Original Contributions

First-in-Human Safety and Efficacy Study on Combination of High-Intensity Focused Ultrasound Sonication and Micellar Nanoparticle-Encapsulated Epirubicin, K-912; a Novel Sonodynamic Therapy for the Treatment of Refractory Abdominal Cancers

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Abstract

Objective: Aims of this first-in-human clinical trial were to evaluate the safety and efficacy of sonodynamic therapy (SDT) using a newly developed triggered-pulse high-intensity focused ultrasound (HIFU) device, MS-2, and micellar nanoparticle-encapsulated epirubicin, K-912, in patients with unresectable refractory abdominal cancers.

Methods: This was a single center prospective exploratory clinical trial. The HIFU sonication power (75 and 150 W at 1 MHz) and K-912 dose (30 and 80 mg/m^2) were increased incrementally (4 cohorts) according to 3+3 design. Each cohort consisted of three patients. K-912 was administered intravenously one day before HIFU treatment.

Results: A total of 12 patients with stage IV pancreatic cancer (n = 11) and cholangiocellular carcinoma (n = 1) completed the SDT. The mean sonication time and total number of sonication was 22.3 min and 17.4 shots, respectively. No adverse events of grade ≥3 were observed during the trial up to 30 days after HIFU treatment. No adverse events related to K-912 were noted. The HIFU sonication power and K-912 dose considered to be tolerable were 150 W and 80 mg/m^2, respectively. The rate of complete and partial tumor coagulative necrosis was 33.3% and 41.7%, respectively. The primary disease control rate was 66.7%. Pain was improved in 33.3% of patients.

Conclusions: This trial demonstrated that SDT using MS-2 and K-912 was safe and well tolerated in patients with advanced abdominal tumors and showed promising preliminary clinical activity.

Key words:

3+3 design, micellar nanoparticle-encapsulated epirubicin, dose escalation, high-intensity focused ultrasound, pancreatic cancer, sonodynamic therapy, unresectable refractory tumor

Introduction

High-intensity focused ultrasound (HIFU) is a noninvasive method that involves the use of focused ultrasound within the body to induce tissue necrosis through both thermal ablation and cavitation [1]. In Japan, HIFU treatment has been approved for thermal ablation of prostate hypertrophy, uterine adenomyosis, and pain due to bone metastases. Its application in the management of benign and malignant tumors has been widely investigated [2-4]. However, HIFU requires high levels of thermal energy to cauterize living tissues and tumors, and ultrasound waves bend in the presence of air and are deflected by bone, consequently, there is a risk that non-targeted sites may be damaged unintentionally [5,6]. In particular, it has been difficult to use HIFU aggressively in the treatment of lesions near the gastrointestinal tract due to the risk of perforation and other contingencies [7].

Sonodynamic therapy (SDT) combines the use of HIFU and a sonosensitizer [8] in an approach designed to reduce the HIFU sonication power and sonosensitizer dose compared with conventional monotherapy [9]. The precise mechanism of SDT is not well understood, but ultrasound cavitation triggers an increase in cell membrane permeability through microjets generated by microbubbles [10], in conjunction with sonosensitizers inducing the production of cytotoxic reactive oxygen species (ROS) [11-13]. We developed a novel HIFU sonication sequence method consisting of a triggered pulse and heating burst for generating and maintaining cavitation bubbles in the body [14-16]. Furthermore, a new triggered-pulse HIFU device, MS-2, has been developed. MS-2 can expand the sonication range by utilizing high-speed scanning sonication with six points, corresponding to circles arranged at each vertex of a hexagon, resulting in reduced treatment time [14].

K-912 (NC-6300) is an epirubicin-conjugated polyethylene glycol polyaspartate block copolymer, which is designed to accumulate in the tumor through tumor-infiltrating permeability and to release epirubicin into the acidic environment of tumor tissue

[17,18]. Epirubicin is currently used for the treatment of various hematological and solid malignancies [19]. K-912 showed a more potent antitumor effect and lower toxicity compared with epirubicin in animal models [20,21]. In the phase 1 trials, K-912 was well tolerated in patients with various solid tumors and exhibited lower toxicity than epirubicin itself [22]. The maximum tolerated dose (MTD) and recommended phase 2 dose were estimated to be 170 mg/m^2, which is higher than the epirubicin doses, and preliminary clinical activity was observed in patients with breast cancer and angiosarcoma [23]. A combination of HIFU MS-2 and K-912 increased cytotoxic ROS generation to a greater extent than HIFU sonication alone [24]. In preclinical studies, this SDT system was safe and effective compared with treatment with HIFU MS-2 or K-912 alone [9,25].

Here, we report the first-in-human clinical trial of a novel form of SDT in combination with HIFU MS-2 and K-912 in patients with refractory abdominal cancers to evaluate the safety, tolerability, and efficacy of treatment.

Materials and methods

Study design and patients

This was a first-in-human, single-center, non-randomized, non-comparative, dose escalation trial in cohorts of adult patients with advanced abdominal solid tumors who had exhausted standard treatment options.

This dose escalation trial was conducted at Tokyo Medical University, Department of Gastroenterology and Hepatology in Japan from May 2017 to October 2017. The HIFU power (75 and 150 W) and K-912 dose (30 and 80 mg/m^2) were escalated incrementally according to the conventional 3 + 3 design using a total four cohorts: cohort 1 (30 mg/m^2 of K-912 and 75 W of HIFU sonication power), cohort 2 (30 mg/m^2 and 150 W), cohort 3 (80 mg/m^2 and 75 W), and cohort 4 (80 mg/m^2 and 150 W). Each cohort consisted of 3 patients until MTD was determined.

