A standardized extract of cultured Lentinula edodes mycelia (AHCC®) as immune modulator in cancer patients treated with immunotherapy: a phase 2 double-blind, randomized, placebo-controlled trial

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1. Abstract

This is a prospective double-blind, randomized, placebo-controlled trial aims to investigate whether add-on of a standardized extract of cultured Lentinula edodes mycelia (AHCC®) can enhance the effect of immunotherapy in cancer patients.

AHCC is a newly developed functional food. In vitro studies have demonstrated that AHCC enhances natural killer cell activity and may serve as a potent biological response modifier for cancer treatment. Previous research involving 269 HCC patients found that 113 patients who took AHCC orally after curative surgery (AHCC group) experienced significantly longer recurrence-free survival (hazard ratio [HR], 0.639; 95% confidence interval [CI], 0.429-0.952; P = 0.0277) and overall survival (HR, 0.421; 95% CI, 0.253-0.701; P = 0.0009) compared to the control group.

This trial will adopt a two-arm Bayesian optimal phase 2 (BOP2) design (Zhao, 2020). The study will simultaneously evaluate efficacy and toxicity. The null hypothesis assumes a true response rate of 0.27 and a grade 3/4 toxicity rate of 0.57, while the alternative hypothesis assumes a true response rate of 0.42 and a grade 3/4 toxicity rate of 0.36. The trial will be conducted in two stages. In the first stage, 36 patients will be recruited and randomized using block randomization with a block size of 4. The study will be terminated early if fewer patients respond in the AHCC group than in the control group or if 2 or more patients in the AHCC group experience grade 3/4 acute toxicity compared to the control group. Otherwise, the second stage will proceed with the recruitment of an additional 58 patients, bringing the total sample size to 94. If, among the 47 patients in the AHCC group, at least 2 more patients respond compared to the control group or if 2 or fewer patients experience grade 3/4 toxicity, we will reject the null hypothesis and conclude that the treatment is promising. This design controls the type I error rate at 0.1 and achieves a power of 0.75. Considering a 5% dropout rate, each group will enroll 49 patients, resulting in a total sample size of 98.

Following the Screening period to confirm eligibility, 98 study participants, who meet the entry criteria, will be 1:1 randomized to study intervention (3g of AHCC oral per day or matching placebo) and will be stratified by AFP (<400 vs. ≥400). Enrollment is anticipated to be completed within 2 years, with each participant followed for 6 months since treatment. Therefore, the total duration of the study is expected to be approximately 2.5 years from the time of Institutional Review Board approval. All enrolled participants will receive AHCC or placebo 3g per day until disease progression, intolerance or patient or physician decision to discontinue. Tumor tissue and peripheral blood samples will be collected from all participants for biomarker analysis.

2. Background

Immune checkpoint inhibitors

In recent years, significant progress has been made, particularly in the areas of personalized medicine and cancer therapeutics. Immunotherapy, including adoptive cell transfer and immune checkpoint inhibitors (ICIs), harnesses the body's immune system to combat tumor cells[1]. Both as standalone treatments and in combination with traditional therapies like radiotherapy and chemotherapy, immunotherapy has achieved notable success in treating various cancers. Co-inhibitory receptors, such as programmed cell death 1 (PD-1) and cytotoxic T lymphocyte antigen

4 (CTLA-4), are expressed on T cells and negatively regulate T cell-mediated immune responses. Tumor cells exploit these receptors to induce immune tolerance and T cell exhaustion. ICIs, such as anti-CTLA-4, anti-PD-1, and anti-PD-L1 antibodies, target these receptors to reactivate the immune system against tumors. Three major groups of ICIs—PD-1 inhibitors (Nivolumab, Pembrolizumab, Cemiplimab), PD-L1 inhibitors (Atezolizumab, Durvalumab, Avelumab), and CTLA-4 inhibitors (Ipilimumab)—have been approved by the US Food and Drug Administration (FDA) for the treatment of various cancers. However, only a subset of patients (20–40%) benefit from this therapy, underscoring the need for reliable predictive biomarkers. Factors such as tumor mutational burden (TMB), PD-L1 expression, the microbiome, hypoxia, interferon-γ, extracellular matrix (ECM), and the molecular and cellular composition of the tumor microenvironment (TME) are all associated with immunotherapy outcomes.

The approval of ipilimumab in 2011 marked the beginning of ICIs as a novel treatment option, revolutionizing cancer therapy. These therapies have provided long-lasting results with relatively low toxicity in certain cases. Unlike traditional treatments, ICIs work by reactivating the immune system to combat tumor cells. Immune checkpoints regulate the balance between pro-inflammatory and anti-inflammatory signals in the body's immune system under normal conditions. These checkpoints are a collection of inhibitory and stimulatory pathways that control immune cell activity. In recent years, antibodies targeting immune inhibitory receptors such as CTLA-4, PD-1, and PD-L1 have become the most widely used immunotherapeutic agents.

A standardized extract of cultured Lentinula edodes mycelia (AHCC®)

AHCC® is a standardized extract of cultured shiitake or Lentinula edodes mycelia (AHCC®) which contains a mixture of nutrients including oligosaccharides, amino acids, and minerals obtained through the liquid culture process of shiitake mycelia. It is produced by Amino Up Co., Ltd. (Sapporo, Japan) under the trademark "AHCC®." The shiitake mycelia used for AHCC® are cultured in a liquid medium where the mycelia proliferate and form globular fungal bodies but not fruiting bodies. AHCC® is produced through the unique manufacturing process of culturing the mycelia followed by separation, sterilization, and freeze-drying. The most abundant component of AHCC® is oligosaccharides which comprise about 74% of the dry weight of AHCC®.

Polysaccharides are ubiquitous among fungi from yeast to mushroom, and these compounds impart structural properties to the organisms. Although the polymeric compositions of various fungal polysaccharides are unique and specific to each organism, the structural configurations are highly conserved[2]. Due to the ubiquitous presence of fungus in the environment and diet, mammalian immune systems have developed innate pattern recognition

systems for fungal polysaccharides. The interaction between fungal derived glucans and various cells of the immune system results in immunostimulatory effects, which in-turn prime the immune system for defense against potential invading microorganisms. The unique capacity of fungal derived glucans to act as biological response modifiers of the immune system has stimulated wide-spread research into their uses as functional foods. The immunological effects of AHCC have been investigated in numerous publications, and the product has been utilized as an immunostimulatory food for over 15 years in Japan. AHCC has been reported to improve the prognosis of patients with postoperative hepatocellular carcinoma[3], and improvements in the quality of life of patients with advanced liver cancer also have been reported. In studies conducted in rodents, AHCC has been shown to reduce metastasis of rat mammary adenocarcinoma[2], and to ameliorate the side-effects evoked by cisplatin chemotherapy in tumor-bearing mice. AHCC also may find utility against viral and microbial infections, and has been reported to increase survival in rodents following various viral and bacterial challenges.

