study entry, with the exception of alopecia; 14) Negative HIV test at screening.

The key exclusion criteria are as follows: 1) History of the leptomeningeal disease; 2) Active or history of autoimmune disease or immune deficiency; 3) History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), drug-induced pneumonitis, or idiopathic pneumonitis, or evidence of active pneumonitis on screening chest computed tomography (CT) scan. History of radiation pneumonitis in the radiation ield (fibrosis) is permitted; 4) Active tuberculosis; 5) Significant cardiovascular disease (such as New York Heart Association Class II or greater cardiac disease, myocardial infarction, or cerebrovascular accident) within 3 months prior to initiation of study treatment, unstable arrhythmia, or unstable angina; 6) History of congenital long QT syndrome or corrected QT interval > 500 ms (calculated with the use of the Fridericia method) at screening; 7) History of uncorrectable electrolyte disorder affecting serum levels of potassium, calcium, or magnesium; 8) The major surgical procedure, other than for diagnosis, within 4 weeks prior to initiation of study treatment, or anticipation of the need for a major surgical procedure during the study; 9) History of malignancy other than HCC within 5 years prior to screening; 10) Severe infection within 4 weeks prior to initiation of study treatment; 11) Treatment with therapeutic oral or intravenous antibiotics within 2 weeks prior to initiation of study treatment; 12) Prior allogeneic stem cell or solid organ transplantation; 13) Untreated or incompletely treated esophageal and/or gastric varices that are bleeding or have a high risk for bleeding; 15) Co-infection of HBV and HCV; 16) Symptomatic, untreated, or actively progressing central nervous system (CNS) metastases.

Treatment plan

Patients will initially receive intensity-modulated radiotherapy (IMRT) with each cycle for 28 days. A treatment plan will be established after CT location. The clinical target volume (CTV) includes tumors and tumor thrombi. The planning target volume (PTV) will be expanded by 0.5-1.0 cm, with conventional fractionation at a single dose of 180-220 cGy. Total DT will be 40 Gy/20 f/26 d. CT scan will be performed at the end of every cycle, and the treatment plan will be reformulated after field constriction. The treatment will continue until the total DT is up to 54-56 Gy/27-28 f.

The treatment with atezolizumab plus bevacizumab will be started at 3±1 d after the initiation of IMRT. Each treatment cycle will last for 21 days. On the first day of each cycle, atezolizumab injection will be intravenously administered at a fixed dose of 1,200 mg (initial intravenous injection for 60 min, and subsequent intravenous injections for 30 min if well-tolerated). Bevacizumab at a dose of 15 mg/kg will be intravenously administered at least 5 min apart (initial intravenous injection for 90 min, and subsequent intravenous injections reduced to 60 min and 30 min if well-tolerated). The treatment was discontinued when there was no additional clinical benefit, as judged by the investigator (based on imaging, biochemical indicators, and clinical status of the patient). Then, the patient received the guideline-recommended second-line therapy.

Delayed treatment or dose reduction will be determined by the clinical team, and the specific adjustment plan will be determined by the investigator based on the patient's clinical condition. In case of severe toxicity, the administration will be delayed, and/or the dose will be reduced. If any serious adverse event (SAE) occurs during the radiotherapy, or the radiotherapy is delayed for more than 4 weeks for any reason, radiotherapy sessions will be terminated. The doses of atezolizumab and bevacizumab will be adjusted according to the drug's instructions.

Endpoints

The primary endpoint is ORR, which is defined as the proportion of patients with Complete Response (CR) and Partial Response (PR). CR or PR is assessed in accordance with Response Evaluation Criteria In Solid Tumors (RECIST) 1.1. The responses will be independently evaluated by three to six experienced senior physicians who are not investigators in this study.

Secondary endpoints include (1) OS, defined as the time between the first study treatment and death (for any reason). (2) Disease control rate (DCR), defined as the percentage of subjects with the best response as CR, PR, or stable disease (SD). (3) Progression-free survival (PFS), defined as the time between the first treatment and the first tumor progression or appearance of a new lesion or death

due to any cause, whichever occurs first. (4) Time to progression (TTP), defined as the time between the first treatment to first tumor progression. (5) Duration of response (DOR), defined as the time from first tumor remission to first tumor progression or death due to any cause. The DCRs, PFSs, TTPs, and DORs will be calculated in accordance with the RECIST version 1.1, modified RECIST (mRECIST), and immune-modified RECIST (imRECIST), respectively. (6) The rate of surgical conversions is defined as the proportion of subjects who receive the study treatment and are assessed to be viable for surgical resection. The resectability criteria include successful down-staging of the tumor, sufficient future liver remnant (FLR), and technically resectable assessed by the investigator. The definition of successful downstaging was based on the CSCO guideline, which was 1) stage Ia, Ib, and IIa, 2) IIb, if the tumor is confined to the same segment or the same side of the liver or intraoperative radiofrequency ablation can be performed at the same time to treat the lesions outside the resection range, and 3) IIIa/b, if the tumor is confined to the hemi-liver, and it is expected that the tumor thrombus can be completely removed during the operation, surgical resection of the tumor and thrombectomy through the portal vein can be considered.

Adverse events (AEs) are assessed in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE V5.0). Monitoring will be performed from the date on which the patient signs the informed consent to 90 days after the last treatment.

Biomarkers for exploratory endpoints include metagenomic sequencing of fecal organisms, five gene indexes of hepatocellular carcinoma (HN1, RAN, RAMP3, KRT19, TAF9), immune correlation detection (programmed death-ligand 1 [PD-L1] expression, CD8 expression; microsatellite instability [MSI], and tumor mutational burden [TMB], immune-related gene expression, RNA-seq) and other checkpoint proteins and cell surface markers.

Participant timeline

Table 1 lists the time points for assessing efficacy, adverse events, laboratory safety assessments (hematology, coagulation, and chemistry), physical examination, electrocardiogram (ECG), and tumor measurements.

Table 1. Schema of Single-arm Clinical Trial

Item	Screening Period		Treatment Period			Perioperative Period	End of Trial	Survival Follow-up
	-28 - 0d	-7 - 0d	4 weeks after the first treatment session	7 weeks after the first treatment session	n cycles		30 days after checkout	
Window period (days)	——	——					±7	
Informed consent	×							
Demographics								
Past medical history	×							
History of tumor treatment								
Physical examination		×	×	Every 3 weeks		×	×	
Vital signs		×	×	Every 3 weeks		×	×	
ECOG		×	×	Every 3 weeks ×			×	
Pregnancy test		×					×	
Infection screening	×						×	
Imaging	×		×	Every 6 weeks			×	×
Tumor markers		×	×	Every 6 weeks			×	
Echocardiography	×						×	
Tumor tissue	×							
12-lead electrocardiogram		×	When clinically indicated				×	
Blood biochemistry		×	×	Every 3 weeks			×	
Routine blood test		×	×	Every 3 weeks			×	

Routine urine test		×	×	Every 3 weeks		×	
Routine stool test		×	×	Every 3 weeks		×	
Thyroid function, pituitary function, coagulation		×	×	Every 3 weeks		×	
Gut microbiome testing for the stool sample		×		×		×	
Biomarker testing for the blood sample		×		×		×	
Surgical resection					×		
Surgical complications					×		
Concomitant medication	×	×	×	Every 3 weeks		×	
Adverse events	×	×	×	Every 3 weeks		×	
Compliance evaluation	×	×	×	Every 3 weeks			
Dispensing of drugs			×	Every 3 weeks			
Recovery of drugs			×	Every 3 weeks		×	
Follow-up on survival status and antitumor treatment							×

Furthermore, for the testing of biomarkers as exploratory endpoints, stool and blood samples will be collected from patients prior to the first treatment, at 7 weeks after the first treatment, and after treatment discontinuation, respectively.