Patients of either sex aged 20 to 75 years diagnosed with refractory cancer including pancreatic cancer, biliary tract cancer, bone tumors including bone metastases, which were detectable by ultrasonography, were enrolled in this trial. Eligibility criteria are shown in Supplementary Table S1.

The study protocol and informed consent form were approved by the institutional review board of Tokyo Medical University. All patients gave written informed consent before initiation of any study-specific procedures. The trial was conducted in accordance with ethical principles originating in or derived from the Declaration of Helsinki, and Good Clinical Practice guidelines. This study is registered in University Hospital Medical Information (UMIN); UMIN000027283.

Procedure

HIFU device

The triggered-pulse HIFU device used was MS-2. It was developed by a joint consortium comprising Hitachi, Ltd., DENSO CORPORATION, ASAHI Corporation, Japan Probe Co., Ltd., Harata Corporation, Tohoku University, and Tokyo Women's Medical University (Fig. 1). The HIFU output specifications were frequency, 1.0 MHz ± 15%; focal size, <3 mm x 3 mm x 10 mm.

Sonosensitizer

K-912 (prepared by Kowa Company. Ltd.) was used as the sonosensitizer.

Treatment

The assigned dose of K-912 was administered intravenously 24 ± 2 h prior to HIFU treatment. In the case of pancreatic cancer, a protease inhibitor was administered one day before HIFU treatment to prevent pancreatitis. The position, and size of the tumor and its relationship to contiguous organs were determined by B-mode ultrasonography

prior to HIFU treatment. Regarding the abdominal organs, the intestinal tract was cleansed by fasting, if needed, drinking degassed water, and receiving dimethicone or butyl scopolamine. During HIFU treatment, the patient lay supine on the treatment table. A low-viscosity HIFU gel was applied to the treatment area. The HIFU transducer was placed on the treatment area and gentle pressure was applied using a degassed water-filled bag. The imaging confirmation probe and treatment oscillator were aligned on the same axis. The sonication time was five seconds per shot with the planned power and the cauterization area per single sonication with six focal points was approximately 0.5 cm^3. In principle, the treatment area was at least 1 cm away from the surrounding major organs such as the stomach, spleen, liver, duodenum, and bile duct, as well as major blood vessels to avoid unintended damage during the procedure. During HIFU treatment, abdominal pain, back pain, pelvic pain, and skin pain occurring at any time were monitored and assessed. If pain in the abdomen was greater than before treatment commenced or skin pain occurred, the treatment was suspended and not resumed until the pain subsided. It was permissible to reduce the power by 10% depending on the patient's tolerance for pain. The initial dose of 30 mg/m^2 of K-912 was set such that it did not exceed 20% of 170 mg/m^2, which was the MTD determined in the phase 1 study [22]. The initial output power of 75 W was set such that it did not exceed 20% of 900 W, which was a level that was found to be safe in our previous clinical study [26]. If no adverse events (AEs) such as uncontrollable pain caused by SDT were observed in cohort 4, 150 W and 80 mg/m^2 of K-912 were defined as the MTD. Puncture treatment, local therapy, and surgical procedures for lesions were prohibited during the first month following treatment. After the follow-up assessment at one month after SDT, other forms of treatment including chemotherapy were allowed.

Dose escalation

The HIFU sonication power (75 and 150 W) and K-912 dose (30 or 80 mg/m^2) were increased incrementally (Supplementary Table S2).

If no adverse events (AEs) occurred in cohort 1, such as uncontrollable pain due to sonodynamic therapy (SDT), treatment was initiated in cohort 2. If no AEs occurred in cohort 2, treatment was initiated in cohort 3. If no AEs occurred in cohort 3, treatment was initiated in cohort 4. If no AEs were observed in cohort 4, 80 mg/m^2 of K-912 + HIFU at 150 W was estimated to be the maximum tolerated dose (MTD).

If one patient developed an AE at a specific dose, three patients were added to the same cohort. If AEs did not occur in the additional three patients, the procedure advanced to the next cohort. In the case of cohort 4, 80 mg/m^2 + 150 W was estimated to be the MTD.

If AEs occurred in two or more patients at a specific dose, the MTD was estimated to be one level lower. If AEs occurred in two or more patients in cohort 1, the dose of K-912 was reduced by 50% and resumed at 15 mg/m^2 + 75 W.

Patients who were discontinued for reasons other than AEs were not included in the MTD analysis and additional patients were registered in the relevant step.

The decision to step up treatment levels after resumption was made by the study leader and the principal investigator.

Study endpoints and assessment

The primary objective was to evaluate the safety of SDT in combination with HIFU MS-2 and K-912, and to estimate the MTD of SDT. The safety and tolerability were evaluated during sonication, and at weeks 1 and 4 after SDT based on AEs as per the Common Terminology Criteria for Adverse Events version 4.03. Secondary endpoints were the tumor reduction effect and pain improvement. The tumor reduction effect was assessed by diagnostic imaging using ultrasound (US), and computer tomography (CT) before SDT and at week 4 after SDT. Tumor coagulative necrosis was assessed by

change in the blood flow infiltration into capillaries and tumor stroma diagnosed using contrast-enhanced US and contrast-enhanced CT, respectively. If both imaging modalities confirmed there was no blood flow to the entire tumor, the outcome was defined as complete tumor coagulative necrosis, and if it was partial, it was defined as partial tumor coagulative necrosis. In patients who had pain at baseline, pain was assessed using a visual analogue scale (VAS) prior to, and on the day of SDT, and at days 1, 3, 7, and 30 after SDT. The VAS scores before and after SDT were compared in patients whose VAS was ≥5 at baseline, and evaluated in relation to the following three stages: very much improved (the post-treatment VAS value is 0 - 2, or it has decreased by 5 or more from before treatment), improved (it has decreased by 2 or more and less than 5 from before treatment), and no improvement.