The mechanism(s), and specific receptor mediated interaction(s) by which AHCC affects the immune system are not completely understood; however, studies in healthy subjects administered AHCC daily for a period of 4 weeks indicate that AHCC can modulate dendritic cell number and activity[4]. Thus, the utility of AHCC in cancer subjects and in various infectious models in rodents may be mediated through its ability to stimulate dendritic cells, which are potent antigen presenting cells that are able to prime T-cells. Finally, recent evidence also has suggested that the functional properties of AHCC may be multi-factorial; in addition to direct modulation of the immune system, AHCC also has been observed to attenuate inflammation in rats with hapten-induced colitis via prebiotic effects on the colonic microflora.

Safety profile of AHCC in preclinical model[2]

Figure 1. Body weight changes in male rats treated orally with AHCC for 90 days.

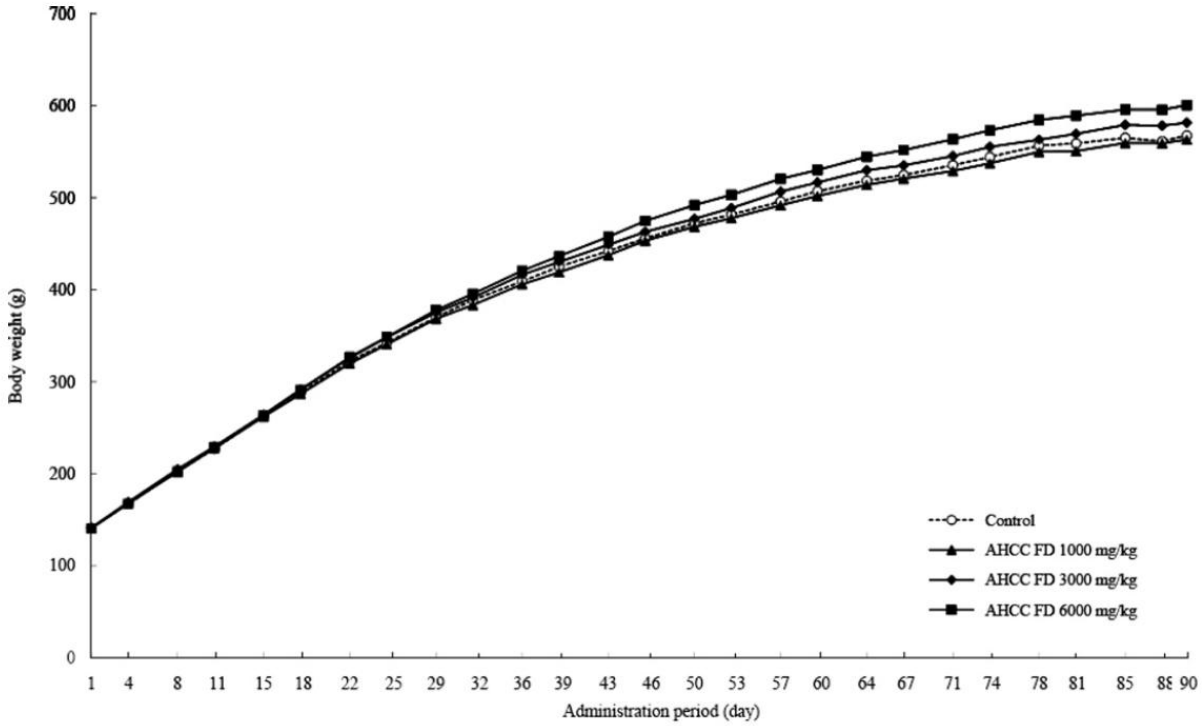


Table 1. Hematology results for male and female rats following daily gavage administration of AHCC-FD for a period of 90 days.

Parameter (units)	Dose group (mg/kg body weight/day)							
	Male (n = 10)				Female (n = 10)			
	0	1000	3000	6000	0	1000	3000	6000
RBC ($\times 10^4/\mu L$)	927.7 ± 32.5	924.4 ± 13.6	930.4 ± 25.6	910.7 ± 30.6	888.5 ± 26.0	861.5 ± 21.6	883.4 ± 32.3	864.1 ± 40.0
Ht (%)	46.71 ± 1.07	46.54 ± 1.17	46.82 ± 1.02	46.36 ± 1.44	47.36 ± 1.00	46.15 ± 1.25	46.75 ± 1.86	45.94 ± 1.79
Hb (g/dL)	15.72 ± 0.27	15.58 ± 0.43	15.66 ± 0.38	15.45 ± 0.54	16.39 ± 0.32	15.95 ± 0.51	16.12 ± 0.60	15.88 ± 0.59
MCV (fL)	50.38 ± 1.33	50.37 ± 1.32	50.33 ± 0.80	50.94 ± 1.61	53.33 ± 0.74	53.60 ± 1.23	52.93 ± 1.03	53.19 ± 0.92
MCH (pg)	16.96 ± 0.48	16.85 ± 0.43	16.85 ± 0.46	16.97 ± 0.54	18.47 ± 0.29	18.50 ± 0.49	18.25 ± 0.34	18.39 ± 0.43
MCHC (g/dL)	33.65 ± 0.27	33.49 ± 0.30	33.47 ± 0.54	33.34 ± 0.29	34.62 ± 0.36	34.56 ± 0.35	34.49 ± 0.21	34.57 ± 0.49
WBC ($\times 10^2/\mu L$)	87.6 ± 16.7	87.2 ± 11.9	85.0 ± 11.4	95.7 ± 16.2	53.0 ± 10.0	56.9 ± 12.8	53.8 ± 11.2	60.8 ± 8.0
Platelet count ($10^4/\mu L$)	95.69 ± 10.51	104.59 ± 9.37	102.11 ± 7.26	103.65 ± 11.11	103.24 ± 11.35	104.13 ± 9.37	104.98 ± 3.67	105.95 ± 11.26
Reticulocyte count (%)	28.0 ± 3.1	28.3 ± 2.4	25.7 ± 1.8	26.1 ± 2.8	24.4 ± 2.8	23.7 ± 4.6	22.0 ± 4.3	22.5 ± 2.5
PT (s)	17.01 ± 1.22	16.63 ± 1.01	16.83 ± 1.17	16.33 ± 0.67	16.57 ± 0.54	16.35 ± 0.39	16.79 ± 0.55	17.08 ± 0.43
APTT (s)	25.98 ± 2.11	24.79 ± 1.61	24.91 ± 2.07	24.56 ± 1.83	20.06 ± 1.12	20.02 ± 1.53	20.07 ± 1.21	19.57 ± 0.87
Differential WBC counts (%)								
Neutrophil (stab form)	1.60 ± 0.98	1.24 ± 0.72	2.04 ± 0.55	1.08 ± 0.91	1.16 ± 0.30	1.36 ± 0.43	1.12 ± 0.77	1.32 ± 0.68
Neutrophil (segmented)	15.00 ± 6.08	13.60 ± 3.35	16.12 ± 5.56	13.64 ± 4.02	17.52 ± 3.73	17.48 ± 5.72	18.52 ± 5.57	14.44 ± 3.03
Eosinophil	1.48 ± 0.71	2.00 ± 1.80	2.00 ± 1.08	0.96 ± 0.63	2.36 ± 1.12	1.72 ± 0.73	2.20 ± 1.51	1.24 ± 1.26
Basophil	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocyte	2.36 ± 0.97	2.40 ± 0.88	2.56 ± 1.18	2.12 ± 1.13	1.88 ± 0.96	2.56 ± 1.38	2.12 ± 1.03	1.24 ± 0.89
Lymphocyte	79.56 ± 7.33	80.76 ± 5.34	77.28 ± 6.70	82.20 ± 4.46	77.08 ± 3.79	76.88 ± 5.79	76.04 ± 6.68	81.76 ± 3.25
Others	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

All values represent the mean ± SD.
APTT, activated partial thromboplastin time; Hb, hemoglobin; Ht, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PT, prothrombin time; RBC, red blood cell count; WBC, white blood cell count.