Data collection and management

The completed CRFs will be submitted to the respective sites for archiving. All data will be recorded in an electronic case report form (eCRF) by investigators or clinical research coordinators. All study documents will be confidential. All study data, including confirmation of all patients (effective check on recorded information, such as study cases), all original copies of signed ICFs, as well as detailed original records of drug distribution, shall be kept uniformly by the study institution until 5 years after the end of the trial.

Statistical methods

According to the results of IMbrave150,[6] the ORR of atezolizumab plus bevacizumab in unresectable HCC patients was 27.3%. Assuming an ORR of 47%, with a two-sided alpha error of 0.1, 90% power, and 10% dropout rate, the required number of evaluable patients is 42.

The efficacy assessment is based on the full analysis set (FAS). The intention-to-treat (ITT) efficacy analysis will be performed for subjects who received at least one session of the study treatment based on the FAS. No supplementation will be made for missing values. The safety analysis set (SAS) includes all enrolled subjects who have received at least one session of study treatment and have a post-treatment safety record. This dataset will be used for safety analysis.

Monitoring

An independent Data and Safety Monitoring Committee has been established to assess the safety data if serious adverse events occur. Any adverse events will be registered. A qualified and independent auditor is appointed to audit the trial systems, and the audit will be conducted before and during the

study following a written procedure. The stopping rules for the trial are 1) the investigators find serious safety issues, or 2) the administrative department cancels the trial. The termination of the trial can be temporary or permanent. When terminating the trial, all records will be kept for future reference.

Patient and public involvement

The patients and general public were not involved in the trial design.

Discussion

Atezolizumab plus bevacizumab is the standard first-line therapy for unresectable HCC patients. Although the combination can significantly improve the prognosis in patients, the ORR is still not satisfactory.[6] Improving the therapeutic effect in patients is still a great clinical challenge. For patients with HCC, vascular invasion and metastasis are important reasons these patients cannot undergo surgical resection, as well as poor prognostic factors. Radiotherapy has an important role in treating patients with PVTT; however, the therapeutic effects are still limited. Previous clinical studies have shown that radiotherapy combined with targeted therapy, such as sorafenib, tends to prolong the overall survival of patients with HCC complicated by PVTT. In the meanwhile, a retrospective study reported the feasibility of radiotherapy combined with atezolizumab plus bevacizumab in unresectable HCC.[22] However, evidence on whether the combination of radiotherapy with atezolizumab plus bevacizumab can improve the therapeutic effects in unresectable HCC patients with PVTT is still warranted. Accordingly, this open-label, multicenter, single-arm clinical trial was designed to preliminarily explore the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in naive unresectable HCC patients with PVTT.

There are several limitations in the present study that should be considered. First, this is a single-arm clinical trial without a control group that could be used to compare the efficacy and safety between treatment regimens. Second, since this study is an exploratory trial with a small sample size, no

confirmatory efficacy conclusions can be drawn. The conclusions of this study may serve as preliminary evidence for subsequent large-scale, randomized, controlled clinical trials.

As the prognosis in unresectable HCC patients with PVTT remains poor, there is an urgent need to find an efficacious and safe therapeutic regimen. In this study, the efficacy and safety of IMRT combined with atezolizumab plus bevacizumab in native unresectable HCC patients with PVTT will be investigated so as to explore a new therapeutic regimen and further improve the efficacy and prognosis in these patients.

Ethics and dissemination

This study will be conducted according to the standards of Good Clinical Practice and in compliance with the principles of the Declaration of Helsinki. The Ethics Committee of our Hospital has approved the protocol (EHBHKY2021-K-017). All participants are required to provide written informed consent. The results of the trial will be published in peer-reviewed journals and presented at international conferences.

Trial status

The trial has been registered in ChiCTR.org (Registration number: ChiCTR2100049831; registration date: 2021-08-10; http://www.chictr.org.cn/showproj.aspx?proj=126593). Recruitment is ongoing. The protocol version number is 1.0. The study protocol has been reported in accordance with the Standard Protocol Items: Recommendations for Clinical Interventional Trials (SPIRIT) guidelines.

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Data Sharing Statement

We will widely disseminate the results of this clinical trial through conference presentations and publications in relevant journals. All evaluation forms, reports, and other records will be identified in a manner designed to maintain participants' confidentiality. All records will be kept in a secure storage area with limited access. Data during the study are available from the study leader upon reasonable request.

Authors' contributions

Kang Wang and Yan-Jun Xiang drafted the manuscript. Kang Wang, Yan-Jun Xiang, and Hong-Ming Yu contributed equally to this work. Shu-Qun Cheng, Kang Wang, Yan-Jun Xiang, and Hong-Ming Yu designed the trial. Yu-Qiang Cheng, Qian-Zhi Ni, Wei-Xing Guo, Jie Shi, Shuang Feng, and Jian Zhai are responsible for planning the data analysis and analyzing the data resulting from the trial. All named authors adhere to the authorship guidelines of Trials. All authors have agreed to publication. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no conflicts of interest to declare.

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P2/10
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	P10
Funding	4	Sources and types of financial, material, and other support	P14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P14
	5b	Name and contact information for the trial sponsor	P14
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P14
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P3-4

	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	P4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P5/16-21
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P5-6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P6-7
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P7

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P7
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P7
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P8
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P8
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P8
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	P8
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P8
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P8
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P9
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	P10
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P14
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P8
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating hepatocellular carcinoma with portal vein tumor thrombus: A study protocol

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Efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating hepatocellular carcinoma with portal vein tumor thrombus: A study protocol

Short running title: Radiotherapy plus T+A for HCC with PVTT

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Abstract

Introduction: Vascular invasion and metastasis are poor prognostic factors in patients with hepatocellular carcinoma (HCC). The efficacy of available therapeutic regimens for unresectable HCC is not satisfactory in HCC with portal vein tumor thrombosis (PVTT). Therefore, this open-label, single-arm phase II clinical trial aims to investigate the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating HCC patients with PVTT.

Methods and analysis: We plan to enroll patients diagnosed with unresectable hepatocellular carcinoma complicated by PVTT. Intensity-modulated radiotherapy (IMRT) combined with atezolizumab plus bevacizumab will be administered for treatment. Patients will initially receive radiotherapy, with each IMRT cycle lasting for 28 days and the total dose of tumor (DT) of 40 Gy/20 f/26 d. CT scan will be performed again, and the treatment plan will be reformulated after field constriction. The treatment will continue until the total DT is up to 54-56 Gy/27-28 f. The treatment with atezolizumab plus bevacizumab will be started at 3±1 d after the initiation of radiotherapy and will continue until unacceptable toxicity or disease progression. The primary endpoint is objective response rate (ORR), while the secondary endpoints include overall survival, disease control rate, progression-free survival, time to progression, duration of response, and the rate of surgical conversions. Assuming an ORR of 47%, with a two-sided alpha error of 0.1, 90% power, and a 10% dropout rate, the required number of evaluable patients is 42.

Ethics and dissemination: This study will be conducted according to the standards of Good Clinical Practice and in compliance with the principles of the Declaration of Helsinki. The Ethics Committee of our Hospital has approved the protocol (EHBHKY2021-K-017). All participants are required to provide written informed consent. The results of the trial will be published in peer-reviewed journals and presented at international conferences.

