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1 Fast and selective ablation of liver tumors by high-intensity focused ultrasound
2 using a toroidal transducer guided by ultrasound imaging: the results of animal
3 experiments

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1 ABSTRACT

2

3 This study demonstrated that high-intensity focused ultrasound (HIFU) produced with an
4 intra-operative toroidal-shaped transducer causes fast, selective liver tumor ablations in an
5 animal model. The HIFU device is comprised of 256 emitters working at 3 MHz. A 7.5 MHz
6 ultrasound imaging probe centered on the HIFU transducer guided treatment. VX2 tumor
7 segments (25 mg) were implanted into the right lateral liver lobes of 45 New Zealand rabbits.
8 The animals were evenly divided into groups 1 (toroidal HIFU ablation), 2 (surgical
9 resection), and 3 (untreated control). Therapeutic responses were evaluated with gross
10 pathology and histology 11 days post treatment. Toroidal transducer-produced HIFU ablation
11 (average ablation rate 10.5 cc/min) allowed fast and homogeneous tumor treatment.
12 Sonograms showed all ablations. VX2 tumors were completely coagulated and surrounded by
13 safety margins without surrounding-organ secondary HIFU lesions. HIFU group tumor
14 volumes at autopsy (39 mm³) were significantly lower than control group volumes (2610
15 mm³, p<0.0001). HIFU group tumor metastasis (27%) was lower than resected (33%) and
16 control (67%) group metastasis. Ultrasound imaging, gross pathology and histology results
17 supported these outcomes. HIFU procedures had no complications. Rabbit liver tumor
18 ablation using a toroidal HIFU transducer under ultrasound imaging guidance might therefore
19 be an effective intra-operative treatment for localized liver metastases.

20

21 Keywords: Ultrasound, HIFU, therapy, liver, VX2, in vivo, rabbit, animals

INTRODUCTION

Colorectal liver metastases (CLMs) represent a major public health issue (Bray, et al. 2018). Hepatic resection is the gold standard in the treatment of CLMs and is currently the best treatment option for long-term survival. Five-year survival rates of up to 51% have been reported after resection (Nordlinger, et al. 2013). Liver resection and tumorectomy are widely accepted as a potentially curative treatment for liver metastases from the gastro-intestinal tract. However, the indications for liver resection and even more for tumorectomy in the treatment of liver metastases from non-colorectal primary tumors are still unclear (Chen, et al. 2020, Heinrich 2019, Maeda, et al. 2015). This discrepancy may be due to the fact that liver metastases from other cancers typically occur in the setting of widespread systemic failure, whereas tumors arising within the portal system possess a greater proclivity for establishing truly isolated liver metastases. Currently, surgical management of liver metastases primarily involves interventions that focus on achieving potential cure; therefore, patient selection is limited to those in whom the liver is the only site of failure or the dominant site of disease. Despite progress in surgery (Adam, et al. 2000) and downsizing obtained with chemotherapy (Folprecht, et al. 2005), only a minority of patients are eligible for surgery (less than 20%). Patients are eligible for hepatic resection if complete resection of colorectal metastases is possible with tumor-free margins. Additionally, the remaining functional hepatic volume must be sufficient to ensure adequate postoperative liver function (Pawlik, et al. 2008). Combining hepatic resection and intra-operative local destruction, such as radiofrequency ablation, cryosurgery ablation or microwaves is a widely accepted multidisciplinary treatment for unresectable colorectal cancer liver metastases to increase the number of patients treated with a curative intent (Sasaki, et al. 2016). However, all these techniques require intraparenchymal introduction of a probe, which can be a limit in some difficult regions (Meijerink, et al. 2018).

1 Moreover, RFA is frequently limited by perfusion and CSA is limited by heat conduction
2 (Lin, et al. 2016). In addition, local recurrence has been reported especially for tumors with a
3 diameter larger than 3 cm (van Amerongen, et al. 2017, Wu, et al. 2011).

4 High-intensity focused ultrasound (HIFU) has been proven effective in a wide range of
5 clinical applications (Copelan, et al. 2015, Hsiao, et al. 2016). HIFU treatment does not
6 require to puncture the parenchyma and monitoring is possible using B-Mode ultrasound
7 imaging (Parmentier, et al. 2009). As current regional destruction techniques for treating
8 CLMs make it possible to increase the number of patients treated with curative intend we
9 developed a HIFU device that can be used during surgery. Such an approach is invasive but
10 has the advantage to be less invasive than conventional intra-operative local destruction
11 techniques since the HIFU treatment is performed without puncture of the liver and thus
12 preserve the hepatic capsule. We have previously reported that the use of a HIFU transducer
13 with a toroidal geometry can increase the ablation rate by increasing the focal volume
14 (Chenot, et al. 2010, Melodelima, et al. 2007, N'Djin, et al. 2011). Several preclinical studies
15 (in vitro and in vivo) have validated the concept of using toroidal HIFU focusing for inducing
16 large ablations in a short amount of time without the need for mechanical scanning allowing
17 to use the device by hand (Caloone, et al. 2017, Melodelima, et al. 2009, Vincenot, et al.
18 2013). Since this HIFU treatment is planned to be performed during an open procedure
19 (Dupre, et al. 2015, Dupre, et al. 2017), the ultrasound propagation was not dependent on
20 anatomical constraints, such as the position of the ribs or lungs. Therefore, the device was
21 used and evaluated in an open procedure to provide a complementary tool to surgeons for
22 hepatic resection. In normal liver tissues, the toroidal HIFU focusing strategy indeed
23 confirmed promising results in terms of ablations rate, homogeneity and repeatability (Dupre,
24 et al. 2015). To date however, the impact of this focusing method on the ablation quality has
25 not been clearly investigated in the case of heterogeneous target tissues, especially in presence

1 of solid liver tumors. Here, we report an in-vivo evaluation of this unconventional treatment
2 strategy in a rabbit liver tumor model. Particular attention was paid to the evaluation of the
3 HIFU ablations obtained in this tumor model and the short-term efficacy of the treatment with
4 regard to tumor growth.