Table 2. Urinalysis results for of male and female rats following 90-day gavage administration of AHCC-FD.

Parameters measured		Male				Female			
Dose (mg/kg body weight/day)		0	1000	3000	6000	0	1000	3000	6000
Number of animals examined		10	10	10	10	10	10	10	10
pH	6.0	0	0	0	2	0	0	1	3
	6.5	0	0	2	3	0	0	3	6
	7.0	0	0	2	4	0	0	1	1
	7.5	1	1	1	0	0	3	2	0
	8.0	3	3	2	0	1	2	1	0
	8.5	6	6	3	1++	9	5	2++	0++
Protein	–	1	0	0	0	3	0	0	0
	±	1	3	0	0	5	7	4	2
	1+	8	6	6	3	2	2	4	7
	2+	0	1	4	6	0	1	2	1
	3+	0	0	0	1++	0	0	0+	0++
Glucose	–	10	10	10	10	10	10	10	10
Ketone body	–	10	10	10	10	10	10	10	10
Urobilinogen	0.1 EU/dL	10	10	10	10	10	10	10	10
Bilirubin	–	10	10	10	10	10	10	10	10
Occult blood	–	4	4	8	7	9	7	9	8
	±	3	4	2	3	0	3	1	2
	1+	1	1	0	0	0	0	0	0
	2+	2	0	0	0	1	0	0	0
	3+	0	1	0	0	0	0	0	0
Specific gravity	1.000–1.010	0	0	0	0	0	1	0	0
	1.011–1.020	0	0	0	0	0	0	0	1
	1.021–1.030	1	0	0	0	2	2	2	0
	1.031–1.040	7	5	3	1	4	3	3	4
	1.041–1.050	1	4	4	5	2	4	3	4
	1.050<	1	1	3	4++	2	0	2	1
Urine volume	mL/21 h	17.05 ± 4.05	14.55 ± 2.15	14.40 ± 3.91	14.90 ± 3.44	10.35 ± 3.46	15.75 ± 13.21	12.20 ± 6.33	13.50 ± 8.82

Values are number of animals with findings.
–, normal; ±, slight; 1+, moderate; 2+, severe; 3+, very severe.
+., significantly different from the control groups ($p \leqslant 0.05$, Mann–Whitney's *U*-test).
++, significantly different from the control groups ($p \leqslant 0.01$, Mann–Whitney's *U*-test).

Table 3. Results of Ames reverse mutation experiments conducted with AHCC-FD in the absence (-S9) and presence of metabolic activation (+S9).

Metabolic activation	Concentration (μg/plate)	Number of revertant colonies per plate (number in parentheses represent the mean number of colonies)											
		TA102		TA1535		TA1537		TA100		TA104		TA98	
–S9	0 (negative control)	384		7		8		134		442		17	
		324		7		6		122		498		22	
		333	(347)	11	(8)	7	(7)	114	(123)	527	(489)	20	(20)
	156	383		11		8		–		–		–	
		386	(385)	10	(11)	7	(8)	–		–		–	
	313	403		7		5		118		118		15	
		379	(391)	7	(7)	7	(6)	112	(115)	588	(576)	9	(12)
	625	377		8		7		109		513		19	
		403	(390)	6	(7)	8	(8)	108	(109)	527	(520)	16	(18)
	1250	422		9		6		118		482		15	
		411	(417)	10	(10)	5	(6)	115	(117)	535	(509)	16	(16)
	2500	411		8		5		116		534		20	
		450	(431)	5	(7)	7	(6)	103	(110)	547	(541)	17	(19)
	5000	461		12		11		136		550		15	
		398	(430)	7	(10)	7	(9)	130	(133)	656	(603)	24	(20)
+S9	0 (negative control)	364		7		6		141		566		34	
		402		7		10		140		605		35	
		384	(383)	9	(8)	10	(9)	130	(137)	554	(575)	40	(36)
	156	395		13		8		–		–		–	
		402	(399)	7	(10)	12	(10)	–		–		–	
	313	424		7		7		150		556		29	
		392	(408)	11	(9)	8	(8)	140	(145)	451	(504)	25	(27)
	625	391		11		8		158		475		27	
		389	(390)	8	(10)	10	(9)	151	(155)	567	(521)	32	(30)
	1250	407		12		11		144		581		35	
		390	(399)	7	(10)	10	(11)	136	(140)	522	(552)	31	(33)
	2500	400		9		7		157		611		35	
		412	(406)	6	(8)	6	(7)	136	(147)	697	(654)	28	(32)
	5000	438		5		10		182		600		31	
		415	(427)	8	(7)	12	(11)	145	(164)	548	(574)	38	(35)
Positive Controls													
–S9	Positive control	MMC		NaN_3		9-AA		AF-2		AF-2		AF-2	
	Concentration (μg/plate)	0.05		0.5		80		0.01		0.01		0.1	
	No. of revertant colonies/plate	1297		200		194		688		1309		484	
		1343	(1320)	262	(231)	289	(242)	625	(657)	1283	(1296)	470	(477)
+S9	Positive control	2-AA		2-AA		2-AA		2-AA		2-AA		2-AA	
	Concentration (μg/plate)	5		2		2		1		5		0.5	
	No. of revertant colonies/plate	1518		322		182		1387		3072		299	
		1437	(1478)	310	(316)	197	(190)	1333	(1360)	2931	(3002)	344	(322)

2-AA, 2-aminoanthracene; 9-AA, 9-aminoacridine hydrochloride hydrate; AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide; MMC, mitomycin C; NaN_3, sodium azide.

Safety profile of AHCC in phase 1 clinical trial[4]

Table 4. Lab results in 26 health volunteers

Parameter	Baseline	Final
Systolic BP (mmHg)	112±14.8	114±15.8
Diastolic BP (mmHg)	71±9.2	72±13.5
Pulse (per min)	73±11.1	76±12.3
WBC (K/μL)	7±1.7	7±1.4
HCT (%)	41±4.1	42±4.0
HBG (g/dL)	14±1.4	14±1.4
Platelets (u/mL)	259±69.3	269±61.4
PT (s)	11±0.5	10±0.5
PTT (s)	29±1.5	28±1.4
TSH (mL IU/L)	2±0.8	2±1.0
BUN (mg/dL)	15±4.3	14±4.6
Creatinine (mg/dL)	1±0.1	1±0.1
Ca (mg/dL)	9±0.4	9±0.4
Mg (mg/dL)	2±0.1	2±0.1
Glucose (mg/dL)	5±13.5	84±10.3
Na (mmol/L)	139±1.7	139±1.9
K (mmol/L)	4±0.3	4±0.2
Cl (mmol/L)	104±1.9	103±1.6
CO_2 (mmol/L)	24±2.0	24±2.3
Amylase (U/L)	50±13.6	51±16.4
Lipase (U/dL)	38±10.5	37±10.6
ALT (U/L)	19±5.8	22±9.1
AST (U/L)	21±8.8	23±12.5
ALK PHOS (U/L)	64±20.3	64±18.0
Urine analysis	normal	normal
EKG	normal	normal

3. Study objectives

Primary objective:

- To investigate whether add-on of AHCC can enhance the effect of immunotherapy in cancer patients.