Statistical analysis

Patient disposition, demographics, and baseline characteristics were summarized using descriptive statistics. The incidence of all AEs that occurred during SDT and within one month after SDT, and AEs that were reasonably related to SDT were summarized and tabulated. AEs of grade 3 or higher assessed using CTCAE v4.03 – JCOG were reported. SDT was defined as safe if there were no AEs of grade 3 or higher related to HIFU treatment. The maximum diameter of the ablation range as a percentage of the tumor diameter was calculated, and change over time in the ablation range was assessed. Efficacy in reducing pain was assessed by comparing the VAS score before and after SDT as follows: very much improved (post-treatment VAS of 0 - 2 or ≥ 5 lower than before treatment); improved (≥ 2 to < 5 lower than before treatment); or not improved.

3. Results

Patient disposition

A total of 12 patients with stage IV pancreatic cancer (n = 11) and cholangiocellular carcinoma (n = 1) underwent SDT. All patients completed SDT without any need for general anesthesia or sedation. The mean total HIFU sonication time and total number of sonication was 22.3 min and 17.4 shots, respectively. Baseline demographic and disease characteristics are outlined in Tables 1 and 2.

Safety

The summary of SDT is shown in Tables 3 and 4. No AEs related to K-912 were reported. Grade 1 skin burn and grade 2 abnormal laboratory values of the biliary system were observed in one patient of cohort 2. These were causally related to HIFU treatment. No AEs of grade ≥3 were observed during SDT treatment and at weeks 1 and 4 after SDT treatment. SDT combining HIFU sonication power of 150 W and a K-912 dose of 80 mg/m^2 was safe and well tolerated, and this was estimated to be the MTD.

Efficacy

Complete and partial tumor coagulative necrosis was observed in 4 (33.3 %) and 5 (41.7%) patients, respectively (Tables 3 and 4). Tumor coagulation necrosis was reported in five of six patients who were sonicated by HIFU at a power of 150 W during SDT; complete response in four patients and partial response in one patient. Tumor reduction was observed in five patients (41.7%). The disease control rate (%) including partial response and stable disease was 66.7% (8 of 12 patients). Figs. 2 and 3 show the clinical outcomes in two patients. Pain was improved in two (33.3%) of six patients who had pain at baseline. Among three patients whose VAS was ≥5 at baseline, two were very much improved and one was not improved.

Discussion

In this first-in-human trial, our SDT system combining a new triggered-pulse HIFU MS-2

and micellar nanoparticle-encapsulated epirubicin K-912 demonstrated that it was safe and well tolerated in patients with refractory pancreatic cancer and cholangiocellular carcinoma. The HIFU power and K-912 dose were escalated in four cohorts. No AEs of grade ≥3 were observed during SDT treatment and at weeks 1 and 4 after treatment in any cohort. No AEs related K-912 were observed. SDT administered at a HIFU power of 150W and K-912 dose of 80 mg/m^2 was safe and well tolerated, and these levels were confirmed as the MTD, which can be used for reference in future studies. Sehmbi et al. reported [27] that most common AEs after HIFU for hepatobiliary malignancies were skin burns (15%), local pain (5%) and fever (2%). In our previous study on the treatment of pancreatic cancers using HIFU alone, AEs occurred in three (10%) patients; pseudocyst formation in two patients and mild pancreatitis development in one patient [26]. In this trial, abdominal pain, back pain, pelvic pain, and skin pain occurring at any time were monitored and assessed during and after HIFU, but no pain-related AEs were reported. The output power of 75 and 150 W in the HIFU MS-2 was much lower than the previous power of 900 W, resulting in a reduction in the risk of AEs in the gastrointestinal tract due to heating [26].

Preliminary clinical efficacy was observed based on tumor coagulative necrosis and disease control. In one patient with pancreatic cancer in cohort 2, complete tumor coagulative necrosis was observed as early as one week after SDT (Fig. 2). Such a rapid response was not achieved in the previous study in which HIFU alone was used [26]. This was attributed to the concomitant use of K-912. Furthermore, in one patient with intrahepatic cholangiocarcinoma in cohort 2, because of the large tumor diameter of 75 mm, only the horizontal plane in the middle of the tumor was treated as HIFU was unable to cover the entire tumor. Despite this limitation, complete tumor coagulative necrosis was obtained one week after SDT (Fig. 3), suggesting that even if the entire tumor was not ablated, the addition of K-912 enhanced the therapeutic effect throughout the entire tumor. Moreover, the adjacent gallbladder remained unaffected, with no signs

of cholecystitis observed. These findings suggest that K-912 may have enhanced the therapeutic efficacy of HIFU by acting as an enhancer. Additionally, while conventional HIFU treatments have raised concerns about potential thermal damage to adjacent organs, including the gastrointestinal tract (e.g., perforation or obstruction), SDT appears to reduce these risks, indicating its potential as a safe and effective option for cancer treatment.