5

6

MATERIALS AND METHODS

7

8 *Therapeutic ultrasound device*

9 The therapeutic transducer was a piezocomposite material (Imasonic, Voray-sur-
10 l'Ognon, France) operating at a frequency of 3 MHz. The transducer has a toroidal shape 70
11 mm in diameter and was divided into eight radial ultrasound emitters. The radius of curvature
12 was 70 mm, and each of the 8 emitters was divided into 32 individual transducers (Figure 1).
13 Then, the spatial location and intensity of the pressure field were controlled electronically by
14 modulating the amplitude and the phase applied to each of the individual transducers. An
15 ultrasound imaging probe working at a frequency of 7.5 MHz (Vermon, Tours, France) was
16 placed in the center of the device and connected to a BK HAWK 2102 EXL scanner (B-K
17 Medical, Herlev, Denmark) for guiding the treatment. The imaging plane was aligned with the
18 HIFU acoustic axis. The device was in acoustic contact with the liver using an ultrasound
19 coupling fluid (Ablasonic®, EDAP, Vaux-en-Velin, France). The Ablasonic® was contained
20 in a sterile polyurethane envelope (CIV-Flex Transducer cover, CIVCO, Kalona, Iowa, USA)
21 that covered the device, making it possible to use the HIFU system under sterile conditions.
22 Continuous flow (0.3 l/min) maintained the degassed coupling water at 20°C and allowed the
23 cooling of the HIFU transducer during the treatment. A peristaltic Masterflex pump (L/S

1 model 7518-60, Cole-Parmer Instruments Co., Chicago, IL, USA) drove the water around a
2 closed cooling circuit.

3 The HIFU transducer-driving equipment in this experiment was similar to that
4 reported previously (Melodelima, et al. 2005); 32 channels of a 50 channel pattern generator
5 (PG1050 Acute, Hsin Chuang City, Taiwan) generated 32 transistor-transistor logic (TTL)
6 signals at the working frequency (3 MHz). Thirty-two power amplifiers (AHF 855, ADECE,
7 Artannes, France) converted the TTL signals into amplified sine waves. The TTL-signals
8 were AC-coupled into the amplifier which also performed bandwidth-limited signal
9 amplification. The amplified signals were then band-pass filtered by their respective matching
10 networks, resulting into amplified sine-waves that were required to drive the individual
11 transducers. This system was developed to allow electronic beam steering while optimizing
12 the number of power amplifiers. An individual element, i , of the first emitter ($1 \leq i \leq 32$), as
13 well as the seven similar elements located at same position in the other seven radial emitters,
14 were supplied in parallel by only one power amplifier. Inside each amplifier, a directional
15 coupler generated 2 voltages, one that was directly proportional to the direct electrical power
16 and one that was directly proportional to the reflected electrical power. The gain of each
17 amplifier was adjusted using digital-analog output cards to control the acoustic power
18 delivered in tissues during treatment. Finally, the user interface displayed the position of the
19 HIFU focal region superimposed on the sonogram, making it possible to place the location of
20 the ablation in specific tissues and visualize in real time the coagulated zone created during
21 sonication.

22

23 *Animals*

1 Experiments were performed on 45 New Zealand rabbits from Charles River
2 Laboratories (Ecully, France). While no model can truly represent CLM, VX2 tumors
3 implanted in the liver of rabbits provide a good substitute with regard to the size of the animal
4 used to test the HIFU device. Animals weighed between 2.5 and 3.2 kg on the day of arrival.
5 The animals were kept on-site 7 days before the start of the experiments. The experiments
6 were conducted at the Institute of Experimental Surgery of the Léon Bérard Cancer Center
7 after local institutional review board approval. These animal experiments conformed to the
8 requirements of the INSERM Office of Animal Experimentation and were in accordance with
9 the legal conditions of the National Commission on Animal Experimentation.

10

11 *Implantation of VX2 liver tumors*

12 VX2 cells were initially implanted in both hind limbs of a donor rabbit. Frozen VX2
13 cancer was extracted from frozen archives which had been used in previous research. Frozen
14 VX2 tumor tissue kept in -80°C was thawed, minced, and isolated with a $100\ \mu\text{m}$ cell strainer
15 to obtain a uniform single-cell suspension from tissues. A venous catheter was placed in an
16 auricular vein for the premedication, which was performed 15 min prior to anesthesia using a
17 combination of ketamine (1.6 ml) and xylazine (0.1 ml). Anesthesia was performed by
18 intravenous injection of 25% of the premedication dose and was maintained by intravenous
19 injection of the same dose every 15 min. 1.0×10^7 of cells suspended in $500\ \mu\text{l}$ of HBSS
20 solution was injected into donor animals' right lateral quadriceps. After 4 weeks of tumor
21 growth, the hind limb tumors were harvested, and small tumor fragments (approximately 25
22 mg) were dissected from the viable tumor tissue. The abdomen was shaved and cleaned with
23 povidone iodine solution using the anesthesia protocol described above. These tumor portions

1 were then implanted in the lateral right liver lobe of the 45 rabbits during a laparotomy
2 procedure. The liver lobe and the abdomen were then sutured.

3

4 *Treatment procedures*

5 Treatments were performed 10 days after implantation of VX2 tumors. All rabbits
6 were anesthetized using the same protocol as described previously. Fifteen rabbits were
7 treated with toroidal HIFU ablation (Group 1). Treatments were performed under sterile
8 conditions during laparotomy. Adjacent organs were carefully isolated from the liver using
9 pads which also allowed to avoid liver movements during HIFU treatments. The HIFU probe
10 was held by hand at the surface of the liver. The tumor to be treated was located using the
11 integrated ultrasound imaging probe by measuring the dimensions of the tumor into several
12 contiguous planes. The acoustic power was 70 W, and the sonication time was 40 seconds to
13 cover the whole tumor with safety margins (i.e., margins ≥ 5 mm) without resorting to
14 mechanical scanning. If incomplete treatment was identified on ultrasound imaging guidance,
15 up to 3 single HIFU were allowed. Fifteen rabbits were included in group 2 for surgical
16 resection. The liver resection technique was performed after laparotomy using a Kelly clamp
17 claw, a suction device, and metallic clips (SLS-CLIP B2180-A, Vitalitec International) when
18 necessary. The judgement of the HIFU treated area and surgical resected area in Group 1 and
19 2 made by the same surgeon.

20 Fifteen rabbits formed a control group (Group 3). For these animals, only the
21 laparotomy was performed. At the end of the treatment, the laparotomy was closed in all
22 animals with a running suture (2 planes). Groups 1 and 2 were compared to evaluate whether
23 toroidal HIFU treatment increases the risk of tumor dissemination. Groups 1 and 3 were
24 compared to evaluate treatment efficacy.