Secondary objectives:

- To evaluate the progression-free survival of patients who receive AHCC along with immunotherapy.
- To evaluate the overall survival of patients who receive AHCC along with immunotherapy.
- To evaluate the safety profile in cancer patients who receive AHCC along with immunotherapy.
- To evaluate the Quality of life in patients who receive AHCC along with immunotherapy.

4. Study design.

This is a prospective double-blind, randomized, placebo-controlled trial.

5. Patient selection

6.1 Inclusion criteria

6.1.1 Liver cancer patient who will receive immunotherapy

6.1.2 At least one measurable tumor, according to RECIST version 1.1, that has not been treated with any local procedure.

6.1.3 Age ≥ 20 years old.

6.1.4 ECOG performance status 0 or 1.

6.1.5 White blood count ≥ 2,000/μL ; platelet count ≥ 60,000/μL.

6.1.6 Liver transaminases (ALT and AST) ≤ 5 times upper limit of normal values (ULN); total bilirubin ≤ 2 times ULN; creatinine clearance or eGFR > 50 mL/min (either Cockcroft-Gault or MDRD is acceptable, whichever is higher).

6.1.7 Subjects with chronic hepatitis B virus infection (HBV surface antigen (HBsAg) positive) must start antiviral therapy with nucleoside analogs (e.g., entecavir or tenofovir, according to current practice guidelines) before start of study drug treatment [5,6].

6.2. Exclusion criteria

5.2.1 Major systemic diseases that the investigator considers inappropriate for participation.

5.2.2 Any active autoimmune disease or history of known autoimmune disease except for vitiligo, resolved childhood asthma/atopy, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.

5.2.3 Dementia or significantly altered mental status that would prohibit the understanding or rendering of informed consent and compliance with the requirements of this protocol.

5.2.4 Prior therapy with an anti-PD-1, anti-PD-L1, or anti-CTLA-4 antibody (or any other antibody or drug specifically targeting T-cell costimulation or checkpoint pathways).

5.2.5 Requirement of systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses > 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.

5.2.6 Prior organ allograft or allogeneic bone marrow transplantation.

5.2.7 Other severe acute or chronic medical or psychiatric condition, or laboratory abnormality that may increase the risk associated with study participation and in the judgment of the investigator would make the patient inappropriate for entry into this study.

6. Screening and registration

6.1 The following examinations must be done to check the patients' eligibility:

7.1.1. Tumor assessment (according to RECIST 1.1); histological proof of HCC; viral hepatitis serology (HBsAg, anti-HBs, anti-HCV), HBV DNA level (for HBsAg (+) or anti-HBc (+) subjects), HCV RNA level (for anti-HCV (+) subjects).

7.1.2. Within 1 week of registration: physical examination (including performance status), hematology/ biochemistry.

6.2 The following procedures must be done and data recorded at registration: written informed consent, demography, medical history, tumor staging (BCLC, AJCC), tumor assessment (according to RECIST 1.1), Child-Pugh score, hematology/biochemistry, viral hepatitis status.

7. Treatment and follow-up plan

7.1 All enrolled patients will receive AHCC or placebo 3g every day until disease progression, intolerance or patient or physician decision to discontinue.

7.2 During treatment the subjects will be followed according to the follow-up plan summarized in the following table:

7.2.1 Hematology and biochemistry levels will be checked within 72 hours before each administration of study drug treatment.

7.2.2 A window of +/- 7 calendar days is allowable for imaging examination for tumor assessment.

7.3 Prohibited treatment during the study drug treatment period includes the following:

7.3.1 Investigational agents for the treatment of cancer;

7.3.2 Systemic steroids > 10 mg within 14 days of dosing (with a few exceptions eg, steroids for adrenal insufficiency, steroids if taken as part of prophylaxis for CT prep).

	Screen	Treatment Cycles (21-day cycles)					End-of-treatment (EOT) evaluation [a]
Week		C1	C2	C3	C4	C5	4 weeks after last treatment
		C6	C7	C8	C9	C10	
		⋮	⋮	⋮	⋮	⋮	
Informed consent	X						
Inclusion/exclusion criteria	X						
Demography/ tumour staging	X						
Medical history	X						
Vital signs	X	X	X	X	X	X	X
ECG	X						X
Blood test [b]	X		X	X	X	X	X
Urinalysis	X					X	X
HBV/HCV serology markers [c]	X						X
Performance status	X	X	X	X	X	X	X
Chest X-ray	X						X
Tumor assessment [d]	X					X	
Treatment		X	X	X	X	X	
Pregnancy test [e]	X						
Immunological study [f]	X					X	X
Thyroid function [g]	X						X
Tolerability/AE reporting		X	X	X	X	X	X
QOL questionnaire	X			X		X	X

a) The EOT assessment will take place at approximately 1 month after last treatment.
b) Blood tests will include CBC/DC, albumin, total and direct bilirubin, transaminases (AST and ALT), ALP, AC sugar, sodium, blood urea nitrogen (BUN), creatinine, amylase, lipase, Troponin-T(or hs-cTnT), pro-BNP (brain natriuretic peptide), and creatinine kinase (CK) or CK-MB isoform (CK-MB). Other biochemistry tests may be done at the discretion of individual investigators.
c) HBV and HCV serology markers, including HBsAg, anti-HBs, anti-HBc, and anti-HCV, will be checked at baseline only. Subjects who test positive for HBsAg are categorized as chronic HBV infection. Subjects who test positive for anti-HCV are categorized as chronic HCV infection. For subjects with chronic HBV infection, HBV DNA levels should be checked at baseline and at EOT evaluation. For subjects with chronic HCV infection, HCV RNA levels should be checked at baseline and at EOT evaluation.
d) Tumor assessment (according to RECIST 1.1) will be done every 12 weeks. Computed tomography is the preferred imaging modality and should include imaging of chest and abdomen at baseline and follow-up.
e) For women with childbearing potential.
f) In brief, 20ml blood and tumor sample will be collected. Please refer to Sec. 10 for detailed description for collection of each type of samples.
g) Including serum T3, T4 and TSH.
h) Quality of life is assessed using the EORTC QLQ-C30 and QLQ-HCC18 at baseline, every 6 weeks and EOT.

8. Assessment of treatment efficacy and safety

8.1 Assessment of treatment efficacy

8.1.1 All enrolled patients will receive assessment of tumor response by imaging studies every 12 weeks, according to RECIST version 1.1. Computed tomography is the preferred imaging modality and should include imaging of chest and abdomen at baseline and follow-up.