In our previous study on HIFU treatment alone, the output HIFU power was 500 - 1350 W [26]. However, the power in this study was much lower, but higher power yielded better clinical outcomes; 150 W vs. 75 W. The mean number of sonications was 17.4 shots over 5 s, which was much fewer than 110 shots over 3 s using the conventional HIFU system (in-house data). The mean treatment time (22.3 min) was about half of the 45 min required using the conventional HIFU system. Improvements to the HIFU MS-2 device included the sonication sequence method consisting of a triggered-pulse and heating burst, and the introduction of 6-point high-speed scanning sonication, which expanded the ablation area from approximately 0.1 cm^3 to 0.5 cm^3, making it possible to reduce both the number of sonications and the treatment time.

Pancreatic cancer is known to be one of the most lethal cancers due to its highly metastatic nature with a 5-year survival rate of approximately 10% [28,29]. Our SDT system was safe, well tolerated, and just a single treatment had a potent effect in advanced pancreatic cancer, suggesting that this system may represent a novel treatment option for these lethal cancers.

This trial has some limitations. First, dose-limiting toxicity was not reached. Second, there was no evaluation of safety and efficacy with repeated SDT treatment. Third, this was a single-arm feasibility study; therefore, an additional large study is needed.

Conclusions

SDT using MS-2 as a HIFU device and K-912 as a sonosensitizer was safe and well

tolerated in patients with advanced abdominal tumors, and the MTD was a HIFU power of 150 W and a K-912 dose of 80 mg/m^2. A primary clinical response was observed in patients with refractory pancreatic cancer and cholangiocellular carcinoma.

Table legends

Table 1 Patient characteristics
Table 2 Individual characteristics of patients in each cohort
Table 3 Summary of the sonodynamic therapy
Table 4 Summary of sonodynamic therapy for individual patients

Conflict of interests

This study was funded by the Japan Agency for Medical Research and Development; # 17hk0102028h0003. Medical writing was supported by SONIRE Therapeutics Inc., Tokyo, Japan. All authors reported no conflicts of interest.

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Data availability statement

The protocol will be shared with those who request data sharing. Requests for data should be directed to the corresponding author. Requests will be reviewed, and scientifically sound proposals will be approved by the authors. In addition, an agreement for data

sharing needs to be contracted between data requestors and the corresponding author. Data will be shared two years after article publication.

Supplementary materials

Table S1 Inclusion and exclusion criteria

Table S2 Dose escalation criteria

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Figure legends

Figure. 1 HIFU MS-2 device and HIFU sonication sequence. **(A)** Our triggered-pulse HIFU MS-2 device consisted of HIFU sonication, diagnostic imaging, and a robot control system. A HIFU transducer was attached to the distal part of a 6-degree-of-freedom robot and covered with a water bag filled with degassed water to avoid ultrasound attenuation. An ultrasound probe was installed in the central axis of the HIFU transducer, allowing the ultrasonic image to be observed with the ultrasound diagnostic system. The placement of the HIFU transducer was robotically deployed using a dedicated robot control unit. An operator set the target position by moving the HIFU transducer while observing the ultrasound image, and started HIFU sonication. **(B)** The HIFU sonication sequence consisted of a triggered-pulse and a heating burst. The triggered pulse generated cavitation bubbles, and the heating burst sustained the bubbles to enhance the heating effect through friction between tissues. **(C)** In a single sonication sequence, six points arranged in a 6 mm circle are sonicated in sequential order at high speed.

HIFU, high-intensity ultrasound

Figure. 2 Changes in the diagnostic imaging of patient no. 6 with pancreatic cancer in cohort 2. **(A) and (C)** Before SDT, the contrast-enhanced CT portal phase showed that the mass in the body of the pancreas was partially/faintly stained. **(B) and (D)** One week after SDT, the contrast-enhanced CT portal phase showed that the entire mass in the body of the pancreas was not stained at all. Additionally, the blood vessels within the mass were unaffected and remained intact. **(E)** Contrast-enhanced ultrasound pre-SDT showed a hypoechoic mass, but blood flow signals were observed inside the tumor. **(F)** Contrast-enhanced ultrasound one week after SDT showed slight blood flow signals at the tumor margins, but the entire tumor was anechoic, and the blood flow signals previously observed inside the tumor had disappeared.

CT, computed tomography; SDT, sonodynamic therapy

Figure. 3 Changes in the diagnostic imaging of patient no. 5 with intrahepatic

455 cholangiocarcinoma in cohort 2. **(A) and (C)** In the pre-SDT contrast-enhanced CT portal
456 phase, the tumor was partially/faintly stained and was adjacent to the gallbladder. **(B) and**
457 **(D)** One week after SDT, on contrast-enhanced CT portal phase imaging, the entire tumor
458 was not stained at all. Additionally, the adjacent gallbladder was unaffected. **(E)** Contrast-
459 enhanced ultrasound pre-SDT showed a hypoechoic mass, but blood flow signals were
460 observed inside the tumor. **(F)** Contrast-enhanced ultrasound one week after SDT showed
461 slight blood flow signals at the tumor margins, but the entire tumor was anechoic, and the
462 blood flow signals previously observed inside the tumor had almost disappeared. **(G)** A
463 tumor biopsy conducted one week after SDT revealed complete necrosis of the tumor.
464 CT, computed tomography; SDT, sonodynamic therapy
465