1

2 *Pathologic examination*

3 All animals were euthanized 11 days after treatment by a single intravenous injection
4 of 5 ml of Dolethal® (Vetoquinol, Paris, France). A total hepatectomy was then performed to
5 visually inspect the tumor and ablation. Dimensions of the tumor and ablation were measured
6 along thin contiguous planes. The length (L, millimeters), width (W, millimeters), and depth
7 (D, millimeters) of ablated area and of the tumor were measured in each animal, and the
8 volume (V, cubic millimeters) was calculated as $V = \pi/6 L \times W \times D$ (Di Matteo, et al. 2013).

9 Tumor metastasis was also evaluated. The lungs, kidney, abdominal cavity and pelvic
10 cavity were examined by ultrasonography, especially lymph node metastasis. Metastasis was
11 confirmed by anatomic examination. Once metastasis was identified, the metastasis was
12 visually graded as follows: 0, the organ has normal structure and there is no newly generated
13 mass; 1, there are 1-2 newly generated masses, and metastatic VX2 tumor is confirmed in the
14 pelvic lymph nodes by pathological examination (0-1 cm in diameter); 2, there are 3-10 newly
15 generated masses, and metastatic VX2 tumor is confirmed in the pelvic lymph nodes by
16 pathological examination (1-2 cm in diameter); 3, there are more than 10 newly generated
17 masses, and metastatic VX2 tumor is confirmed in the pelvic lymph nodes by pathological
18 examination (>2 cm in diameter).

19 All collected samples were also evaluated microscopically. Representative portions of
20 tissues were immersed in 10% formalin for fixation and set in paraffin. The samples were
21 then stained with hematoxylin and eosin. The samples were treated and analyzed by the
22 Anipath Laboratory (Lyon, France).

23

1 *Statistical analysis*

2 All images obtained from sonograms and gross pathology were interpreted and
3 evaluated independently by three examiners. All examiners were blinded to the results of each
4 other. Lin's Concordance Correlation Coefficient (CCC) was used to evaluate the inter-reader
5 variability (Lin 1989). The CCC was interpreted using the classification of Partik et al. that
6 ranges the CCC from unacceptable (<0.50) to excellent (>0.95) (Partik, et al. 2002).

7 Data were given as the mean values \pm standard deviations (minimum value–maximum
8 value). The studied criteria were the dimensions of the tumors measured via gross pathology.
9 The study was designed to show a difference of at least 25% in the tumor volumes for
10 statistical significance at the 5% level between groups with a power of 95%. The statistical
11 analyses were performed using R® software (The R Foundation for Statistical Computing,
12 Vienna, Austria). Data at different time points were compared with one-way analysis of
13 variance (ANOVA), followed by paired comparison with Student' t test The significance level
14 for all tests was fixed at $p = 0.05$.

15

16

RESULTS

17 Figure 2 shows a VX2 tumor visualized with the integrated ultrasound imaging probe
18 just before (Figure 2a) and just after (Figure 2b) one HIFU ablation. As previously described,
19 HIFU lesions created in the liver with a toroidal transducer were seen as a hypoechoic
20 (Melodelima, et al. 2009), whereas VX2 tumors appear as hyperechoic. Therefore, it was
21 possible to clearly identify whether the treatment was performed with safety margins at the
22 time of ablation and to apply another HIFU sonication when necessary. In this study, 12 VX2
23 tumors were treated with only one HIFU sonication, and 3 VX2 tumors were treated with two
24 HIFU sonications. The total procedure time, including targeting, HIFU ablations and the time

1 to perform ultrasound imaging averaged 14 ± 4 min (9 – 24). The time for resection in group
2 2 was significantly longer and averaged 34 ± 8 min (22 – 47). In all cases, the VX2 tumors
3 were completely coagulated and surrounded by ablated liver tissue without secondary thermal
4 lesions in surrounding organs. The coagulated tissues are clearly distinguishable from
5 untreated tissue; the treated region has an off-white color, which is sometimes dark at the
6 most heated points (Figure 3). The average treatment margins around the VX2 tumor were
7 negative in all directions, with a minimum value of 3 mm. Based on ultrasound imaging, on
8 the day of the treatment, the tumor volumes averaged 162 ± 20 mm³, 150 ± 19 mm³ and $145 \pm$
9 21 mm³ for the HIFU, resected and control groups, respectively. There were no significant
10 differences between the tumor volumes on the day of the treatment of the groups. The
11 correlation between the dimensions of the HIFU treated region measured using ultrasound
12 imaging and the dimensions measured via gross pathology after autopsies was $r = 0.88$, $p <$
13 0.05 (Figure 4). The agreement between measurements made by the three observers ranges
14 from satisfactory (0.765) to excellent (0.989). According to the classification of Partik et al.
15 (Partik, et al. 2002), the CCC for dimensions measured via gross pathology were 0.765
16 (satisfactory), 0.815 (fairly good) and 0.901 (very good) for inter-readers 1 and 2, 1 and 3 and
17 2 and 3 respectively. The CCC for dimensions measured using ultrasound imaging were 0.989
18 (excellent), 0.879 (fairly good) and 0.869 (fairly good) for inter-readers 1 and 2, 1 and 3 and 2
19 and 3 respectively. Figure 5 shows that tumor volume was significantly lower at the time of
20 autopsy (39 ± 9 mm³) in the HIFU group than in the control group (2610 ± 700 mm³,
21 $p < 0.0001$). The tumor and the surrounding tissues treated by HIFU are still visible on gross
22 pathology at the day of autopsy (Figure 3) but complete coagulation necrosis was confirmed
23 in both tissues (normal and tumoral) by histological analysis (Figure 6). The tumor volume
24 measured macroscopically in group 2 (resection) at the day of the resection was on average
25 157 ± 23 mm³. Secondary tumors were observed in 4, 10 and 5 animals in the HIFU group,

1 control group and resected group, respectively. Tumor dissemination was significantly lower
2 in the HIFU group (27%) than in the control group (67%) and all metastases were graded 0
3 (Table 1). There was no significant difference between tumor dissemination in the HIFU
4 group and the resected group (33%). The dissemination sites were the stomach, the lungs and
5 the peritoneum in all groups.

6 Histological findings supported these outcomes. Edges of the coagulated area were
7 clearly delimited. The demarcation between treated and untreated liver was very sharp
8 (approximately 200 μ m). There was a halo of congestive tissues around all HIFU ablations.
9 The microscopic examination confirmed complete homogeneous coagulation necrosis in the
10 tumors and in the safety margins for all HIFU ablations (Figure 6).

11 The animals tolerated the treatment well over the experimental period. The rabbits
12 remained hemodynamically stable during the procedure. They recovered from the procedure
13 within 1 h of termination of anesthesia and quickly resumed eating and normal behavior. The
14 mean weight gain was 200 g (minimum 50 g – maximum 450 g), which is normal according
15 to the age of the animals and the procedure that was conducted. There were no secondary
16 HIFU lesions in adjacent organs.