8.1.2 For subjects who are considered suitable for surgical resection, the actual surgical procedure will be determined by individual treating surgeons and will be recorded. The time frame for holding the study drug prior to surgery will be determined by the treating physician. Participants may resume the study drug after recovery from surgery, at the physician's discretion.

8.2 Management of immune-related adverse events (irAE) [7,8]

8.2.1 The definition of the most irAE and according management recommendation are summarized in the following table.

8.2.2 For the definition of hepatitis grading, only ALT (alanine aminotransferase) level is used because it is more specific to liver inflammation.

8.2.3 Definition of <u>study drug-induced liver injury</u> (DILI) was listed below, according to the amendment #8 of the CA209040 protocol:

8.2.3.1 Concurrent ALT ≥ 10 x ULN AND total bilirubin ≥ 2 x ULN or baseline value (if elevated bilirubin at study entry), AND

8.2.3.2 No other immediately apparent possible causes of ALT elevation and hyperbilirubinemia, including, but not limited to, tumor progression, acute viral hepatitis, cholestasis, pre-existing hepatic disease or the administration of other drug(s), herbal medications and substances known to be hepatotoxic.

8.2.3.3 Management of hepatic events, including dose delay and/or discontinuation as well as intervention with steroid treatment, will follow the recommendation listed below, will not be impacted by the definition of DILI.

Types of irAE	Grade 1	Grade 2	Grade 3	Grade 4
Hepatitis	ALT up to 3 x ULN AND > 2x baseline • Monitor weekly • Look for other causes of hepatitis (e.g., viral infections other than HBV/HCV, hepatotoxic drugs)	ALT 3 to 5 x ULN AND > 2x baseline • If patient is well, re-check liver function every 2-3 days. Steroid treatment (e.g., prednisolone 1–2 mg/kg/day or IV equivalent) if no improvement or worsening. • Taper steroids over 4 weeks once liver function G1 or at baseline	ALT 5 to 20 x ULN: • As per Grade 2 • Delay study drug treatment if ALT≤ 8X ULN and bilirubin ≤ 5X ULN. Discontinue if worse • Steroid treatment (e.g., prednisolone 1–2 mg/kg/day or IV equivalent) • Consider liver biopsy • Consider prophylactic antibiotics for opportunistic infection	ALT >20 x ULN: • Discontinue study drug administration • As per Grade 3 (Steroid treatment at prednisolone 2 mg/kg/day or IV equivalent). • Consult immunologists for additional immunosuppressive therapy (e.g. infliximab, mycophenolate, immunoglobulins).
Diarrhea/ enterocolitis	<4 bowel actions per day over baseline; mild: • Supportive measures such as increasing • Oral fluid • Anti-motility agents such as loperamide	4–6 bowel actions per day over baseline; moderate: • Delay stud drug administration. • As per Grade 1 if patient is well. • Steroid treatment (e.g., prednisolone 0.5–1 mg/kg/day or IV equivalent) if no improvement in 5-7 days, or if worsening of symptoms; continue until symptoms improve to G1. Steroids can be tapered over 2–4 weeks. • If no improvement > 3-5 days with oral steroids, manage as per G3. • Consider sigmoidoscopy and biopsy	≥7 bowel actions per day over baseline; severe symptoms: • Discontinue study drug administration. • Hospitalization and intravenous hydration • Steroid treatment (e.g., prednisolone 1–2 mg/kg/day or IV equivalent. • If no improvement in 2–3 days, consult immunologists for additional immunosuppressive therapy (e.g. infliximab, mycophenolate, immunoglobulins). • If improving, taper steroids over minimum 1 month (up to 3 months for severe cases). • Consider sigmoidoscopy and biopsy	Life threatening consequences (e.g., perforation) • Urgent intervention indicated: as per G3. • Involve gastroenterologist and surgeon in management.
Pneumonitis	Asymptomatic; • Clinical observations; • Consider delaying study drug administration. • Consider steroids (e.g. prednisone 1 mg/kg/day PO or methylprednisolone 1 mg/kg/day IV). • Re-image at least	Symptomatic; limits instrumental ADLs: • Delay study drug administration. • Consider hospitalization, daily monitoring of symptoms. • Steroid treatment (prednisone 1 mg/kg/day PO or IV	Severe symptoms; limits self-care ADLs; oxygen indicated: • Discontinue study drug administration. • Hospitalization. • Steroid treatment (prednisone 2-4 mg/kg/day PO or IV equivalent).	Life-threatening respiratory compromise; • Urgent intervention indicated (eg intubation): as per Grade 3. • Intensive care support required

	every 3 weeks.	equivalent). • Consider empiric antibiotics • Follow-up: reassess management every 1–3 days. If improving taper steroids. • May continue study drug treatment if symptoms resolve completely.	• Consider high-dose steroid treatment (e.g. methyl-prednisolone 1 g/day IV) (Spain L, et al. *Cancer Treat Rev* 2016). • Add prophylactic antibiotics for opportunistic infections. • Consider bronchoscopy with biopsy. • Reassess management daily. If not improving after 48 h or worsening, consult immunologists for additional immunosuppressive therapy (e.g. infliximab, mycophenolate, immunoglobulins). If improving, taper steroids.	
Endocrine dysfunction	Asymptomatic • Monitor only • Consider endocrinology consultation	Symptomatic • Consult endocrinologist • Levothyroxine replacement for hypothyroidism • Cortisol replacement for hypoadrenalism • Consider β-blockers for hyperthyroidism-related symptoms • Consider steroids or carbimazole for hyperthyroidism	Severe symptoms: • As per grade 2. • Delay study drug administration. • Steroid treatment (e.g., prednisolone 1–2 mg/kg/day or IV equivalent)	Life threatening consequences • As per Grade 3. • Delay or discontinue study drug administration.
Nephritis	Creatinine > 1X ULN and ≤1.5 X baseline; proteinuria 1+, <1.0g / 24 h: • Monitor renal function weekly; hydration	Creatinine > 1.5–3.0 X baseline; proteinuria 2+, 1.0–3.4 g/24 h: • Steroid treatment (e.g., prednisolone 0.5–1 mg/kg/day or IV equivalent) • Delay study drug administration. Monitor renal function every 2-3 days; If worsens, manage as per grade 3 • Exclude non-immune causes	Creatinine > 3.0 X baseline to ≤ 6X ULN; proteinuria ≥ 3.5 g/24 h: • As per Grade 2 • Consider renal biopsy • If elevations persist > 7 days or worsens, manage as per grade 4	Creatinine > 6.0 X ULN: • Steroid treatment (e.g., prednisolone 1–2 mg/kg/day or IV equivalent) • Consider renal biopsy • Discontinue study drug treatment
Skin rash	<10% BSA:	10–30% BSA:	>30% BSA:	Life threatening

	• Antihistamines and topical steroid for pruritus	• As per G1 if tolerable • If intolerable, steroid treatment (eg prednisolone 0.5–1 mg/kg/day with a 1–2 week wean) • Delay study drug treatment until G1 and steroids <10 mg/ day • Consider skin biopsy if symptoms persist or recur	• Consult dermatologist and consider skin biopsy • Steroid treatment (e.g., prednisolone 1 mg/kg/day or IV equivalent). • Delay study drug treatment until G1 and steroids <10 mg/ day	consequences • As per Grade 3.