Registration details: ChiCTR.org, registration number: ChiCTR2100049831.

Keywords: *Hepatocellular carcinoma, portal vein tumor thrombus, radiotherapy, atezolizumab, bevacizumab*

Strengths and limitations of this study

- This study was a single-arm trial without a control group.
- This study is an exploratory trial with a small sample size.
- The conclusions of this study may serve as preliminary evidence for subsequent large-scale, randomized, controlled clinical trials.

Introduction

Liver cancer is the seventh most common cancer and the second most deadly cancer, with more than 900,000 new cases being diagnosed worldwide every year.[1] In China, the age-standardized annual incidence of liver cancer is about 26.67/100,000,[2] while the 5-year survival rate is only around 12%.[3] Hepatocellular carcinoma (HCC) is the most common histological subtype of primary liver cancer,[4] and surgery is the preferred and most efficacious approach for the treatment of HCC. However, some patients lose the chance for surgery at the time of diagnosis due to their intermediate to advanced tumors, liver dysfunction, etc. [5] Local therapies (including radiotherapy) and systemic therapies are important options for prolonging the survival of HCC patients who cannot undergo surgical resection. Due to the promising antitumor activity and favorable safety profile in the IMbrave150 trial, with the mortality risk being decreased by 42% compared with sorafenib,[6] atezolizumab plus bevacizumab have become the standard first-line therapy for unresectable HCC. Yet, the objective response rate (ORR) with atezolizumab plus bevacizumab in unresectable HCC patients was reported to be only 27.3%.[6] Hence, improving the therapeutic effect in patients is still a great clinical challenge.

Vascular invasion and metastasis are commonly seen in patients with intermediate to advanced-stage HC, as 44%-62% of patients with advanced HCC have been reported to develop portal vein tumor thrombosis (PVTT).[7] In addition, the range and severity of PVTT have been significantly associated with the prognosis of unresectable HCC patients, with the median overall survival being just 2.7 to 4 months in naive HCC patients with PVTT.[8] However, there are significant differences between eastern and western countries in the treatment strategies used for HCC patients with PVTT.[9] Guidelines from western countries argue that HCC complicated with PVTT represents the advanced stage of HCC, advocating sorafenib as the only treatment option.[10-12] On the contrary, eastern countries adopt positive strategies, such as surgical resection, radiotherapy, transcatheter arterial chemoembolization (TACE), and surgery combined with multidisciplinary therapies, which are expected to improve the long-term survival of these patients.[13-16]

Radiotherapy is recommended for unresectable HCC patients with types I, II, and III PVTT, whose liver function is graded as Child-Pugh A and B. In addition, previous studies have shown that radiotherapy can synergistically enhance the antitumor effect of immunotherapy and targeted therapy by changing the tumor microenvironment.[17] [18] At present, radiotherapy combined with immunotherapy and targeted therapy is currently one of the dominant research topics within the tumor area. A number of studies have also suggested that radiotherapy combined with sorafenib alone improves OS in patients with HCC complicated by PVTT,[19] and the feasibility of concurrent radiotherapy and sorafenib.[20] [21] In addition, the results of a previous retrospective study showed that radiotherapy combined with atezolizumab plus bevacizumab was feasible in unresectable HCC.[22] However, whether the combination of radiotherapy and immunotherapy plus targeted therapy can improve the efficacy in patients with HCC complicated by PVTT needs to be further verified.

Therefore, we proposed an open-label, multicenter, single-arm phase II clinical trial. Patients diagnosed with locally advanced or metastatic and/or unresectable HCC complicated with PVTT will be preliminarily enrolled to explore the efficacy and safety of radiotherapy combined with

atezolizumab plus bevacizumab, which is expected to provide an efficacious therapeutic regimen for HCC patients with PVTT and further improve the treatment response and prognosis. Here, we present the protocol in accordance with the SPIRIT reporting checklist.

Methods and analysis

Study design and objective

This open-label, multicenter, single-arm clinical trial will enroll patients diagnosed with locally advanced or metastatic and/or unresectable HCC complicated with PVTT from the departments of hepatobiliary surgery of our Hospitals between August 2021 and December 2021 so as to initially exploring the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in naive patients with unresectable HCC complicated by PVTT.

Eligibility criteria

The key inclusion criteria are as follows: 1) Males or non-pregnant females aged between 18 and 70 years old; 2) Signed informed consent form; 3) Able to comply with the study protocol according to the investigator's judgment; 4) Histologically, cytologically or clinically diagnosed with hepatocellular carcinoma (HCC); 5) Confirmed portal vein tumor thrombus by imaging findings; 6) Not suitable for radical surgical treatment; 7) Have not received any antitumor treatment; 8) At least one measurable untreated lesion (per RECIST 1.1); 9) Pre-treatment tumor tissue sample (if available); 10) ECOG Performance Status of 0 or 1 within 7 days prior to initiation of study treatment; 11) Child-Pugh class A within 7 days prior to initiation of study treatment; 12) Adequate hematologic and organ functions, based on the following laboratory test results obtained within 7 days prior to initiation of study treatment; 13) Resolution of any acute, clinically significant treatment-related toxicity from prior therapy to grade < 1 prior to study entry, with the exception of alopecia; 14) Negative HIV test at screening.

The key exclusion criteria are as follows: 1) History of the leptomeningeal disease; 2) Active or history of autoimmune disease or immune deficiency; 3) History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), drug-induced pneumonitis, or idiopathic pneumonitis, or evidence of active pneumonitis on screening chest computed tomography (CT) scan. History of radiation pneumonitis in the radiation ield (fibrosis) is permitted; 4) Active tuberculosis; 5) Significant cardiovascular disease (such as New York Heart Association Class II or greater cardiac disease, myocardial infarction, or cerebrovascular accident) within 3 months prior to initiation of study treatment, unstable arrhythmia, or unstable angina; 6) History of congenital long QT syndrome or corrected QT interval > 500 ms (calculated with the use of the Fridericia method) at screening; 7) History of uncorrectable electrolyte disorder affecting serum levels of potassium, calcium, or magnesium; 8) The major surgical procedure, other than for diagnosis, within 4 weeks prior to initiation of study treatment, or anticipation of the need for a major surgical procedure during the study; 9) History of malignancy other than HCC within 5 years prior to screening; 10) Severe infection within 4 weeks prior to initiation of study treatment; 11) Treatment with therapeutic oral or intravenous antibiotics within 2 weeks prior to initiation of study treatment; 12) Prior allogeneic stem cell or solid organ transplantation; 13) Untreated or incompletely treated esophageal and/or gastric varices that are bleeding or have a high risk for bleeding; 15) Co-infection of HBV and HCV; 16) Symptomatic, untreated, or actively progressing central nervous system (CNS) metastases.

Treatment plan

Patients will initially receive intensity-modulated radiotherapy (IMRT) with each cycle for 28 days. A treatment plan will be established after CT location. The clinical target volume (CTV) includes tumors and tumor thrombi. The planning target volume (PTV) will be expanded by 0.5-1.0 cm, with conventional fractionation at a single dose of 180-220 cGy. Total DT will be 40 Gy/20 f/26 d. CT scan will be performed at the end of every cycle, and the treatment plan will be reformulated after field constriction. The treatment will continue until the total DT is up to 54-56 Gy/27-28 f.