17

18

DISCUSSION

19

20 The data from the present study demonstrated that successful rabbit liver tumor
21 ablation can be achieved using an intraoperative toroidal HIFU transducer under ultrasound
22 imaging guidance and therefore could be an effective treatment for localized liver metastases.
23 All tumors treated by HIFU were completely coagulated and therefore irreversibly destroyed,

1 as confirmed by histological analyses. There was no complication related to the HIFU
2 procedure.

3 A potential risk of focused ultrasound therapy was the possible enhancement of
4 metastatic development if viable tumoral cells penetrate damaged blood vessels and then into
5 the circulation. In our study on VX2 liver tumors, the metastatic rate observed in control
6 rabbits (67%) was significantly higher than the metastatic rate observed in HIFU treated
7 animals (27%). There was no significant difference between tumor dissemination in the HIFU
8 group and the resected group (33%). These findings suggested that HIFU therapy with a
9 toroidal transducer and applied to liver tumors should not create a risk of tumor
10 dissemination. The recurrence rate observed in the HIFU treated animals provides convincing
11 evidence that this approach is competitive with invasive surgical resection.

12 In previous work, we have shown that a conventional ultrasound imaging probe
13 integrated in the center of the toroidal HIFU transducer can accurately visualize the
14 coagulated zone created in the liver (Melodelima, et al. 2009). This result was confirmed in
15 the present study. VX2 tumors were clearly visible as a hyperechoic region, and HIFU
16 ablations appeared hypoechoic, leading to an accurate evaluation of the lesion size as well as
17 the safety margins. This allowed to decide whether additional ablations were required in cases
18 of incomplete ablation around tumors. Due to this reliable evaluation of the treatment,
19 quantitative thermometry was not needed to achieve and validate the therapeutic endpoint.

20 The choice of developing a HIFU device dedicated to intraoperative use was supported
21 by oncological necessities and technological challenges that remain unmet in HIFU
22 technology. The thermal power of this device allows treatments that were shown to be not
23 very dependent on perfusion. This is mainly due to the relatively high frequency (3 MHz) and
24 to the small penetration depth when using an intra-operative approach (Melodelima, et al.
25 2009). Using HIFU during an open procedure facilitates the protection of adjacent viscera with

1 protective gauzes that can be placed all around the liver. Gauzes inhibit ultrasound propagation
2 and unintended HIFU damage to adjacent organs. An open procedure also facilitates the intra-
3 abdominal staging of malignancies. Despite improvements in imaging, liver metastases not
4 detected via preoperative imaging are found in approximately 15% of patients during surgery
5 (Arita, et al. 2015). Additionally, the use of ablation in conjunction with resection appears to
6 be the best current strategy in curative treatment of liver metastases from colorectal cancer
7 (Tsitskari, et al. 2019). Our clinical objective is not to use this HIFU device as a replacement
8 for resection but as a complementary tool to surgery. This approach is currently used for RFA
9 and CSA (Pawlik, et al. 2003). Like RFA, CSA and microwaves, combining hepatic resection
10 with intra-operative HIFU ablation could also expand the number of patients who may be
11 candidates for liver-directed surgical therapy but with a less invasive technique since the HIFU
12 treatment is performed without puncture of the liver. Intraoperative ablations are widely
13 employed in the management of CLM, regardless of whether they are associated with surgery.

14 The main limitation to the widespread clinical use of HIFU has been the length of time
15 required to treat tumors several cubic centimeters in volume (Knuttel, et al. 2017, Leslie, et al.
16 2012). This fact compromises the technique for the most prominent applications, i.e., cancers
17 where tumors may be very large and where therapeutic options are limited. Previous studies
18 have been conducted to enlarge the ablated region using, for example, cavitation (Melodelima
19 and Cathignol 2004, Sokka, et al. 2003), phased array systems (Held, et al. 2006), a split focus
20 (Sasaki, et al. 2003) or an optimization algorithm for the focus pattern (Lu, et al. 2005). The
21 use of a toroidal HIFU transducer may fill this therapeutic gap, since it can rapidly induce
22 significantly larger lesions. Because of its toroidal geometry, the transducer focal zone and the
23 lesion induced were shown to be cone-shaped (Caloone, et al. 2019, Dupre, et al. 2017,
24 Vincenot, et al. 2013). Under these settings, the focal zone observed in the focal plane is a ring
25 of 10 mm in diameter. Each of the eight emitters can focus on a distinct arc of circle (1/8 of the

1 ring) and can contribute to the formation of approximately 1/8 of a conical single HIFU lesion.
2 In this study, all the transducers were working in phase in order to use the natural focusing of
3 the torus. HIFU lesions were generated using a total acoustical power of 70 W by activating
4 the eight emitters at the same time. Due to the geometrical characteristics of a torus, the
5 ultrasound beams coming from each of the eight emitters intersect beyond the principal focal
6 ring and form a secondary focal zone, which contributes to reinforce the homogeneity of the
7 lesion at its center (N'Djin, et al. 2011) to create the HIFU lesion of 2 cm in diameter by 2.5 cm
8 on its major axis. Larger lesions can even be produced with this device by using an electronic
9 beam steering up to 45 cm³ in 130 seconds (Vincenot, et al. 2013). Therefore, the treatment
10 duration is significantly shortened using this new transducer geometry, and importantly,
11 without resorting to mechanical scanning.

12 The coagulated zone is large enough for simply placement of the transducer at the right
13 location to target the tumor and produce an ablation with safety margins in 40 to 370 seconds
14 (Dupre, et al. 2019). Compared with surgery, this method of local treatment could allow the
15 treatment of inaccessible juxtavascular metastases and therefore may have a role in treating
16 unresectable colorectal liver metastases and may also be used in conjunction with resection to
17 extend its limits and thus significantly increase the number of patients eligible for curative
18 surgery.

