Prospective surveillance for very early hepatocellular carcinoma (PRECAR): a study protocol for an observational multicenter cohort trial

NCT number: 03588442

Date:2020.4.30

Version: v1.0

Abstract

Background: Approximately 20% patients are diagnosed to hepatocellular carcinoma at early stage, when effective treatments are feasible, and benefit from curative therapeutic approaches, such as hepatic resection and liver transplantation. Biannual ultrasound is recommended for patients with cirrhosis for early diagnosis of hepatocellular carcinoma. However, limited sensitivity and false-positivity call for development of a new surveillance tool with prior accuracy. This study aims to build a biosignature to stratify high risk population for developing hepatocellular carcinoma and to diagnosis hepatocellular carcinomas at very early stage using biological signature.

Methods: This investigator-initiated, multicenter, observational, cohort trial will enroll 5000 patients with liver cirrhosis and 5000 patients with hepatitis B virus infection. Exclusion criteria include human immunodeficiency virus infection, previous diagnosis of active pulmonary tuberculosis, and pregnancy. Patients will be recruited for 1 year and follow-up investigation will be performed biannually for 3 years to collect demographic and clinical characteristics, blood samples, disease status, and outcome data. Development of hepatocellular carcinoma and overall survival are the primary endpoints, which will be contributed to the development of the biosignature for risk stratification for incidence, diagnosis at very early stage, and survival prediction. The secondary endpoints include development of non-hepatocellular carcinoma hepatic malignancy, liver resection rate, postresection survival, and relapse-free survival.

Discussion: The biosignature will be useful in clinical practice by stratifying patients with cirrhosis or hepatitis B virus infection at high risk for development of hepatocellular carcinoma and diagnosing at very early stage.

Trial registration: ClinicalTrials.gov identifier, NCTxx, first received xx, xx 2018.

Key words: liver cancer, diagnosis, early stage, observational cohort trial, biosignature, prediction model

Introduction

China has among the highest incidence and mortality of hepatocellular carcinoma (HCC) in the world with increasing trend.[1,2] Prognosis of HCC is variable according to different stages due to feasibility of therapeutic options.[3] Radical resection of tumor is recommended for patients with a solitary tumor and preserved liver function without extrahepatic metastasis, whereas liver transplantation provides survival benefits for unresectable cases within Milan criteria (solitary tumor ≤ 5 cm or up to three nodules ≤ 3 cm).[4,5] In addition, image-guided ablation is the most frequently applied strategy with limitations of size and localization of the tumor.[6] For multifocal disease without vascular invasion and extrahepatic metastasis, chemoembolization has been suggested to be promising therapy for improvement of prognosis.[7] Despite candidate selection criteria according to the disease status, recent advances revealed that diagnosis at an early stage is the optimum approach for to achieve favorable prognosis by offering curative treatment options.[8]

To date, approximately 20% of patients with HCC are diagnosed at early stage and benefit from potentially curative ablative treatments.[9] According to a study involving 5 Dutch centers, surveillance (≥ 2 screening tests during 3 preceding years and at least once of imaging test within 18 months before development of HCC) was found to be an independent prognostic factor that predicts favorable outcomes.[10] A randomized controlled trial including 18,816 patients with hepatitis B virus (HBV) infection demonstrated that surveillance by biannual α-fetoprotein test and ultrasonography examination ameliorated HCC-related mortality from 83.2/100,000 (screening group) to 131.5/100,000 (control group) with a hazard ratio (HR) of 0.63 (95% CI, 0.41 to 0.98), which indicates 37% of decrease in HCC-related mortality.[11]

Although a validation trial is yet to be carried out, since the only opportunity to provide effective treatments with long-term survival is the detection of the tumor at an early stage, surveillance is crucial predictor for favorable prognosis.

Justification of inclusion criteria for a surveillance program is determined by the risk of HCC life expectancy, and cost-effectiveness.[12] Regarding absence of experimental data, the decision for patient inclusion is based on its efficacy evaluated by heterogeneous designs, indicating that the efficacy of a surveillance program is dependent to incidence of HCC. Accordingly, populations with liver cirrhosis and HBV infection are currently recommended for surveillance.[13]

Ultrasonography and serum tumor markers are the preferred tests for surveillance of HCC based on toleration, availability, non-invasiveness, and sensitivity. Ultrasonography has a reported sensitivity of 60 to 80%, whereas α-fetoprotein was approximately 60% with the most efficient cut-off (10 to 20 ng/mL).[14,15] Combination of ultrasonography and α-fetoprotein may increase accuracy, but also increases false-positivity and cost. Regarding importance of diagnosis of HCC at early stage, there is an unmet need for a well-made surveillance model to detect early HCC with priority in accuracy and sensitivity; identification of populations at high risk before development of HCC may be ideal if high sensitivity is achieved. In addition, it may prevent up to 6 months of disease progression and improve diagnostic sensitivity, ultimately improving prognosis.