The treatment with atezolizumab plus bevacizumab will be started at 3±1 d after the initiation of IMRT. Each treatment cycle will last for 21 days. On the first day of each cycle, atezolizumab injection will be intravenously administered at a fixed dose of 1,200 mg (initial intravenous injection for 60 min, and subsequent intravenous injections for 30 min if well-tolerated). Bevacizumab at a dose of 15 mg/kg will be intravenously administered at least 5 min apart (initial intravenous injection for 90 min, and subsequent intravenous injections reduced to 60 min and 30 min if well-tolerated). The treatment was discontinued when there was no additional clinical benefit, as judged by the investigator (based on imaging, biochemical indicators, and clinical status of the patient). Then, the patient received the guideline-recommended second-line therapy.

Delayed treatment or dose reduction will be determined by the clinical team, and the specific adjustment plan will be determined by the investigator based on the patient's clinical condition. In case of severe toxicity, the administration will be delayed, and/or the dose will be reduced. If any serious adverse event (SAE) occurs during the radiotherapy, or the radiotherapy is delayed for more than 4 weeks for any reason, radiotherapy sessions will be terminated. The doses of atezolizumab and bevacizumab will be adjusted according to the drug's instructions.

Endpoints

The primary endpoint is ORR, which is defined as the proportion of patients with Complete Response (CR) and Partial Response (PR). CR or PR is assessed in accordance with Response Evaluation Criteria In Solid Tumors (RECIST) 1.1. The responses will be independently evaluated by three to six experienced senior physicians who are not investigators in this study.

Secondary endpoints include (1) OS, defined as the time between the first study treatment and death (for any reason). (2) Disease control rate (DCR), defined as the percentage of subjects with the best response as CR, PR, or stable disease (SD). (3) Progression-free survival (PFS), defined as the time between the first treatment and the first tumor progression or appearance of a new lesion or death

due to any cause, whichever occurs first. (4) Time to progression (TTP), defined as the time between the first treatment to first tumor progression. (5) Duration of response (DOR), defined as the time from first tumor remission to first tumor progression or death due to any cause. The DCRs, PFSs, TTPs, and DORs will be calculated in accordance with the RECIST version 1.1, modified RECIST (mRECIST), and immune-modified RECIST (imRECIST), respectively. (6) The rate of surgical conversions is defined as the proportion of subjects who receive the study treatment and are assessed to be viable for surgical resection. The resectability criteria include successful down-staging of the tumor, sufficient future liver remnant (FLR), and technically resectable assessed by the investigator. The definition of successful downstaging was based on the CSCO guideline, which was 1) stage Ia, Ib, and IIa, 2) IIb, if the tumor is confined to the same segment or the same side of the liver or intraoperative radiofrequency ablation can be performed at the same time to treat the lesions outside the resection range, and 3) IIIa/b, if the tumor is confined to the hemi-liver, and it is expected that the tumor thrombus can be completely removed during the operation, surgical resection of the tumor and thrombectomy through the portal vein can be considered.

Adverse events (AEs) are assessed in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE V5.0). Monitoring will be performed from the date on which the patient signs the informed consent to 90 days after the last treatment.

Biomarkers for exploratory endpoints include metagenomic sequencing of fecal organisms, five gene indexes of hepatocellular carcinoma (HN1, RAN, RAMP3, KRT19, TAF9), immune correlation detection (programmed death-ligand 1 [PD-L1] expression, CD8 expression; microsatellite instability [MSI], and tumor mutational burden [TMB], immune-related gene expression, RNA-seq) and other checkpoint proteins and cell surface markers.

Participant timeline

Table 1 lists the time points for assessing efficacy, adverse events, laboratory safety assessments (hematology, coagulation, and chemistry), physical examination, electrocardiogram (ECG), and tumor measurements.

Table 1. Schema of Single-arm Clinical Trial

Item	Screening Period		Treatment Period			Perioperative Period	End of Trial	Survival Follow-up
	-28 - 0d	-7 - 0d	4 weeks after the first treatment session	7 weeks after the first treatment session	n cycles		30 days after checkout	
Window period (days)	——	——					±7	
Informed consent	×							
Demographics								
Past medical history	×							
History of tumor treatment								
Physical examination		×	×	Every 3 weeks		×	×	
Vital signs		×	×	Every 3 weeks		×	×	
ECOG		×	×	Every 3 weeks ×			×	
Pregnancy test		×					×	
Infection screening	×						×	
Imaging	×		×	Every 6 weeks			×	×
Tumor markers		×	×	Every 6 weeks			×	
Echocardiography	×						×	
Tumor tissue	×							
12-lead electrocardiogram		×	When clinically indicated				×	
Blood biochemistry		×	×	Every 3 weeks			×	
Routine blood test		×	×	Every 3 weeks			×	

Routine urine test		×	×	Every 3 weeks		×	
Routine stool test		×	×	Every 3 weeks		×	
Thyroid function, pituitary function, coagulation		×	×	Every 3 weeks		×	
Gut microbiome testing for the stool sample		×		×		×	
Biomarker testing for the blood sample		×		×		×	
Surgical resection					×		
Surgical complications					×		
Concomitant medication	×	×	×	Every 3 weeks		×	
Adverse events	×	×	×	Every 3 weeks		×	
Compliance evaluation	×	×	×	Every 3 weeks			
Dispensing of drugs			×	Every 3 weeks			
Recovery of drugs			×	Every 3 weeks		×	
Follow-up on survival status and antitumor treatment							×

Furthermore, for the testing of biomarkers as exploratory endpoints, stool and blood samples will be collected from patients prior to the first treatment, at 7 weeks after the first treatment, and after treatment discontinuation, respectively.

Data collection and management

The completed CRFs will be submitted to the respective sites for archiving. All data will be recorded in an electronic case report form (eCRF) by investigators or clinical research coordinators. All study documents will be confidential. All study data, including confirmation of all patients (effective check on recorded information, such as study cases), all original copies of signed ICFs, as well as detailed original records of drug distribution, shall be kept uniformly by the study institution until 5 years after the end of the trial.

Statistical methods

According to the results of IMbrave150,[6] the ORR of atezolizumab plus bevacizumab in unresectable HCC patients was 27.3%. The lack of evidence of atezolizumab, bevacizumab and radiotherapy in patients with HCC and PVTT led to the difficulty of an evidence-based estimated ORR. Instead, we searched for literatures of other systemic regimens combined with radiotherapy and found out the ORR was 61.1% in HCC patients with PVTT treated with sorafenib and MIRT in a retrospective study.[23] Hence, assuming an ORR of 47% after discussion among investigators based on the experience, with a two-sided alpha error of 0.1, 90% power, and 10% dropout rate, the required number of evaluable patients is 42.

The efficacy assessment is based on the full analysis set (FAS). The intention-to-treat (ITT) efficacy analysis will be performed for subjects who received at least one session of the study treatment based on the FAS. No supplementation will be made for missing values. The safety analysis set (SAS) includes all enrolled subjects who have received at least one session of study treatment and have a post-treatment safety record. This dataset will be used for safety analysis.

Monitoring

An independent Data and Safety Monitoring Committee has been established to assess the safety data if serious adverse events occur. Any adverse events will be registered. A qualified and independent auditor is appointed to audit the trial systems, and the audit will be conducted before and during the study following a written procedure. The stopping rules for the trial are 1) the investigators find serious safety issues, or 2) the administrative department cancels the trial. The termination of the trial can be temporary or permanent. When terminating the trial, all records will be kept for future reference.

Patient and public involvement

The patients and general public were not involved in the trial design.