19 There are some limitations to this study. This work was performed on rabbits to find a
20 compromise between the size of the animal and the use of a tumor model. However, the
21 volume of rabbit livers is far away from the size of human livers but physiology is similar.
22 Safety outcomes following surgery in a small animal model might vary from what would be
23 expected with surgical technique in a person with liver metastases and many additional
24 comorbidities. This study helps to provide convincing evidence that this approach is
25 competitive with invasive surgical resection but not to evaluate the creation of ablations in

1 depth. This point was previously validated in a healthy pig model (Melodelima, et al. 2009).
2 Moreover, VX2 tumors provide an excellent model for hepatic tumors with the ability to
3 evaluate the metastatic rate under different treatment conditions. It is the most used tumor
4 model for large animals but VX2 is a rabbit squamous cell cancer line and not a human liver
5 metastatic model limiting the biological evaluation that can be made. However, this model is
6 the closest possible and the most commonly used to evaluate image-guided interventions,
7 particularly in cancer treatment (Aravalli and Cressman 2015). In this study there was no
8 group combining HIFU and resection. As mentioned previously this animal model allows to
9 demonstrate successful liver tumor ablation using toroidal focusing intra-operatively but not
10 to evaluate a complete multimodal management protocol as it would be performed in Humans
11 including chemotherapy, surgical resection, radiation therapy... However, demonstrating the
12 efficacy of this device to ablate hepatic tumors under sterile conditions with adequate
13 ultrasonic guidance for targeting and determining the interventional end-point without
14 promoting the metastatic rate makes it possible to consider clinical application. The main
15 advantage of this treatment during an open procedure is to make it possible to consider
16 associating the HIFU destruction technique with hepatic resection, which seems to be a
17 relevant strategy in curative treatment methods for resecting accessible metastases and
18 clearing residual disease with HIFU.

19 In conclusion, use of a toroidal HIFU transducer allows successful liver tumor ablation
20 and therefore could be an effective treatment for localized liver metastases. The ultrasonic
21 guidance was found to be adequate for targeting and determining the interventional end-point.
22 Quantitative thermometry was not needed to achieve and validate the therapeutic endpoint.
23 The recurrence rate provides convincing evidence that this approach is competitive with
24 invasive surgical resection. The metastatic rate for this invasive (but less invasive than
25 surgery and intra-operative local destruction techniques) approach was more favorable when

1 compared with surgical resection. Based on these results and other preclinical work, a clinical
2 study is ongoing using this device for the treatment of liver metastases.

3

4

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5

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22

23

FIGURES CAPTIONS

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2

3 Figure 1. (a) Toroidal HIFU transducer with an ultrasound imaging probe in the center of the
4 transducer. (b) Simulated pressure field in water emitted by the toroidal transducer. (c)
5 Simulated pressure field in water emitted by a spherical transducer with the same frequency,
6 radius of curvature and diameter.

7

8 Figure 2. VX2 tumor visualized with the integrated ultrasound imaging probe (a) just before
9 and (b) just after one HIFU sonication. (a) The location of the VX2 tumor is indicated by the
10 dotted circle. (b) HIFU ablation appears as a hypoechoic region (indicated by the dotted lines)
11 surrounding the hyperechoic VX2 tumor (indicated by the dotted circle).

12

13 Figure 3. Examples of treated and untreated VX2 tumors. (a) Example of a HIFU lesion
14 observed the day of the treatment. (b), (c) and (d) Examples of three HIFU treatments of VX2
15 liver tumors observed the day of autopsy after the liver was sliced along an axis passing
16 through the tumor. The tumor and the surrounding tissues treated by HIFU were still visible
17 on gross pathology but complete coagulation necrosis was confirmed in both tissues (normal
18 and tumoral) by histological analysis. (e) and (f) Examples of two VX2 tumors that have not
19 been treated and were observed the day of autopsy. One newly generated mass in the liver is
20 visible in (e).

21

22 Figure 4. Correlation between dimensions measured on sonograms using the integrated
23 ultrasound imaging probe and gross pathology. All images obtained from sonograms and
24 gross pathology were interpreted and evaluated independently by three examiners. All

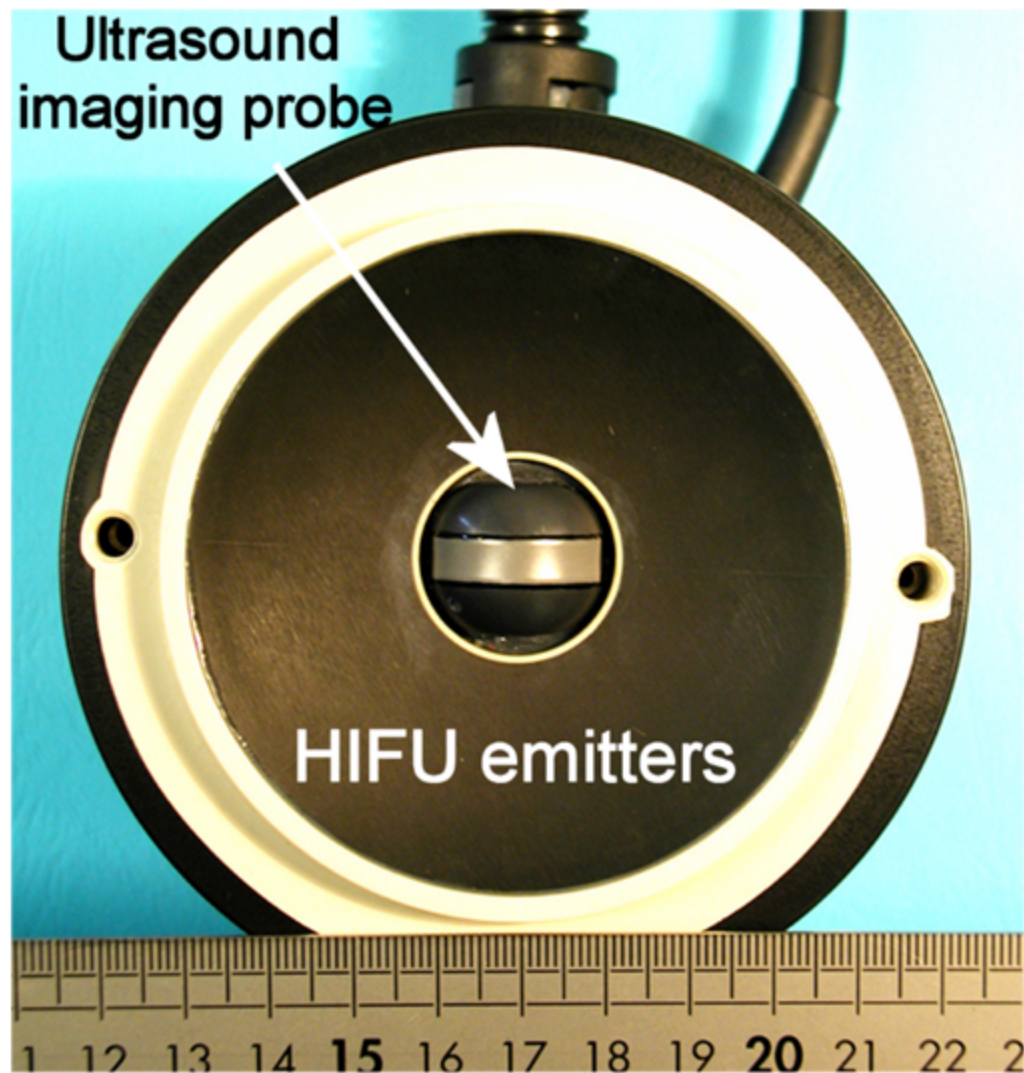
1 examiners were blinded to the results of each other. Measurements performed by each
2 examiner were marked differently using white, black and grey dots.