Table 1 Patient characteristics

	All Patients (N = 12)	HIFU power	
		75W (N = 6)	150W (N = 6)
Age, years			
Mean (range)	63.7 (47 - 74)	64.5 (58 - 74)	62.8 (47 - 73)
Sex, n (%)			
Male	5 (41.7)	3 (50.0)	2 (33.3)
Female	7 (58.3)	3 (50.0)	4 (66.7)
ECOG performance status score, n (%)			
0	6 (50.0)	4 (66.7)	2 (33.3)
1	6 (50.0)	2 (33.3)	4 (66.7)
Clinical stage, n (%)			
IV	12 (100)	6 (100)	6 (100)
Tumor location, n (%)			
Pancreatic head	5 (41.7)	3 (50.0)	2 (33.3)
Pancreatic body	5 (41.7)	2 (33.3)	3 (50.0)
Pancreatic body and tail	1 (8.3)	1 (16.7)	0 (0.0)
Cholangiocellular carcinoma	1 (8.3)	0 (0.0)	1 (16.7)
Tumor diameter, mm			
Median (range)	34.5 (20 - 78)	43.5 (20 - 78)	32.5 (20 - 75)
Metastatic site, n (%)			
Peritoneum	12 (100)	6 (100)	6 (100)
Lymph node	11 (91.7)	6 (100)	5 (83.3)
Liver	8 (66.7)	3 (50.0)	5 (83.3)
Ascites	4 (33.3)	1 (16.7)	3 (50.0)
VAS score			
Mean (range)	2.3 (0 - 10)	2.7 (0 - 8)	2.0 (0 - 10)
Therapeutic history			
Chemotherapy	11 (91.7)	6 (100)	5 (83.3)
None	1 (8.3)	0 (0.0)	1 (16.7)

ECOG, Eastern Cooperative Oncology Group; HIFU, high-intensity focused ultrasound; VAS, visual analog scale

Table 2 Individual characteristics of patients in each cohort

	No.	Dosage of K-912	HIFU Power	Age	Sex	Disease	Location	Metastasis, ascites	Stage	PS	Tumor size	VAS score	Therapeutic history
Cohort 1	1	30 mg/m^2 (47.8 mg)	75 W	61	M	PC	Head	LN, P	IV	1	78 mm	7	Chemo (TS1), HIFU
	2	30 mg/m^2 (53.5 mg)	75 W	62	M	PC	Head	L, LN, P	IV	0	32 mm	0	Chemo (GEM/nab-PTX, mFOLFIRINOX), HIFU
	3	30 mg/m^2 (41.3 mg)	75 W	58	F	PC	Body	L, LN, P, A	IV	1	65 mm	8	Chemo (GEM/nab-PTX), HIFU
Cohort 2	4	30 mg/m^2 (41.5 mg)	150 W	73	F	PC	Body	P	IV	0	20 mm	0	Chemo (GEM)
	5	30 mg/m^2 (56.1 mg)	150 W	47	M	CCC	-	L, LN, P, A	IV	0	75 mm	0	Chemo (GEM+CDDP)
	6	30 mg/m^2 (41.6 mg)	150 W	49	F	PC	Body	L, LN, P	IV	1	28 mm	10	Chemo (GEM/nab-PTX), HIFU
Cohort 3	7	80 mg/m^2 (125.1 mg)	75 W	68	F	PC	Head	LN, P	IV	0	55 mm	0	Ope, chemo (GEM/nab-PTX, S1), HIFU
	8	80 mg/m^2 (104.8 mg)	75 W	74	F	PC	Body	L, LN, P	IV	0	23 mm	0	Chemo (GEM/nab-PTX)
	9	80 mg/m^2 (132.2 mg)	75 W	64	M	PC	Body and tail	LN, P	IV	0	20 mm	1	Chemo (GEM/nab-PTX, TS1)

	10	80 mg/m^2 (109.5 mg)	150 W	72	F	PC	Body	L, LN, P, A	IV	1	27 mm	1	BSC
Cohort 4	11	80 mg/m^2 (103.9 mg)	150 W	71	F	PC	Head	L, LN, P, A	IV	1	37 mm	1	Chemo (GEM)
	12	80 mg/m^2 (131.3 mg)	150 W	65	M	PC	Head	L, LN, P	IV	1	49 mm	0	Chemo (GEM/nab-PTX, mFOLFIRINOX)

A, ascites; BSC, best supportive care; CCC, cholangiocellular carcinoma; chemo, chemotherapy; F, female; GEM, gemcitabine; HIFU, high-intensity focused ultrasound; L, liver; LN, lymph node; M, male; mFOLFIRINOX, 5-flurouracil, irinotecan, and oxaliplatin; nab-PTX, nanoparticle albumin-bound paclitaxel; ope, surgery; PC, pancreatic cancer; P, peritoneum; PS, performance status; TS1, tegafur-gimeracil-oteracil potassium; VAS, visual analogue scale

475 **Table 3** Summary of the sonodynamic therapy

	All Patients (N = 12)
Number of sonications, times	
Mean ± SD (range)	17.4 ±9.5 (6 - 40)
Treatment time, minutes	
Mean ± SD (range)	22.3 ± 10.0 (10 - 40)
Tumor coagulative necrosis rate	
Complete response, n (%)	4 (33.3)
Partial response, n (%)	5 (41.7)
Proportion of tumor reduction, n (%)	5 (41.7)
Disease control rate, n (%)	8 (66.7)
Pain relief effect, n (%) †	2 (33.3)
Adverse events (≥ Grade 3), n (%)	0 (0.0)