Discussion

Atezolizumab plus bevacizumab is the standard first-line therapy for unresectable HCC patients. Although the combination can significantly improve the prognosis in patients, the ORR is still not satisfactory.[6] Improving the therapeutic effect in patients is still a great clinical challenge. For patients with HCC, vascular invasion and metastasis are important reasons these patients cannot undergo surgical resection, as well as poor prognostic factors. Radiotherapy has an important role in treating patients with PVTT; however, the therapeutic effects are still limited. Previous clinical studies have shown that radiotherapy combined with targeted therapy, such as sorafenib, tends to prolong the overall survival of patients with HCC complicated by PVTT. In the meanwhile, a retrospective study reported the feasibility of radiotherapy combined with atezolizumab plus bevacizumab in unresectable HCC.[22] However, evidence on whether the combination of radiotherapy with atezolizumab plus bevacizumab can improve the therapeutic effects in unresectable HCC patients with PVTT is still warranted. Accordingly, this open-label, multicenter, single-arm clinical trial was

designed to preliminarily explore the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in naive unresectable HCC patients with PVTT.

There are several limitations in the present study that should be considered. First, this is a single-arm clinical trial without a control group that could be used to compare the efficacy and safety between treatment regimens. Second, since this study is an exploratory trial with a small sample size, no confirmatory efficacy conclusions can be drawn. The conclusions of this study may serve as preliminary evidence for subsequent large-scale, randomized, controlled clinical trials.

As the prognosis in unresectable HCC patients with PVTT remains poor, there is an urgent need to find an efficacious and safe therapeutic regimen. In this study, the efficacy and safety of IMRT combined with atezolizumab plus bevacizumab in native unresectable HCC patients with PVTT will be investigated so as to explore a new therapeutic regimen and further improve the efficacy and prognosis in these patients.

Ethics and dissemination

This study will be conducted according to the standards of Good Clinical Practice and in compliance with the principles of the Declaration of Helsinki. The Ethics Committee of our Hospital has approved the protocol (EHBHKY2021-K-017). All participants are required to provide written informed consent. The results of the trial will be published in peer-reviewed journals and presented at international conferences.

Trial status

The trial has been registered in ChiCTR.org (Registration number: ChiCTR2100049831; registration date: 2021-08-10; http://www.chictr.org.cn/showproj.aspx?proj=126593). Recruitment is ongoing. The protocol version number is 1.0. The study protocol has been reported in accordance with the Standard Protocol Items: Recommendations for Clinical Interventional Trials (SPIRIT) guidelines.

Acknowledgments

The authors would like to thank the patients, their families, and all the investigators who will participate in the present study.

Data Sharing Statement

We will widely disseminate the results of this clinical trial through conference presentations and publications in relevant journals. All evaluation forms, reports, and other records will be identified in a manner designed to maintain participants' confidentiality. All records will be kept in a secure storage area with limited access. Data during the study are available from the study leader upon reasonable request.

Authors' contributions

Kang Wang and Yan-Jun Xiang drafted the manuscript. Kang Wang, Yan-Jun Xiang, and Hong-Ming Yu contributed equally to this work. Shu-Qun Cheng, Kang Wang, Yan-Jun Xiang, and Hong-Ming Yu designed the trial. Yu-Qiang Cheng, Qian-Zhi Ni, Wei-Xing Guo, Jie Shi, Shuang Feng, and Jian Zhai are responsible for planning the data analysis and analyzing the data resulting from the trial. All named authors adhere to the authorship guidelines of Trials. All authors have agreed to publication. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no conflicts of interest to declare.

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SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P2/10
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	P10
Funding	4	Sources and types of financial, material, and other support	P14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P14
	5b	Name and contact information for the trial sponsor	P14
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P14
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P3-4

	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	P4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P5/16-21
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P5-6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P6-7
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P7

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P7
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P7
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P8
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P8
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P8
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	P8
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P8
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P8
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P9
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	P10
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P14
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P8
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating hepatocellular carcinoma with portal vein tumor thrombus: A study protocol

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Efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating hepatocellular carcinoma with portal vein tumor thrombus: A study protocol

Short running title: Radiotherapy plus T+A for HCC with PVTT

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Abstract

Introduction: Vascular invasion and metastasis are poor prognostic factors in patients with hepatocellular carcinoma (HCC). The efficacy of available therapeutic regimens for unresectable HCC is not satisfactory in HCC with portal vein tumor thrombosis (PVTT). Therefore, this open-label, single-arm phase II clinical trial aims to investigate the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in treating HCC patients with PVTT.

Methods and analysis: We plan to enroll patients diagnosed with unresectable hepatocellular carcinoma complicated by PVTT. Intensity-modulated radiotherapy (IMRT) combined with atezolizumab plus bevacizumab will be administered for treatment. Patients will initially receive radiotherapy, with each IMRT cycle lasting for 28 days and the total dose of tumor (DT) of 40 Gy/20 f/26 d. CT scan will be performed again, and the treatment plan will be reformulated after field constriction. The treatment will continue until the total DT is up to 54-56 Gy/27-28 f. The treatment with atezolizumab plus bevacizumab will be started at 3±1 d after the initiation of radiotherapy and will continue until unacceptable toxicity or disease progression. The primary endpoint is objective response rate (ORR), while the secondary endpoints include overall survival, disease control rate, progression-free survival, time to progression, duration of response, and the rate of surgical conversions. Assuming an ORR of 47%, with a two-sided alpha error of 0.1, 90% power, and a 10% dropout rate, the required number of evaluable patients is 42.

Ethics and dissemination: This study will be conducted according to the standards of Good Clinical Practice and in compliance with the principles of the Declaration of Helsinki. The Ethics Committee of our Hospital has approved the protocol (EHBHKY2021-K-017). All participants are required to provide written informed consent. The results of the trial will be published in peer-reviewed journals and presented at international conferences.

Registration details: ChiCTR.org, registration number: ChiCTR2100049831.

Keywords: *Hepatocellular carcinoma, portal vein tumor thrombus, radiotherapy, atezolizumab, bevacizumab*

Strengths and limitations of this study

- This study was a single-arm trial without a control group.
- This study is an exploratory trial with a small sample size.
- The conclusions of this study may serve as preliminary evidence for subsequent large-scale, randomized, controlled clinical trials.

Introduction

Liver cancer is the seventh most common cancer and the second most deadly cancer, with more than 900,000 new cases being diagnosed worldwide every year.[1] In China, the age-standardized annual incidence of liver cancer is about 26.67/100,000,[2] while the 5-year survival rate is only around 12%.[3] Hepatocellular carcinoma (HCC) is the most common histological subtype of primary liver cancer,[4] and surgery is the preferred and most efficacious approach for the treatment of HCC. However, some patients lose the chance for surgery at the time of diagnosis due to their intermediate to advanced tumors, liver dysfunction, etc. [5] Local therapies (including radiotherapy) and systemic therapies are important options for prolonging the survival of HCC patients who cannot undergo surgical resection. Due to the promising antitumor activity and favorable safety profile in the IMbrave150 trial, with the mortality risk being decreased by 42% compared with sorafenib,[6] atezolizumab plus bevacizumab have become the standard first-line therapy for unresectable HCC. Yet, the objective response rate (ORR) with atezolizumab plus bevacizumab in unresectable HCC patients was reported to be only 27.3%.[6] Hence, improving the therapeutic effect in patients is still a great clinical challenge.