3

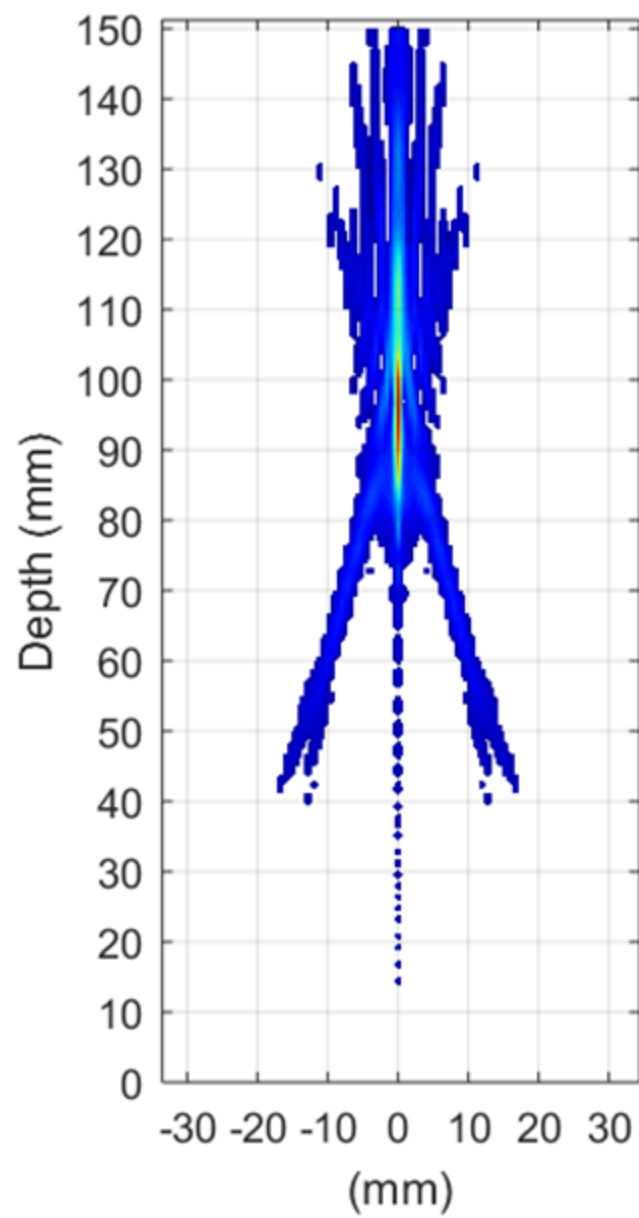
4 Figure 5. Differences in tumor volume between groups in log scale. Statistical differences
5 between groups were marked by a star. The tumor was removed at day 0 in group 2, no value
6 can be shown at day 21 for this group.

7

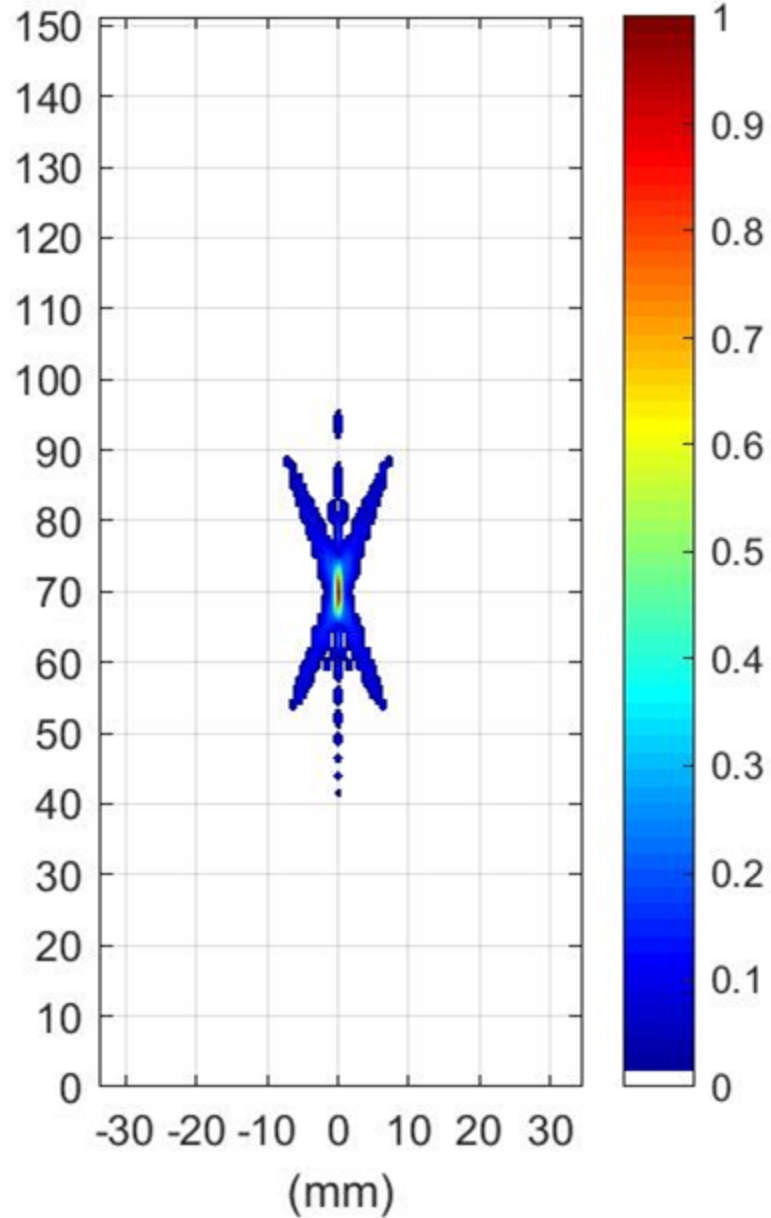
8 Figure 6. Microscopic examination confirming homogeneous necrosis with all HIFU ablations
9 in the tumors and with safety margins. (a) Sharp transition from coagulated area to normal
10 tissue. (b) Treated VX2 tumor in coagulated liver from HIFU ablation.