476 † Six patients who had pain at baseline

477 SD, standard deviation

478

Table 4 Summary of sonodynamic therapy for individual patients

	No.	Dosage of K-912 (mg/m^2)	HIFU Power (W)	Disease	Number of HIFU sonication	Treatment time (min)	VAS		Tumor size (mm)		RECIST (local)	Tumor coagulative necrosis response	Survival time (days)
							Pre-treatment	1 month after treatment	Pre-treatment	1 month after treatment			
Cohort 1	1	30	75	PC	19	25	7	2	78	74	SD	Partial	210
	2	30	75	PC	16	30	0	0	32	23	SD	Partial	349
	3	30	75	PC	31	27	8	1	65	54	SD	Partial	121
Cohort 2	4	30	150	PC	6	10	0	0	20	20	SD	None	677
	5	30	150	CCC	40	40	0	0	75	69	SD	Complete	31
	6	30	150	PC	8	10	10	9	28	37	PD	Complete	74
Cohort 3	7	80	75	PC	10	15	0	0	55	58	SD	Partial	358
	8	80	75	PC	12	20	0	0	23	31	PD	None	72
	9	80	75	PC	14	15	1	1	20	25	PD	None	140
Cohort 4	10	80	150	PC	15	15	1	1	27	-	-	Complete	37
	11	80	150	PC	14	20	1	1	37	37	SD	Partial	44
	12	80	150	PC	24	40	0	0	49	46	SD	Complete	170

CCC, cholangiocellular carcinoma; HIFU, high-intensity focused ultrasound; PC, pancreatic cancer; PD, progressive disease; RECIST, Response Evaluation Criteria in Solid Tumours; SD, stable disease; VAS, visual analogue scale

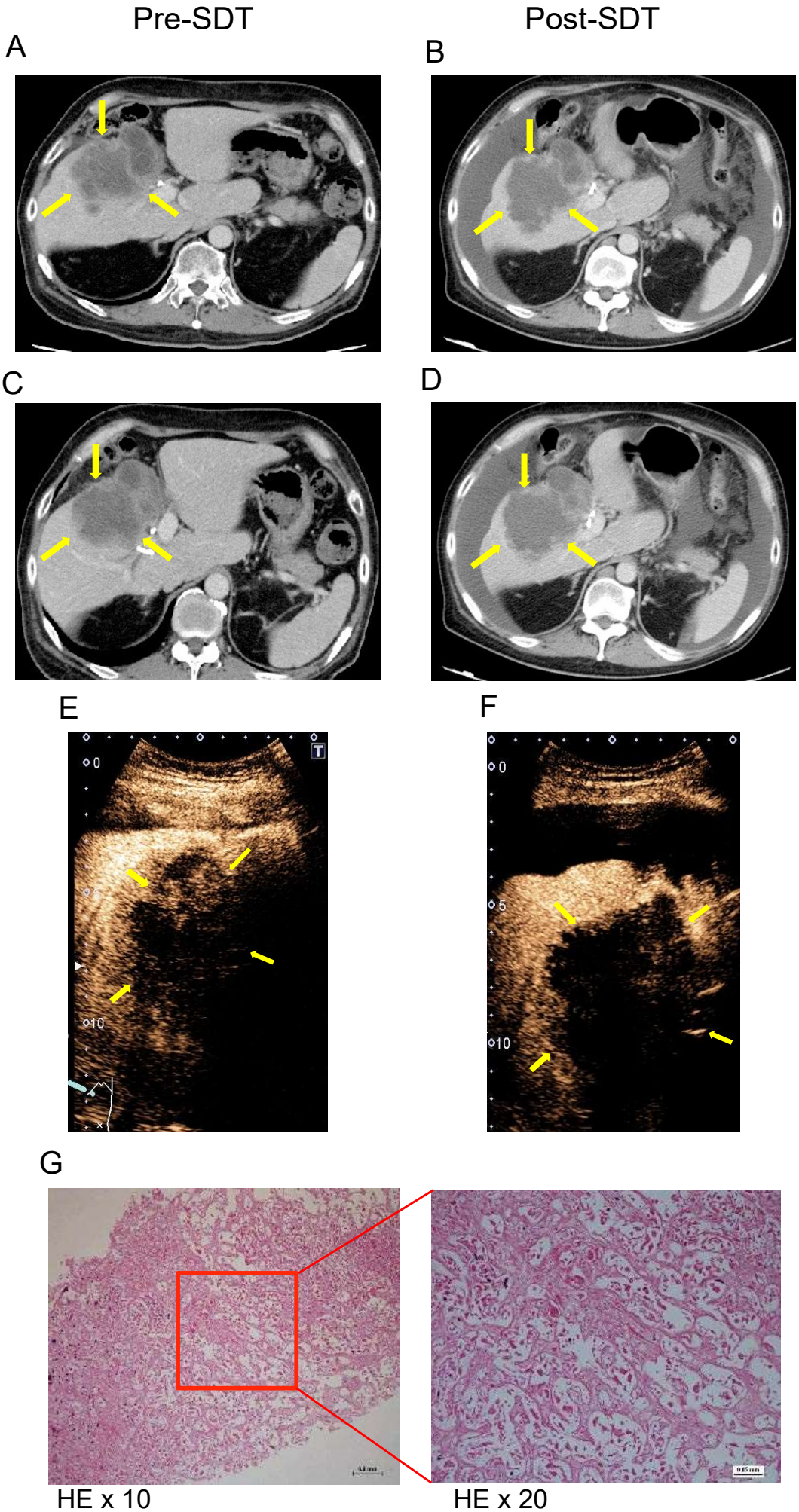
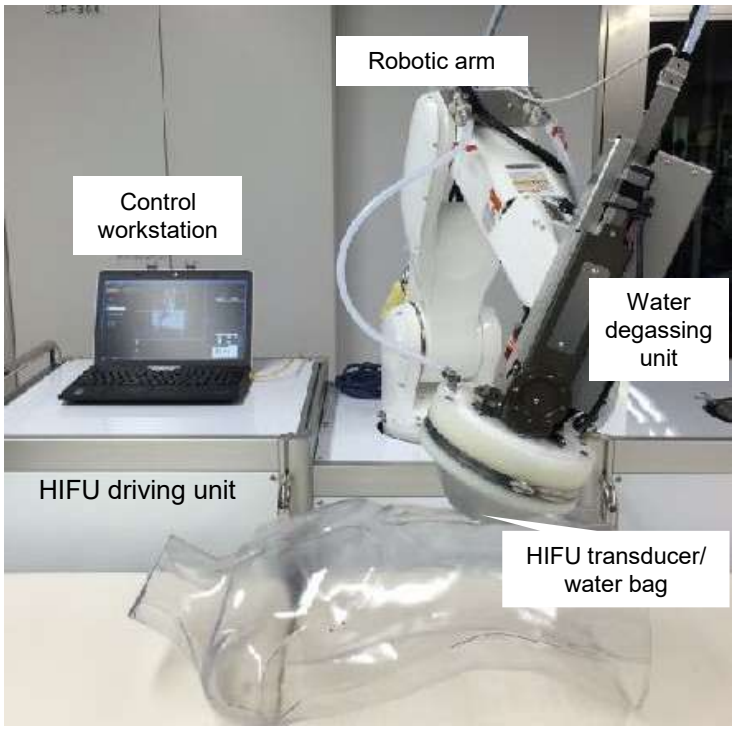
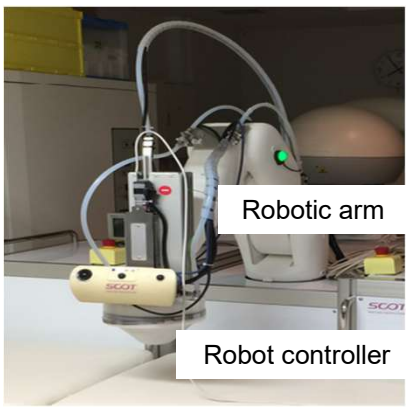
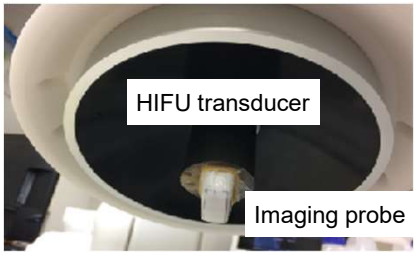