The Prospective Surveillance for Very Early Hepatocellular Carcinoma (PRECAR) trial is designed as an observational multicenter cohort trial to conduct a signature to detect and define very early HCC and individuals at high risk of HCC by analyzing genome sequencing, plasma-

free DNA sequencing, and high-throughput protein expression screening.

Methods and Design

Ethics

The PRECAR trial will be conducted in accordance with the Declaration of Helsinki and current Good Clinical Practice guidelines. The relevant ethics committee approval will be obtained before enrolment of patients. Informed consents will be obtained from all participants before enrollment.

Eligible population

The inclusion and exclusion criteria for the PRECAR trial are described in Table 1. Briefly, two independent cohorts (cirrhosis cohort and HBV infection cohort) will be recruited for surveillance of HCC. The cirrhosis cohort will be consisted of patients with biopsy diagnosis or distinctive clinical characteristics of cirrhotic liver regardless etiology of cirrhosis. For HBV infection cohort, seropositivity of hepatitis B surface antigen is the basic condition for inclusion.

Overview of trial design

PRECAR is a prospective, observational, multicenter trial surveilling development of HCC in patients with cirrhosis or HBV infection. In PRECAR, the aim will be to recruit a total of 10,000 patients (5000 each for the two cohorts) from a minimum of 10 sites. Follow-up will be carried out for eligible patients for collection of demographic, clinical, and serological characteristics and samples for analyses. For those who developed HCC, hepatic resection will be the standard

of care unless unresectable. Data of interest and schedule for the PRECAR trial are summarized in Table 2.

Outcome measures

The primary endpoints of the PRECAR trial include development of HCC, overall survival (OS), and liver-related disease progression (LDP). Secondary endpoints are development of non-HCC malignant neoplasm, such as intrahepatic cholangiocarcinoma, liver resection rate (LRR), and postoperative overall (POS) and relapse-free survival (PRFS) after development of liver malignant neoplasm.

Outcome definitions

HCC – the time from the date of inclusion to development of HCC.

OS – the time from the date of inclusion to death from any cause.

LDP – the time from the date of inclusion to non-malignancy-associated liver-related disease progression or death, whichever is earlier.

Non-HCC – the time from the date of inclusion to development of non-HCC malignant neoplasm.

LRR – the number of malignancy-developed patients whose tumor is resectable without vascular invasion and extrahepatic metastasis.

POS – the time from the date of liver resection to death from any cause.

PRFS – the time from the date of liver resection to tumor progression in the liver or death, whichever is earlier.

Blood collection for biosignature analysis

Peripheral blood samples (25 ml) will be collected from all consenting patients at first 3 follow-up (0, 6th, and 12th month), which will be used to develop a biosignature to stratify patients at high risk for development of HCC. After development of a biosignature, 20 ml of peripheral blood samples will be collected from patients at high risk for HCC at 4th to 7th follow-up for modification and validation of the biosignature.

Statistical analysis

All statistical analyses will be performed using R (http://www.r-project.org/) or SPSS (Chicago, IL). Cox hazards proportional model and logistic regression will be applied to screen factors for the inclusion in a biosignature. Correlation test will be performed between results of multivariate Cox and logistic regressions, and false-positive factors will be deleted. After screening covariates, receiver operating characteristic (ROC) curves will be constructed to calculate optimum threshold for setting of cut-off value to define high risk for HCC. Independent demographic and clinical prognostic factors may be combined with the biosignature to improve performance sensitivity and specificity. Predictive signatures for cirrhosis cohort and HBV infection cohort will be developed independently. A correction for multiple testing may be applied.

Discussion

The PRECAR trial will identify predictors for development of HCC and diagnosis of very early

HCC by comparing HCC cases arising from cirrhosis or HBV infection with non-HCC developing cases. To the best of our knowledge, no prospective observational cohort trial has been published with intent to develop a biosignature to stratify patients at high risk for HCC. However, a case control study with similar design (miRNA as a diagnostic tool for HCC) is ongoing in South Africa, where HIV is hyper endemic, thus the etiology of HCC is different from the PRECAR.[16] In addition, ideal aim of the PRECAR trial is to successfully stratify patients who have high potential to develop HCC and carry out intensive follow-up to prevent up to 6 months of disease progression, as well as to offer curative therapeutic options at very early stage to improve prognosis.