Vascular invasion and metastasis are commonly seen in patients with intermediate to advanced-stage HC, as 44%-62% of patients with advanced HCC have been reported to develop portal vein tumor

thrombosis (PVTT).[7] In addition, the range and severity of PVTT have been significantly associated with the prognosis of unresectable HCC patients, with the median overall survival being just 2.7 to 4 months in naive HCC patients with PVTT.[8] However, there are significant differences between eastern and western countries in the treatment strategies used for HCC patients with PVTT.[9] Guidelines from western countries argue that HCC complicated with PVTT represents the advanced stage of HCC, advocating sorafenib as the only treatment option.[10-12] On the contrary, eastern countries adopt positive strategies, such as surgical resection, radiotherapy, transcatheter arterial chemoembolization (TACE), and surgery combined with multidisciplinary therapies, which are expected to improve the long-term survival of these patients.[13-16]

Radiotherapy is recommended for unresectable HCC patients with types I, II, and III PVTT, whose liver function is graded as Child-Pugh A and B. In addition, previous studies have shown that radiotherapy can synergistically enhance the antitumor effect of immunotherapy and targeted therapy by changing the tumor microenvironment.[17 18] At present, radiotherapy combined with immunotherapy and targeted therapy is currently one of the dominant research topics within the tumor area. A number of studies have also suggested that radiotherapy combined with sorafenib alone improves OS in patients with HCC complicated by PVTT,[19] and the feasibility of concurrent radiotherapy and sorafenib.[20 21] In addition, the results of a previous retrospective study showed that radiotherapy combined with atezolizumab plus bevacizumab was feasible in unresectable HCC.[22] However, whether the combination of radiotherapy and immunotherapy plus targeted therapy can improve the efficacy in patients with HCC complicated by PVTT needs to be further verified.

Therefore, we proposed an open-label, multicenter, single-arm phase II clinical trial. Patients diagnosed with locally advanced or metastatic and/or unresectable HCC complicated with PVTT will be preliminarily enrolled to explore the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab, which is expected to provide an efficacious therapeutic regimen for

HCC patients with PVTT and further improve the treatment response and prognosis. Here, we present the protocol in accordance with the SPIRIT reporting checklist.

Methods and analysis

Study design and objective

This open-label, multicenter, single-arm clinical trial will enroll patients diagnosed with locally advanced or metastatic and/or unresectable HCC complicated with PVTT from the departments of hepatobiliary surgery of our Hospitals between August 2021 and December 2021 so as to initially exploring the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in naive patients with unresectable HCC complicated by PVTT.

Eligibility criteria

The inclusion criteria are as follows:

1. Men or non-pregnant women aged 18 to 70 years. 2. Sign the informed consent. 3. The investigator believes that the patient has the ability to comply with the research protocol. 4. Hepatocellular carcinoma (HCC) is diagnosed by histology or cytology or clinically. Patients with liver cirrhosis are clinically diagnosed by AASLD criteria, and patients without liver cirrhosis need to be diagnosed by histology. 5. Imaging examination confirmed the existence of portal vein tumor thrombus. 6. The disease is not suitable for radical surgery. 7. Have not received any anti-tumor therapy before. 8. At least 1 measurable (measurable according to RECIST1.1), untreated lesions. 9. Pre-treatment tumor tissue samples (if available). If tumor tissue is available, submit 1 formalin-fixed, paraffin-embedded (FFPE) tumor sample in a paraffin block (preferred), or approximately 10-15 slides containing unstained, freshly cut, serial sections radiographs, together with a relevant pathology report within 4 weeks of enrollment. If the FFPE samples described above are not available, any type of sample (including fine needle aspiration biopsy samples, cell mass samples [eg, from pleural

effusions], and lavage samples) is acceptable. A relevant pathology report should be provided with the sample. If tumor tissue was not available (e.g., exhausted due to previous diagnostic testing), the patient was still eligible to participate in the study. 10. ECOG performance status score of 0 or 1 within 7 days before enrollment. 11. Child-Pugh Grade A within 7 days before enrollment. 12. Adequate hematology and organ function, based on the following laboratory test results obtained within 7 days prior to enrollment (unless otherwise stated): (1) Absolute neutrophil count $\geq 1.5 \times 10^9$/L (1500/μL), without granulocyte colony-stimulating factor support; (2) Lymphocyte count $\geq 0.5 \times 10^9$/L (500/μL); (3) Platelet count $\geq 75 \times 10^9$/L (75000/μL), no blood transfusion; hemoglobin ≥90 g/L, in order to meet this criterion, blood transfusion is allowed to the patient; (4) Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase ≤5 times the upper limit of normal (ULN); (5) Serum bilirubin ≤3 times the ULN; (6) Serum creatinine ≤1.5 times the ULN or calculated creatinine clearance ≥50 mL/min (calculated using the Cockcroft-Gault formula); (7) Serum albumin ≥28 g/L (2.8 g/dL); (8) Patients not receiving anticoagulation therapy: international normalized ratio or activated partial thromboplastin time ≤2 times the ULN; (9) Proteinuria < 2+ in urine cellulose test strip (performed within 7 days before the initiation of study treatment); (10) Patients with a baseline cellulose dipstick urine test result of ≥2+ proteinuria should collect 24-hour urine, and then must confirm that the urine protein content within 24 hours is less than 1 g. 13. Any acute, clinically significant treatment-related toxicity (due to previous treatment) must have been alleviated to ≤grade 1 before enrolling in the study, except for alopecia; 14. Human immunodeficiency virus antibody test results are negative at the time of screening; 15. Patients with active hepatitis B virus (HBV) infection: HBV-DNA <500IU/mL obtained within 28 days before the start of study treatment, and received anti-HBV treatment for at least 14 days before entering the study (treatment according to local standard treatment, such as entecavir) and are willing to continue receiving treatment during the study; 16. Women of childbearing age must have a negative pregnancy test before starting treatment, and women of childbearing age and men (having sex with women of childbearing age) must agree to use

without interruption during treatment and for 6 months after the last therapeutic dose is administered to be effective Contraception.

The exclusion criteria are as follows:

1. History of leptomeningitis. 2. Current or past autoimmune disease or immunodeficiency, including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid antibody syndrome, Wegener's granulomatosis, Sjgre's syndrome, Guillain-Barré syndrome, or multiple sclerosis, with the following exceptions: patients with prior autoimmune-related hypothyroidism receiving thyroid hormone replacement therapy are eligible for study participation; patients receiving insulin therapy Patients with controlled type 1 diabetes were eligible for study participation; only patients with dermatologically manifested eczema, psoriasis, chronic lichen simplex, or vitiligo (eg, excluding patients with psoriatic arthritis), provided All of the following conditions are eligible to participate in the study: (1) The rash area must be less than 10% of the body surface area; (2) Good disease control at baseline, requiring only low-efficiency topical glucocorticoid therapy; (3) In the past 12 months, the pre-existing conditions did not require psoralen plus A-band UV radiation, methotrexate, vitamin A acid, biological agents, oral calcineurin inhibitors, or highly effective or Acute exacerbations of oral glucocorticoid therapy. 3. Idiopathic pulmonary fibrosis, organizing pneumonia (eg, bronchiolitis obliterans), drug-induced pneumonia, or idiopathic pneumonia or evidence of active pneumonia on chest computed tomography (CT) images during screening. Radiation pneumonitis in the permissible radiation zone (fibrosis). 4. Known active tuberculosis. 5. Major cardiovascular disease within 3 months prior to initiation of study treatment (such as New York Heart Association class II or more severe heart disease, myocardial infarction or cerebrovascular accident within 3 months prior to initiation of study treatment), unstable Arrhythmia or unstable angina. 6. History of congenital long QT syndrome or corrected QT interval at screening >500ms (calculated using Fridericia method). 7. History of uncorrectable serum potassium, calcium or magnesium electrolyte disturbances. 8.