Fig. 3

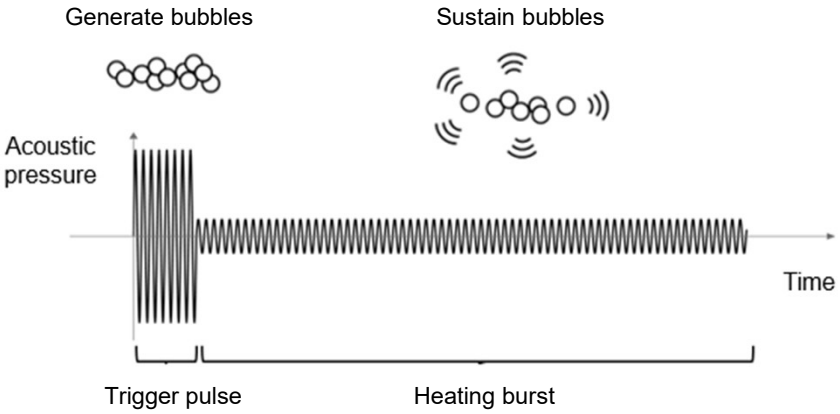
A


Robotic arm
Control workstation
Water degassing unit
HIFU driving unit
HIFU transducer/ water bag


Robotic arm
Robot controller


HIFU transducer
Imaging probe

B


Generate bubbles
Sustain bubbles
Acoustic pressure
Time
Trigger pulse
Heating burst