Surveillance for HCC has been introduced as an effective strategy to improve prognosis of patients with HCC.[17] However, this field remains to be considered immature due to absence of validation of previous studies and limited accuracy that reduced cost-effectiveness. In recent years, the development of powerful tools, such as genome sequencing, plasma-free DNA sequencing, and high-throughput protein expression screening, suggests that the establishment of a biosignature could improve sensitivity and specificity of current surveillance method. The current surveillance, which is consisted of biannual ultrasonography and α-fetoprotein, reveals limited accuracy, false-positivity, and not efficient in a timely manner. Especially, these are confined to detect already-developed HCC, indicating that if HCC occurred a month after routine surveillance, the tumor will progress for 5 followed months before the next surveillance. Regarding aggressive characteristics and rapid progression, advancement of the tumor is likely to derive patient a worse candidate for curative treatments and deteriorates survival outcomes.

The biosignature developed from PRECAR trial will improve predictive and diagnostic

accuracy for HCC in settings with cirrhosis and HBV infection. During the establishment of the PRECAR biosignature, developmental process of HCC will be further elucidated and translated to improve predictive and diagnostic accuracy. We believe that the PRECAR biosignature will be useful in stratifying patients with cirrhosis or HBV infection at high risk for HCC, as well as in diagnosing HCCs at very early stage.

Trial status

The PRECAR trial is currently ongoing. Patient recruitment will be closed on 15 July 2019 with estimated participant number of 10,000 (5000 for cirrhosis cohort and 5000 for HBV infection cohort). The final analysis will be triggered after 3 years of additional follow-up for enrolled patients for validation of the PRECAR biosignature.

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Table 1. Patient eligibility criteria for PRECAR trial

	Cirrhosis cohort	**HBV infection cohort**
Inclusion	Aged between 35 and 70 years	Aged between 40 to 70 years
	Diagnosis of liver cirrhosis within recent 6 months	Chronic HBV infection (≥6 months)
	Satisfying one of below conditions:	ECOG performance status 0 to 1
	1. Metavir score of 4	Written informed consent provided
	2. Ishank score of 5 to 6	
	3. Ascites, hepatic encephalopathy, or variceal hemorrhage	
	4. Satisfying ≥ 2 of below conditions	
	● Imaging study indicating	
	● characteristics of liver cirrhosis	
	● Platelet count < 200×10^9/L	
	● ALT < 5 folds and liver hardness > 12 kPa	
	● Gastoesophageal varices from endoscopy	
	Child-Pugh score A to B	
	ECOG performance status 0 to 1	
	Written informed consent provided	
Exclusion	Hereditary metabolic liver diseases	Hereditary metabolic and autoimmune liver diseases
	HIV infection	HCV, HDV, HEV, or HIV infection
	Previous diagnosis of active pulmonary tuberculosis	Previous diagnosis of active pulmonary tuberculosis
	Diagnosis of malignant tumor before or during hospitalization	Diagnosis of malignant tumor before or during hospitalization
	Allogenic blood transfusion or cell therapy within recent 1 year	Allogenic blood transfusion or cell therapy within recent 1 year
	Pregnancy	Pregnancy
		Liver cirrhosis

ALT, alanine aminotransferase; ECOG, Eastern Cooperative Oncology Group; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus.

Table 2. PRECAR trial assessment schedule

Schedule	Follow-up						
	1st (0 m)	2nd (6th m)	3rd (12th m)	4th (18th m)	5th (24th m)	6th (30th m)	7th (36th m)
Informed consent	x						
Demographics	x						
General habits	x						
Medical and surgical history	x						
Concurrent diseases	x						
Concomitant medications	x						
Physical and clinical assessment							
Height	x						
Weight	x	x	x	x	x	x	x
Blood pressure	x	x	x	x	x	x	x
Body temperature	x	x	x	x	x	x	x
Routine blood test	x	x	x	x	x	x	x
Liver function test	x	x	x	x	x	x	x
Renal function test	x	op	op	op	op	op	op
Electrolyte test	x	op	op	op	op	op	op
Blood glucose	x						
Glycosylated hemoglobin	x						
Blood lipid	x						
Hepatitis virus	x						
HBV DNA[a]	x	x	x	x	x	x	x
HIV antibody	x						
Serum tumor markers	x	x	x	x	x	x	x
Ascites[b]	op	op	op	op	op	op	op
INR	x	x	x	x	x	x	x
Ultrasonography	x	x	x	x	x	x	x
Contrast enhanced CT or MRI[c]	x	op	op	op	op	op	op
Liver hardness	x	x	x	x	x	x	x
Child-Pugh score	x	x	x	x	x	x	x
Endpoints evaluation		x	x	x	x	x	x
Sample collection	x	x	x	x	x	x	x

HBV, hepatitis B virus; HIV, human immunodeficiency virus; INR, international normalized ratio; CT, computed tomography; MRI, magnetic resonance imaging.

[a]required for patients with HBV infection.

[b]required for patients with ultrasonography results indicating ascites.

[c]required for patients with ultrasonography results indicating hepatic nodules.