8.2.4 When irAE improved to Grade 1 after steroid treatment, may taper steroid dosage over at least 1 month.

8.2.5 For other grade 3-4 toxicities (according to CTCAE version 4) that are considered to be related to study drug treatment, study drug treatment should be held if the toxicities last for 3 days or more under best supportive care. Further study drug treatment may be considered when toxicity has resolved to ≤ grade 1, after discussion with the principal investigator.

8.2.6 Recent review of irAE indicated the clinical significance of the rare but potentially fatal cardiac toxicity for patients who received combination immunotherapy [9,10]. Cardiac exam (echocardiography or radionuclide ventriculography (MUGA scan)) will be done at baseline, at the first imaging assessment, and at the end-of-treatment (EOT) evaluation. Troponin-T(or hs-cTnT), pro-BNP (brain natriuretic peptide), and creatinine kinase (CK) or CK-MB isoform (CK-MB) will be checked every 3 weeks before each administration of study drug treatment and at EOT evaluation.

8.2.7 When cardiac toxicity is suspected, cardiologists will be consulted for further diagnostic work-up. High-dose steroid and other immunosuppressive treatment will be used according to the most recent expert recommendation.

8.3 Patients will be followed every 3 weeks to evaluate the occurrence and severity of adverse events, according to CTCAE version 4.

8.4 The Institution and the Principal Investigator shall report any Adverse Events arising out of or in connection with the Study to the Regulatory Authority in accordance with applicable laws and regulations.

8.5 Serious adverse events (SAE) must be reported in written forms to the institutional review boards of the participating centers and Department of Health, Taiwan, within 7 working days. SAE is defined as any event that

8.5.1 Results in death;

8.5.2 Life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);

8.5.3 Results in hospitalization or prolong inpatient hospitalization;

8.5.4 Results in persistent or significant disability/incapacity;

8.5.5 Results in congenital anomaly of offspring;

8.5.6 Requires treatments for permanent injuries;

8.5.7 Results in development of second malignancy. Second malignancy includes any second primary malignancy, regardless of causal relationship to the study drug, occurring at any time for the duration of the study, from the time of signing the informed consent up to 3 months after withdrawal from the study drug.

8.5.8 An important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.

8.5.9 Results in suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.

8.5.10 Although pregnancy, overdose, potential drug-induced liver injury (DILI) and cancer are not always serious by regulatory definition, these events must be handled as SAEs. Overdose is defined as the accidental or intentional administration of any dose of study drugs that is considered both excessive and medically important.

8.6 If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to IRB. All SAEs should be followed to resolution or stabilization.

8.6.1 An SAE report should be completed for any event where doubt exists regarding its seriousness.

8.6.2 If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

8.6.3 If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

8.6.4 Definition of SAE is listed in Section 10.5. Additional description of SAE, as required by the study drug supplier, is listed below.

8.6.4.1 Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

8.6.4.2 The following hospitalizations are not considered SAEs in this study:

8.6.4.3 A visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)

8.6.4.4 Elective surgery, planned prior to signing consent

8.6.4.5 Admissions as per protocol for a planned medical/surgical procedure

8.6.4.6 Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)

8.6.4.7 Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases

8.6.4.8 Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

8.6.5 An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

8.6.6 The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

8.6.6.1 Related: There is a reasonable causal relationship between study drug administration and the AE.

8.6.6.2 Not related: There is not a reasonable causal relationship between study drug administration and the AE.

8.6.6.3 The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

8.6.6.4 Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

8.6.6.5 Nonserious AE information should also be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects.

8.6.6.6 The collection of nonserious AE information should begin at initiation of study drug. All nonserious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 30 days following the last dose of study treatment.

8.6.6.7 Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

8.6.7 All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported as such. The following laboratory abnormalities should be documented and reported appropriately:

8.6.7.1 any laboratory test result that is clinically significant or meets the definition of an SAE

8.6.7.2 any laboratory abnormality that required the participant to have study drug discontinued or interrupted

8.6.7.3 any laboratory abnormality that required the subject to receive specific corrective therapy.

8.6.8 Other Safety Considerations: Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a nonserious or serious AE, as appropriate, and reported accordingly.

9. Analysis of predictive markers

9.1 Biomarker study of the pre-treatment biopsy tumor tissue

9.1.1 Five 10-micrometer-thick slides will be obtained from paraffin-embedded tumor specimens for DNA/RNA extraction and subsequent whole exome sequencing and RNA sequencing.

9.1.2 Ten 4-micrometer-thick slides will be obtained from paraffin-embedded tumor specimens for tissue staining. Immunohistochemistry or multiplex staining will be done in paraffin-embedded tumor specimens if enough tissue samples are available to confirm the proportion and distribution of immune cells in different tumor microenvironment.

9.2 Biomarker study of peripheral blood samples during study drug treatment

9.2.1 For the immunology study, peripheral blood samples will be collected before the start of the first cycle of study drug treatment, at the time of tumor response assessment, and at the end-of-treatment assessment.

9.2.2 Blood samples will be put in BD Vacutainer®CPT™ cell preparation tubes and EDTA tube respectively, and centrifuged within 6 hours after sampling.

9.2.3 The mononuclear cell layer will be stored in liquid nitrogen for flow cytometry and DNA/RNA extraction studies.

9.2.4 Flow cytometry and transcriptomic analysis will be done to analyze the proportion of different immune cells in peripheral blood and the results will be correlated with efficacy of study drug treatment.

9.2.5 Plasma will be used for blood biomarker research including but not limited to cfDNA, cytokine and lipid profiling.

9.3 Biomarker study of the resected tumor tissue from subjects who undergo surgery

9.3.1 Samples from the same tumor(s) will be obtained from formalin-fixed, paraffin-embedded tumor specimen for relevant studies

9.3.2 The genetic study to be done include but not limited to whole exome sequencing.

9.3.3 Transcriptomic analysis will be done to analyze the composition (e.g., the proportion of different immune cells) of the immune microenvironment by using RNA-Seq and the results will be correlated with efficacy of study drug treatment.

9.3.4 The biomarker studies of tumors and peripheral blood samples will be done at the National Health Research Institutes. The relevant information of the responsible laboratory is as follows:

Name of the institution/laboratory: National Institute of Cancer Research.

Mailing address: No. 367, Sheng-Li Road, Tainan 704, Taiwan (R.O.C.)

Name of the person responsible for specimens storage: Yung-Yeh Su
Place of specimens storage: National Institute of Cancer Research, National Health Research Institutes.
Estimated period of time for specimens storage: 10 years

10. Criteria for discontinuation of study drug treatment

Discontinuation of study drug treatment is when a patient no longer receives study drug but continues to be followed up for tumor status and survival according to local treatment guidelines for up to half year, unless consent is withdrawn. Patients may be discontinued from study drug treatment and assessments at any time. When study drug treatment is discontinued due to the reasons listed below, no further AHCC/placebo treatment will be provided by this study and further anticancer therapy will be decided by the investigators according to current treatment guidelines. Specific reasons for discontinuation of study drug treatment include:

10.1 Voluntary discontinuation by the subject who is at any time free to discontinue his/her participation in this study, without prejudice to further treatment.