C

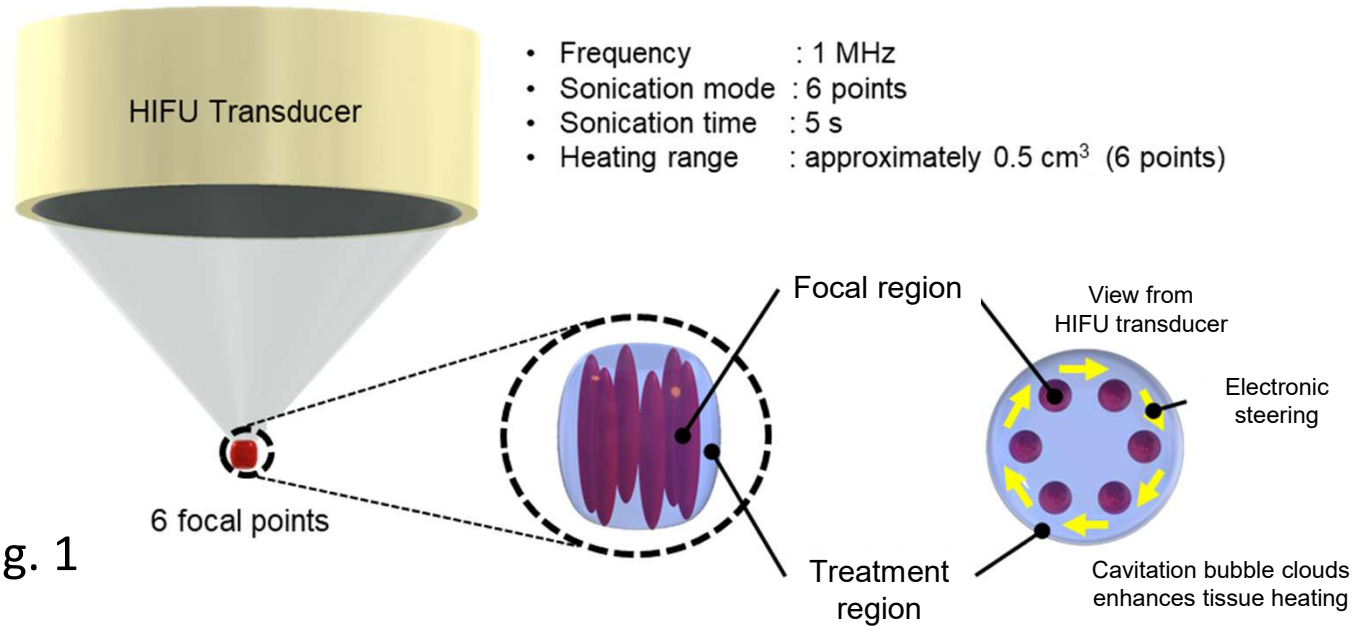
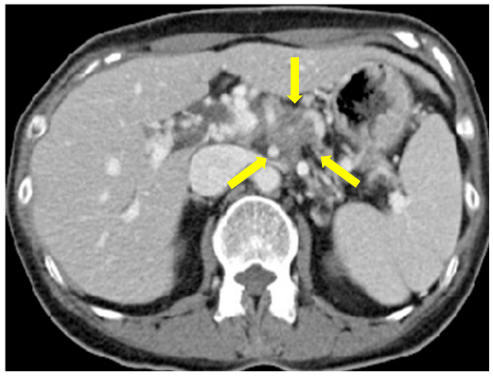

HIFU Transducer
• Frequency : 1 MHz
• Sonication mode : 6 points
• Sonication time : 5 s
• Heating range : approximately 0.5 cm3 (6 points)
Focal region
View from HIFU transducer
Electronic steering
6 focal points
Treatment region
Cavitation bubble clouds enhances tissue heating

Fig. 1

Pre-SDT

Post-SDT

A



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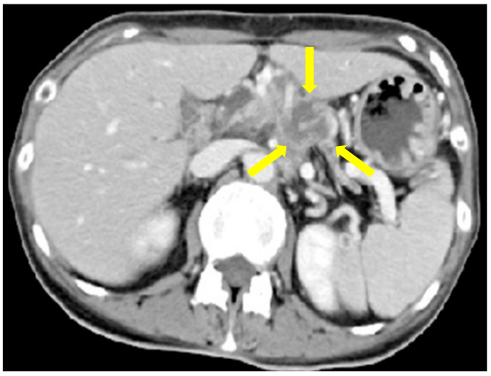
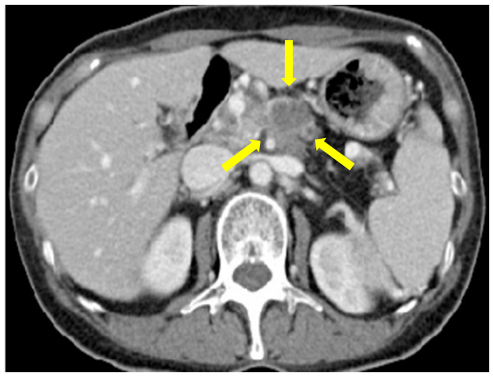


C



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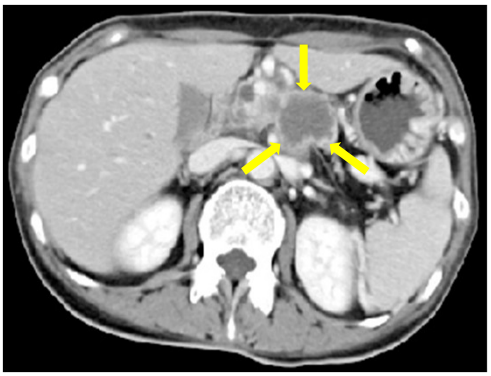

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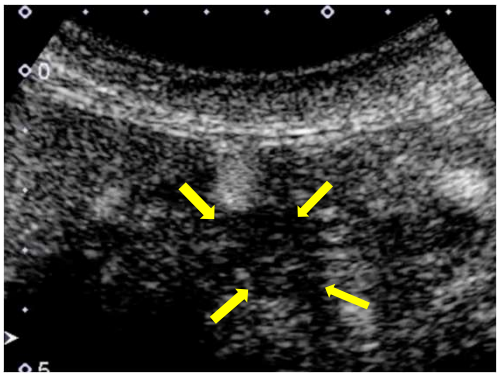

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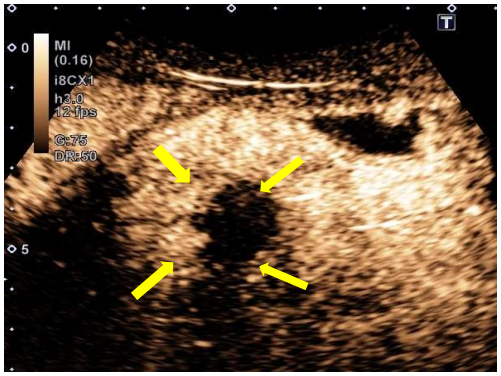

Fig. 2