Received major surgery (except for diagnosis) within 4 weeks before starting the study treatment or is expected to undergo major surgery during the study period. 9. Malignant tumors other than HCC within 5 years prior to screening, except for malignancies with negligible risk of metastasis or death (eg, 5-year OS rate >90%), such as adequately treated in situ cervical cancer, Non-melanoma skin cancer, localized prostate cancer, ductal carcinoma in situ, or stage I uterine cancer. 10. Severe infection within 4 weeks prior to initiation of study treatment, including but not limited to hospitalization due to complications of infection, bacteremia or severe pneumonia. 11. Oral or intravenous therapeutic antibiotics within 2 weeks prior to initiation of study treatment. Patients receiving prophylactic antibiotics (eg, to prevent urinary tract infections or exacerbations of chronic obstructive pulmonary disease) are eligible to participate in the study. 12. Previous allogeneic stem cell or solid organ transplantation. 13. Received a live attenuated vaccine within 4 weeks before starting study treatment, or is expected to receive such a vaccine during atezolizumab treatment or within 5 months after the last dose of atezolizumab. 14. Patients with untreated or incompletely treated esophageal and/or gastric varices with associated bleeding or at high risk of bleeding. Before enrollment, patients must undergo B-ultrasound, CT, MRI, or liver elastography to assess the size of all varicose veins (small to large) and treat according to local standard of care. Patients who have received corresponding examinations within 6 months before starting study treatment do not need to be reexamined. 15. Co-infection with HBV and hepatitis C virus (HCV). Patients with a history of HCV infection but negative PCR results for HCV RNA can be considered HCV-uninfected. 16. Symptomatic, untreated or progressively progressive central nervous system (CNS) metastases. Asymptomatic patients with treated CNS lesions were eligible for study participation as long as all of the following criteria were met: Must have disease outside the CNS measurable according to RECIST v1.1; patients had no history of intracranial or intraspinal hemorrhage; metastases limited to the cerebellum or supratentorial (ie, no midbrain, pons, medulla, or spinal cord metastases); no evidence of progression between completion of CNS-directed therapy and initiation of study treatment; patients not receiving stereotaxic within 28 days prior to initiation of study treatment

Targeted, whole-brain radiotherapy, and/or neurosurgical resection; patients did not require continuous glucocorticoid therapy for CNS disease. Dose-stabilized anticonvulsant therapy is permitted. Asymptomatic patients with newly detected CNS metastases at screening are eligible to participate in the study after radiotherapy or surgery without repeat screening brain scan results. 17. The patient cannot receive follow-up or is participating in other clinical trials. 18. Subjects deemed unsuitable for inclusion in this study by the investigator..

Treatment plan

Patients will initially receive intensity-modulated radiotherapy (IMRT) with each cycle for 28 days. A treatment plan will be established after CT location. The clinical target volume (CTV) includes tumors and tumor thrombi. The planning target volume (PTV) will be expanded by 0.5-1.0 cm, with conventional fractionation at a single dose of 180-220 cGy. Total DT will be 40 Gy/20 f/26 d. CT scan will be performed at the end of every cycle, and the treatment plan will be reformulated after field constriction. The treatment will continue until the total DT is up to 54-56 Gy/27-28 f.

The treatment with atezolizumab plus bevacizumab will be started at 3±1 d after the initiation of IMRT. Each treatment cycle will last for 21 days. On the first day of each cycle, atezolizumab injection will be intravenously administered at a fixed dose of 1,200 mg (initial intravenous injection for 60 min, and subsequent intravenous injections for 30 min if well-tolerated). Bevacizumab at a dose of 15 mg/kg will be intravenously administered at least 5 min apart (initial intravenous injection for 90 min, and subsequent intravenous injections reduced to 60 min and 30 min if well-tolerated). The treatment was discontinued when there was no additional clinical benefit, as judged by the investigator (based on imaging, biochemical indicators, and clinical status of the patient). Then, the patient received the guideline-recommended second-line therapy.

Delayed treatment or dose reduction will be determined by the clinical team, and the specific adjustment plan will be determined by the investigator based on the patient's clinical condition. In

case of severe toxicity, the administration will be delayed, and/or the dose will be reduced. If any serious adverse event (SAE) occurs during the radiotherapy, or the radiotherapy is delayed for more than 4 weeks for any reason, radiotherapy sessions will be terminated. The doses of atezolizumab and bevacizumab will be adjusted according to the drug's instructions.

Endpoints

The primary endpoint is ORR, which is defined as the proportion of patients with Complete Response (CR) and Partial Response (PR). CR or PR is assessed in accordance with Response Evaluation Criteria In Solid Tumors (RECIST) 1.1. The responses will be independently evaluated by three to six experienced senior physicians who are not investigators in this study.

Secondary endpoints include (1) OS, defined as the time between the first study treatment and death (for any reason). (2) Disease control rate (DCR), defined as the percentage of subjects with the best response as CR, PR, or stable disease (SD). (3) Progression-free survival (PFS), defined as the time between the first treatment and the first tumor progression or appearance of a new lesion or death due to any cause, whichever occurs first. (4) Time to progression (TTP), defined as the time between the first treatment to first tumor progression. (5) Duration of response (DOR), defined as the time from first tumor remission to first tumor progression or death due to any cause. The DCRs, PFSs, TTPs, and DORs will be calculated in accordance with the RECIST version 1.1, modified RECIST (mRECIST), and immune-modified RECIST (imRECIST), respectively. (6) The rate of surgical conversions is defined as the proportion of subjects who receive the study treatment and are assessed to be viable for surgical resection. The resectability criteria include successful down-staging of the tumor, sufficient future liver remnant (FLR), and technically resectable assessed by the investigator. The definition of successful downstaging was based on the CSCO guideline, which was 1) stage Ia, Ib, and IIa, 2) IIb, if the tumor is confined to the same segment or the same side of the liver or intraoperative radiofrequency ablation can be performed at the same time to treat the lesions outside the resection range, and 3) IIIa/b, if the tumor is confined to the hemi-liver, and it is expected that the

tumor thrombus can be completely removed during the operation, surgical resection of the tumor and thrombectomy through the portal vein can be considered.

Adverse events (AEs) are assessed in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE V5.0). Monitoring will be performed from the date on which the patient signs the informed consent to 90 days after the last treatment.

Biomarkers for exploratory endpoints include metagenomic sequencing of fecal organisms, five gene indexes of hepatocellular carcinoma (HN1, RAN, RAMP3, KRT19, TAF9), immune correlation detection (programmed death-ligand 1 [PD-L1] expression, CD8 expression; microsatellite instability [MSI], and tumor mutational burden [TMB], immune-related gene expression, RNA-seq) and other checkpoint proteins and cell surface markers.

Participant timeline

Table 1 lists the time points for assessing efficacy, adverse events, laboratory safety assessments (hematology, coagulation, and chemistry), physical examination, electrocardiogram (ECG), and tumor measurements.