10.2 When the investigator(s) consider other treatment modalities (surgery, others) are more suitable for the subjects according to assessment of tumor response specified in the protocol.

10.3 Safety reasons as judged by the investigator. Severe non-compliance to protocol as judged by the investigator.

10.4 Incorrect enrollment, in the opinion of the investigator or study delivery team physician, which will compromise the patient's health if study drug treatment or assessments are continued.

10.5 Dose delay or interruption of study drug treatment for more than 3 weeks.

11. Statistical consideration

11.1 The primary endpoint is ORR in AHCC arm compared to placebo arm. The secondary endpoints include, incidence and severity of adverse events according to CTCAE version 4, progression-free survival and overall survival.

11.2 Exploratory endpoints will include correlation of treatment response (tumor shrinkage, objective response rate, etc.) with immune biomarkers in subjects' samples from tumor tissue and peripheral blood.

11.3 We simultaneously monitor efficacy and safety endpoints using the 2-arm Bayesian optimal phase 2 (BOP2) design (Zhao et al., 2020).

11.3.1 Specifically, let n denote the interim sample size and N denote the maximum sample size. Let E and C denote the experimental arm and the control arm, respectively. For arm $t, t = E, C$, let $Y_{eff,t}$ and $Y_{tox,t}$ respectively denote the efficacy and toxic endpoints, with $Y_{eff,t} = 1$ and $Y_{tox,t} = 1$ respectively indicating that patients experience efficacy and toxicity. We assume that the joint distribution of $(Y_{eff,t}, Y_{tox,t})$ follows a multinomial distribution with four elementary outcomes: $(Y_{eff,t}, Y_{tox,t})$ = (1, 1), (1, 0), (0, 1) and (0, 0). Let $\mathbf{p_t} = (P_{11,t}, P_{10,t}, P_{01,t}, P_{00,t})$

denote the probabilities of observing the four outcomes, and let $p_{eff,t} = Pr(Y_{eff,t} = 1)$, $p_{tox,t} = Pr(Y_{tox,t} = 1)$ and $p_{efftox,t} = Pr(Y_{eff,t} = 1, Y_{tox,t} = 1)$.

11.3.2 The experimental arm is deemed as unacceptable if $H_0: p_{eff,E} \leq p_{eff,C}$ or $p_{tox,E} > p_{tox,C}$, i.e., the experimental treatment is inefficacious or overly toxic, with respect to the control. We employ the following Bayesian rule to make a go/no-go decision: (Futility and toxicity stopping) stop enrolling patients and claim that the experimental arm is unacceptable if

$$Pr(p_{eff,E} > p_{eff,C}|data) < \lambda\left(\frac{n}{N}\right)^{\alpha},$$

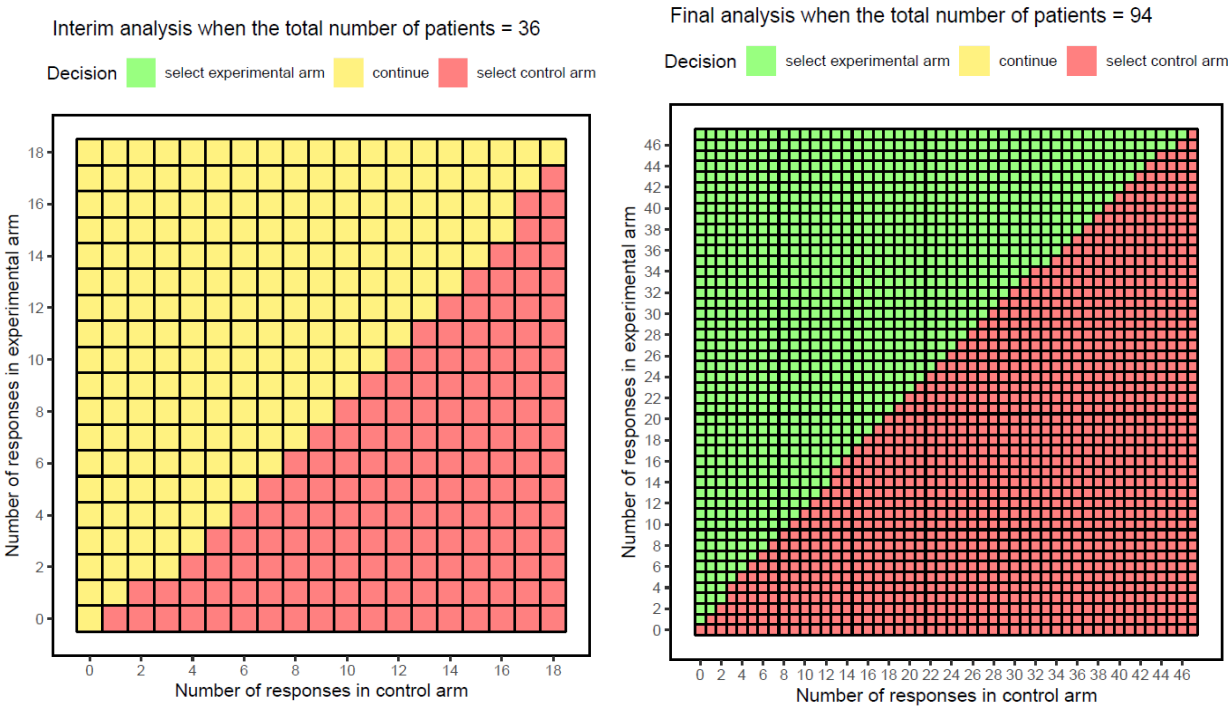
OR

$$Pr(p_{tox,E} \leq p_{tox,C}|data) < \lambda\left(\frac{n}{N}\right)^{\alpha},$$

where λ=0.68 and α=0.84 are design parameters optimized to maximize the probability of correctly concluding an efficacious and safe treatment as acceptable when $p_{eff,E} = 0.42$, $p_{tox,E} = 0.36$ and $p_{efftox,E} = 0.15$, while controlling that the probability of incorrectly claiming an inefficacious and toxic treatment, with $p_{eff,C} = p_{eff,E} = 0.27$, $p_{2,C} = p_{2,E} = 0.57$ and $p_{3,C} = p_{3,E} = 0.15$, as acceptable is 10 %. This optimization is performed assuming a vague Dirichlet prior $Dir(0.25,0.25,0.25,0.25)$ for $\mathbf{p}$.The prior is chosen such that it corresponds to a prior effective sample size of 1. The above decision rule leads to the optimal stopping boundaries shown in following tables. The trial stops when either the efficacy endpoint or the toxicity endpoint crosses the stopping boundaries.