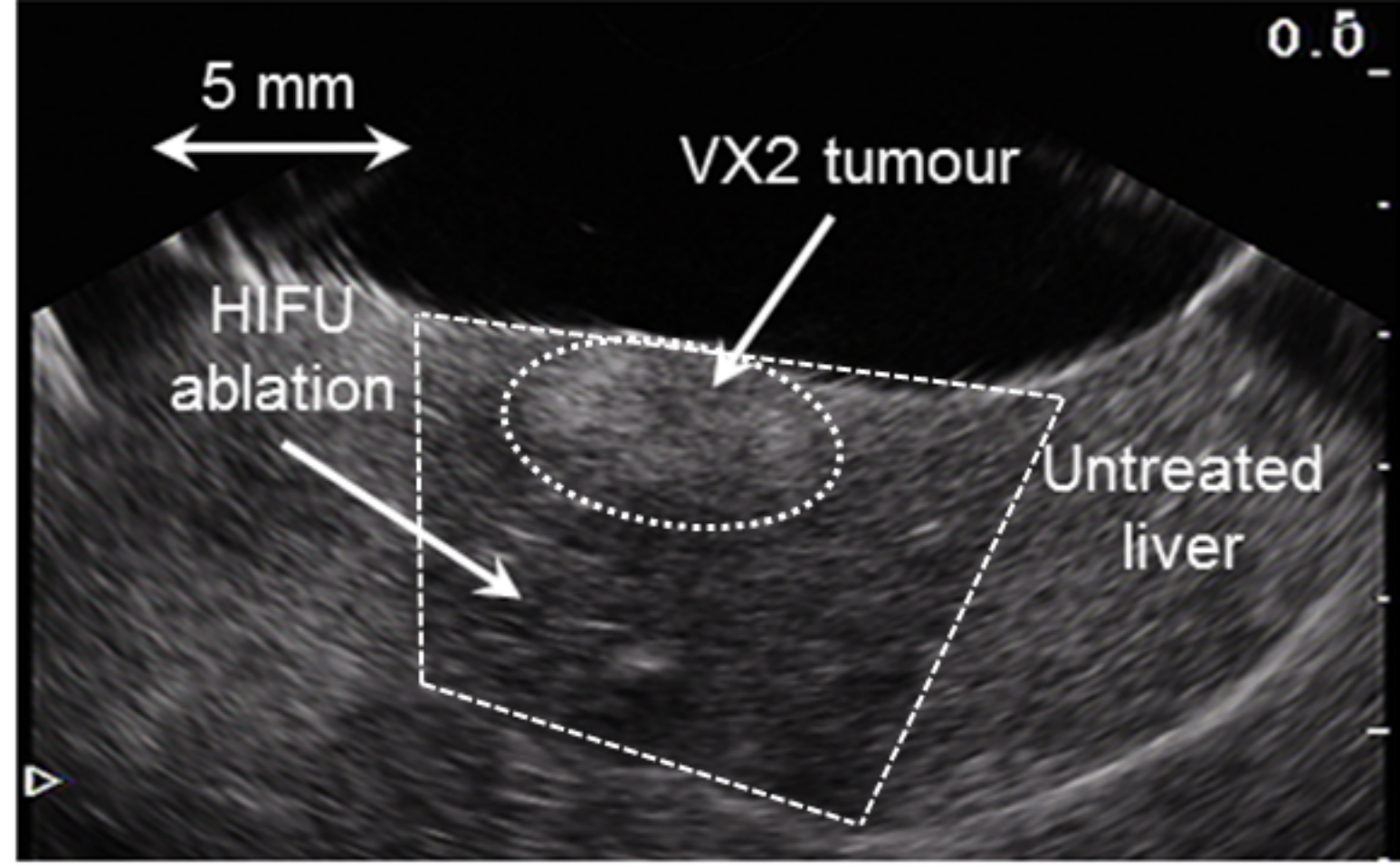
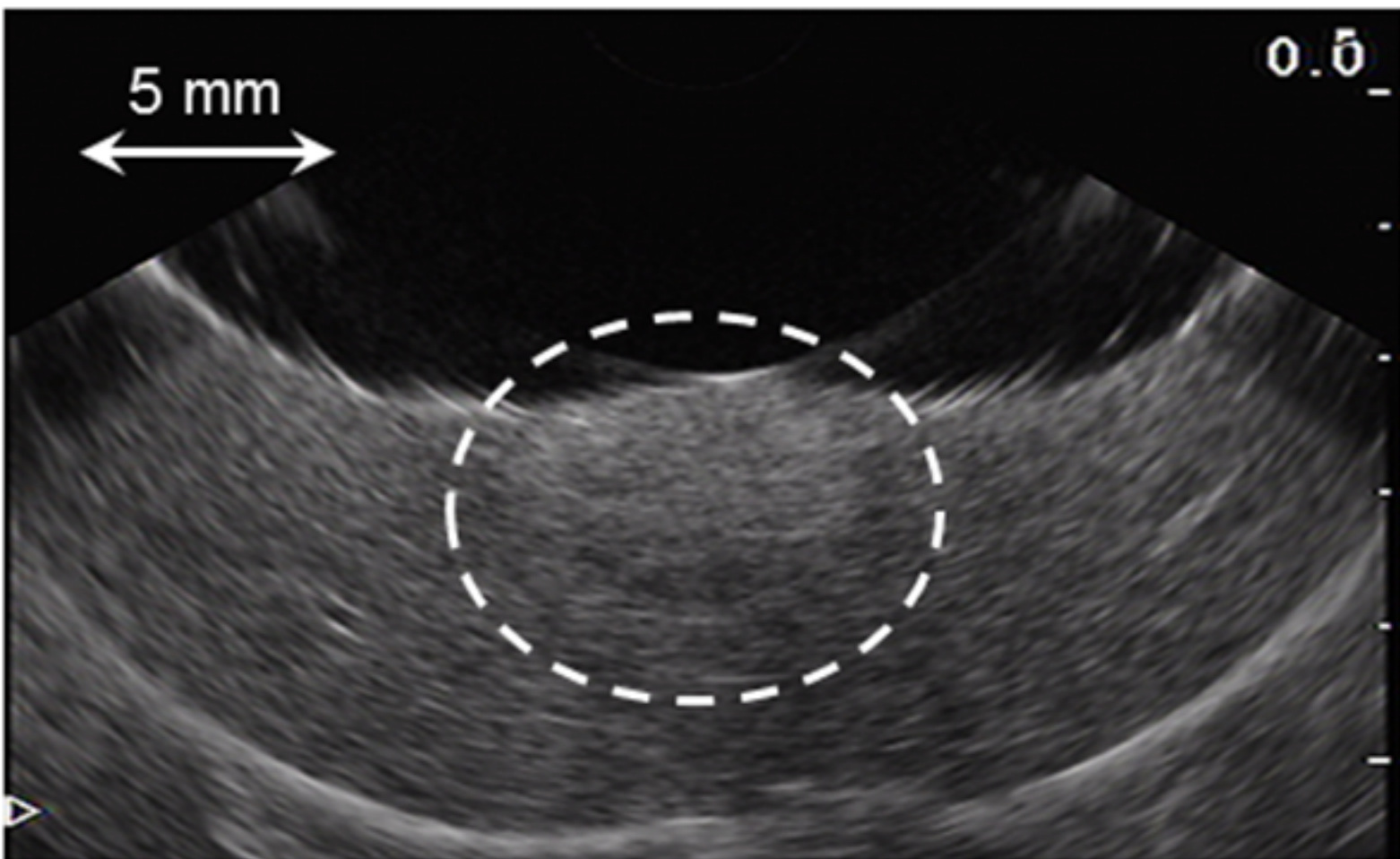
(a)