Table 1. Schema of Single-arm Clinical Trial

Item	Screening Period		Treatment Period			Perioperative Period	End of Trial	Survival Follow-up
	-28 - 0d	-7 - 0d	4 weeks after the first treatment session	7 weeks after the first treatment session	n cycles		30 days after checkout	
Window period (days)	——	——					±7	
Informed consent	×							
Demographics								
Past medical history	×							
History of tumor treatment								
Physical examination		×	×	Every 3 weeks		×	×	
Vital signs		×	×	Every 3 weeks		×	×	
ECOG		×	×	Every 3 weeks ×			×	
Pregnancy test		×					×	
Infection screening	×						×	
Imaging	×		×	Every 6 weeks			×	×
Tumor markers		×	×	Every 6 weeks			×	
Echocardiography	×						×	
Tumor tissue	×							
12-lead electrocardiogram		×	When clinically indicated				×	
Blood biochemistry		×	×	Every 3 weeks			×	
Routine blood test		×	×	Every 3 weeks			×	

Routine urine test		×	×	Every 3 weeks		×	
Routine stool test		×	×	Every 3 weeks		×	
Thyroid function, pituitary function, coagulation		×	×	Every 3 weeks		×	
Gut microbiome testing for the stool sample		×		×		×	
Biomarker testing for the blood sample		×		×		×	
Surgical resection					×		
Surgical complications					×		
Concomitant medication	×	×	×	Every 3 weeks		×	
Adverse events	×	×	×	Every 3 weeks		×	
Compliance evaluation	×	×	×	Every 3 weeks			
Dispensing of drugs			×	Every 3 weeks			
Recovery of drugs			×	Every 3 weeks		×	
Follow-up on survival status and antitumor treatment							×

Furthermore, for the testing of biomarkers as exploratory endpoints, stool and blood samples will be collected from patients prior to the first treatment, at 7 weeks after the first treatment, and after treatment discontinuation, respectively.

Data collection and management

The completed CRFs will be submitted to the respective sites for archiving. All data will be recorded in an electronic case report form (eCRF) by investigators or clinical research coordinators. All study documents will be confidential. All study data, including confirmation of all patients (effective check on recorded information, such as study cases), all original copies of signed ICFs, as well as detailed original records of drug distribution, shall be kept uniformly by the study institution until 5 years after the end of the trial.

Statistical methods

According to the results of IMbrave150,[6] the ORR of atezolizumab plus bevacizumab in unresectable HCC patients was 27.3%. The lack of evidence of atezolizumab, bevacizumab and radiotherapy in patients with HCC and PVTT led to the difficulty of an evidence-based estimated ORR. Instead, we searched for literatures of other systemic regimens combined with radiotherapy and found out the ORR was 61.1% in HCC patients with PVTT treated with sorafenib and MIRT in a retrospective study.[23] Hence, assuming an ORR of 47% after discussion among investigators based on the experience, with a two-sided alpha error of 0.1, 90% power, and 10% dropout rate, the required number of evaluable patients is 42.

The efficacy assessment is based on the full analysis set (FAS). The intention-to-treat (ITT) efficacy analysis will be performed for subjects who received at least one session of the study treatment based on the FAS. No supplementation will be made for missing values. The safety analysis set (SAS) includes all enrolled subjects who have received at least one session of study treatment and have a post-treatment safety record. This dataset will be used for safety analysis.

Monitoring

An independent Data and Safety Monitoring Committee has been established to assess the safety data if serious adverse events occur. Any adverse events will be registered. A qualified and independent auditor is appointed to audit the trial systems, and the audit will be conducted before and during the study following a written procedure. The stopping rules for the trial are 1) the investigators find serious safety issues, or 2) the administrative department cancels the trial. The termination of the trial can be temporary or permanent. When terminating the trial, all records will be kept for future reference.

Patient and public involvement

The patients and general public were not involved in the trial design.

Discussion

Atezolizumab plus bevacizumab is the standard first-line therapy for unresectable HCC patients. Although the combination can significantly improve the prognosis in patients, the ORR is still not satisfactory.[6] Improving the therapeutic effect in patients is still a great clinical challenge. For patients with HCC, vascular invasion and metastasis are important reasons these patients cannot undergo surgical resection, as well as poor prognostic factors. Radiotherapy has an important role in treating patients with PVTT; however, the therapeutic effects are still limited. Previous clinical studies have shown that radiotherapy combined with targeted therapy, such as sorafenib, tends to prolong the overall survival of patients with HCC complicated by PVTT. In the meanwhile, a retrospective study reported the feasibility of radiotherapy combined with atezolizumab plus bevacizumab in unresectable HCC.[22] However, evidence on whether the combination of radiotherapy with atezolizumab plus bevacizumab can improve the therapeutic effects in unresectable HCC patients with PVTT is still warranted. Accordingly, this open-label, multicenter, single-arm clinical trial was

designed to preliminarily explore the efficacy and safety of radiotherapy combined with atezolizumab plus bevacizumab in naive unresectable HCC patients with PVTT.

There are several limitations in the present study that should be considered. First, this is a single-arm clinical trial without a control group that could be used to compare the efficacy and safety between treatment regimens. Second, since this study is an exploratory trial with a small sample size, no confirmatory efficacy conclusions can be drawn. The conclusions of this study may serve as preliminary evidence for subsequent large-scale, randomized, controlled clinical trials.

As the prognosis in unresectable HCC patients with PVTT remains poor, there is an urgent need to find an efficacious and safe therapeutic regimen. In this study, the efficacy and safety of IMRT combined with atezolizumab plus bevacizumab in native unresectable HCC patients with PVTT will be investigated so as to explore a new therapeutic regimen and further improve the efficacy and prognosis in these patients.

Ethics and dissemination

This study will be conducted according to the standards of Good Clinical Practice and in compliance with the principles of the Declaration of Helsinki. The Ethics Committee of our Hospital has approved the protocol (EHBHKY2021-K-017). All participants are required to provide written informed consent. The results of the trial will be published in peer-reviewed journals and presented at international conferences.

Trial status

The trial has been registered in ChiCTR.org (Registration number: ChiCTR2100049831; registration date: 2021-08-10; http://www.chictr.org.cn/showproj.aspx?proj=126593). Recruitment is ongoing. The protocol version number is 1.0. The study protocol has been reported in accordance with the Standard Protocol Items: Recommendations for Clinical Interventional Trials (SPIRIT) guidelines.

Acknowledgments

The authors would like to thank the patients, their families, and all the investigators who will participate in the present study.

Data Sharing Statement

We will widely disseminate the results of this clinical trial through conference presentations and publications in relevant journals. All evaluation forms, reports, and other records will be identified in a manner designed to maintain participants' confidentiality. All records will be kept in a secure storage area with limited access. Data during the study are available from the study leader upon reasonable request.

Authors' contributions

Kang Wang and Yan-Jun Xiang drafted the manuscript. Kang Wang, Yan-Jun Xiang, and Hong-Ming Yu contributed equally to this work. Shu-Qun Cheng, Kang Wang, Yan-Jun Xiang, and Hong-Ming Yu designed the trial. Yu-Qiang Cheng, Qian-Zhi Ni, Wei-Xing Guo, Jie Shi, Shuang Feng, and Jian Zhai are responsible for planning the data analysis and analyzing the data resulting from the trial. All named authors adhere to the authorship guidelines of Trials. All authors have agreed to publication. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no conflicts of interest to declare.

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SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P2/10
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	P10
Funding	4	Sources and types of financial, material, and other support	P14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P14
	5b	Name and contact information for the trial sponsor	P14
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P14
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P3-4

	6b	Explanation for choice of comparators	NA
Objectives	7	Specific objectives or hypotheses	P4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P5/16-21
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P5-6
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P6
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P6
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P6-7
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P7

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P7
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P7
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P8
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P8
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P8
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	P8
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P8
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P8
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P9
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	P10
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P14
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P8
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.