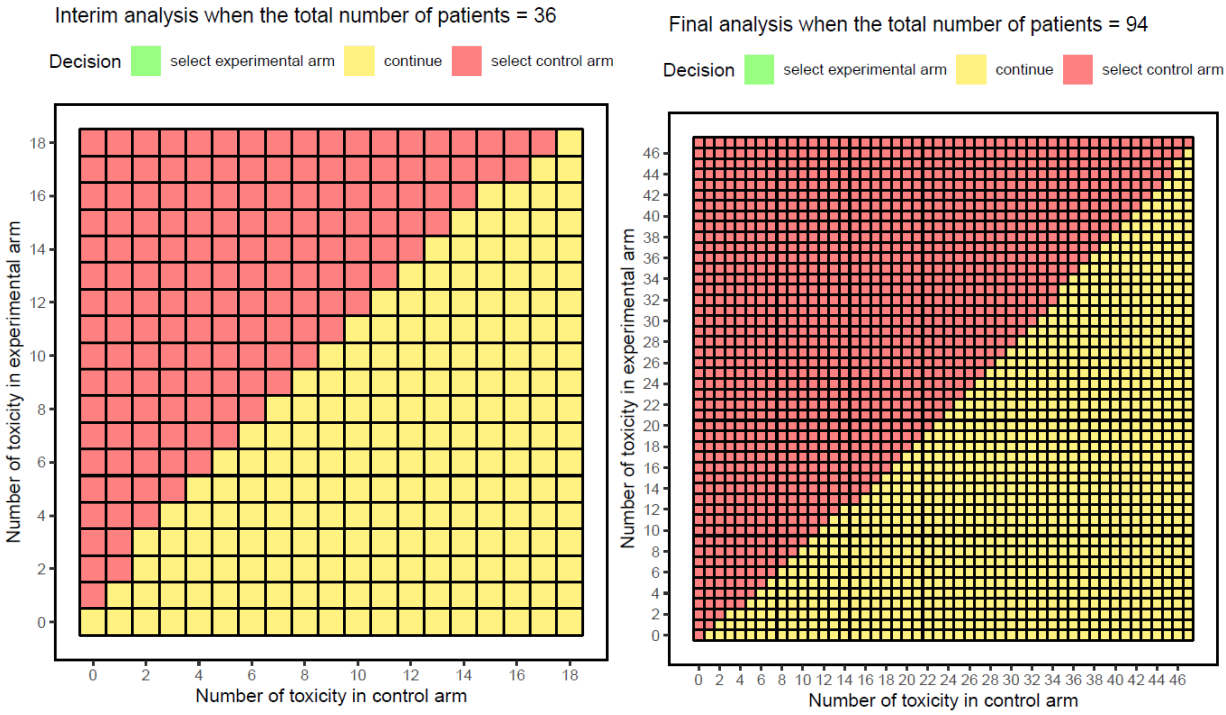
Optimized stopping boundaries for efficacy endpoint

Interim (no. in control)	Interim (no. in experimental)	No. response (eff) in control	Stop for futility if no. of response in experimental - no. of response in control <=
18	18	0	Never
18	18	1~2	-1
18	18	3~16	-2
18	18	17~18	-1
47	47	0~2	0
47	47	3~12	1
47	47	13~32	2
47	47	33~44	1
47	47	45~47	0



Optimized stopping boundaries for toxicity endpoint

Interim (no. in control)	Interim (no. in experimental)	No. toxicity (tox) in control	Stop for unsafety if no. of toxicity in experimental - no. of toxicity in control >=
18	18	0~1	1
18	18	2~15	2
18	18	16~17	1
18	18	18	Never
47	47	0~3	0
47	47	4~14	-1
47	47	15~34	-2
47	47	35~45	-1
47	47	46~47	0



Based on the above tables and figures, we perform the interim analysis when the number of enrolled patients reaches 36. When the total number of patients reaches the maximum sample size of 94, we reject the null hypothesis and conclude that the experimental arm is acceptable, compared to the control, if both the futility and toxicity stopping boundary are not crossed. Below are the operating characteristics of the design based on 10000 simulations using the BOP2 web application, which is available at http://www.trialdesign.org.

Operating characteristics

Scenario	Pr(Eff) for Control	Pr(Tox) for Control	Pr(Eff & Tox) for Control	Pr(Eff) for Experimental	Pr(Tox) for Experimental	Pr(Eff & Tox) for Experimental	Early Futility Stopping (%)	Claim Promising (%)	Average Sample Size
1	0.27	0.57	0.15	0.37	0.47	0.17	23.58	44.40	80.3
2	0.27	0.57	0.15	0.42	0.36	0.15	9.79	75.93	88.3

11.4 The primary endpoint analysis and other secondary endpoints will be based on the intent-to-treat population and per-protocol population, while the safety data will be summarized based on the intent-to-treat population.

11.4.1 Intent-to-treat population refers to all enrolled patients regardless of their compliance with the study drug treatment.

11.4.2 Per-protocol population refers to all patients who have received at least 6 weeks (2 cycles) of study medication and received the first scheduled assessment of tumor response.

11.5 Disease stabilization rate (complete response + partial response by RECIST + stable disease by RECIST that last for ≥ 8 weeks), objective response rate (complete response + partial

response by RECIST), and down-staging rate will be calculated with their 95% confidence intervals.

11.6 Progression-free survival, time-to-tumor progression, and overall survival will be calculated by the Kaplan-Meier method.

11.6.1 Progression-free survival is defined as the duration from the date of the first dose of study drug treatment to the date of documented disease progression or death of any cause.

11.6.2 Time-to-tumor progression is defined as the duration from the date of the first dose of study drug treatment to the date of documented tumor progression by imaging studies.

11.6.3 Overall survival is defined as the duration from the date of the first dose of study drug treatment to the date of death of any cause.

11.7 The baseline and changes in immune-related biomarkers after study drug treatment will be correlated with the patients' treatment outcome, including disease stabilization, best radiographic response, time to tumor progression, progression-free survival, and overall survival.

11.8 For the endpoint of the safety assessment, the tabulations will count the number of subjects reporting individual adverse events. Incidence of adverse events and serious adverse events will be summarized.

11.9 The demographic features of the subjects will be summarized by descriptive statistics. Comparison between sub-groups, for examples,
subjects with vs. without objective tumor response/AFP response/disease stabilization
subjects with vs. without surgery after study drug treatment
by using Mann-Whitney tests/ one-way ANOVA (continuous variables) or chi-square/Fisher's Exact tests (categorical variables).

12. Institutional review board

12.1 The study will be conducted in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects (revised in 2013) the International Conference on Harmonization guideline on Good Clinical Practice (GCP), and applicable local regulatory requirements and laws.

12.2 The investigator of each participating center is responsible for submission and obtaining approval of the study protocol, protocol amendments, informed consent forms, and other relevant documents from the institutional review board or research ethics committee of each participating center.

13. Records to be kept

13.1 The investigator of each participating center is responsible for keeping the following records, according to the GCP guidelines and applicable local regulatory requirements and laws:

13.1.1 Identity of all participating subjects (sufficient information to link the source documents (hospital records) and the case record forms);

13.1.2 All original signed informed consent forms;

13.1.3 Source documents;

13.1.4 Copies of all case record forms;

13.1.5 Serious adverse event forms;

13.1.6 All correspondence with the institutional review board.

14. References

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15. Appendix

15.1 Improved prognosis of postoperative hepatocellular carcinoma patients when treated with functional foods: a prospective cohort study. **J Hepatol. 2002 Jul;37(1):78-86.**

15.2 A Phase I study of the safety of the nutritional supplement, active hexose correlated compound, AHCC, in healthy volunteers. **Nutr Sci Vitaminol (Tokyo). 2007 Dec;53(6):536-9.**