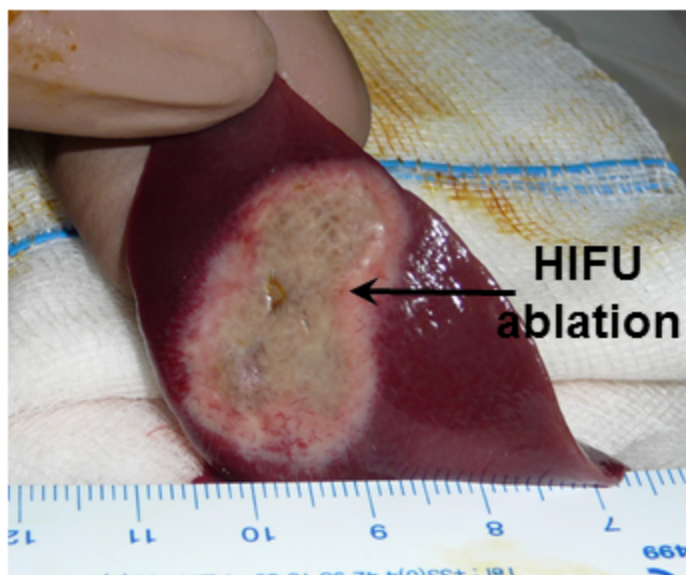


(b)

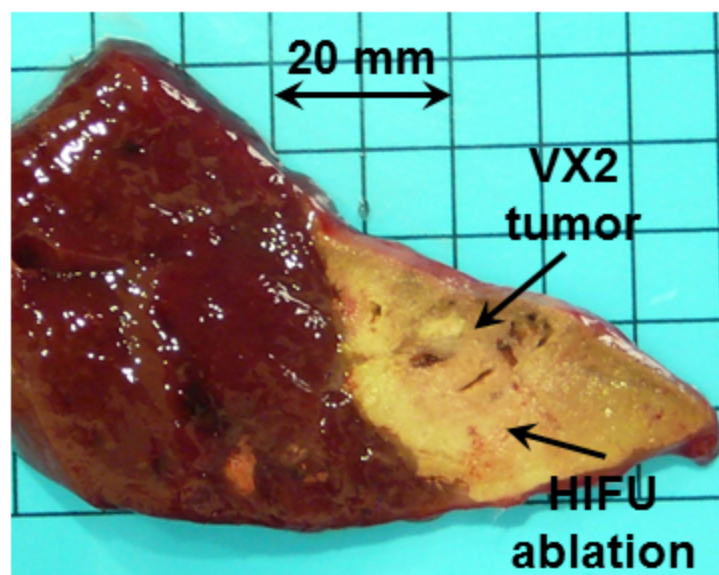


(c)





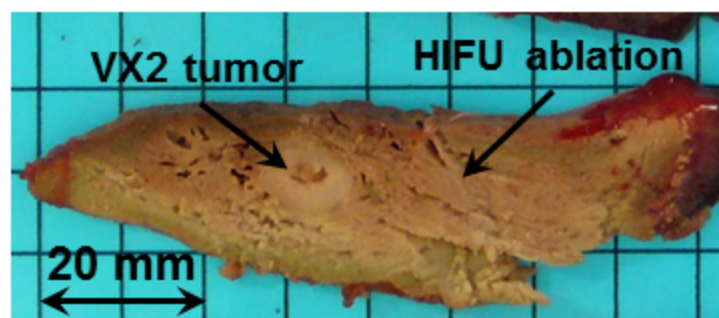
(a)



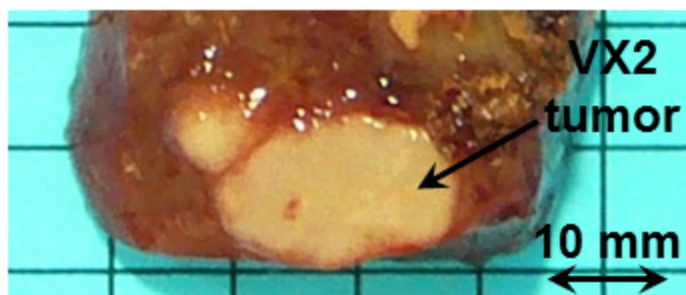
(b)



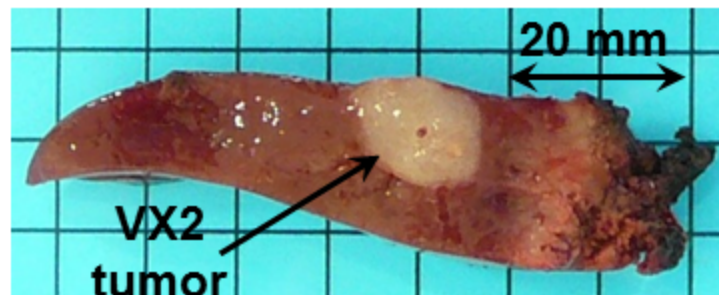
(c)



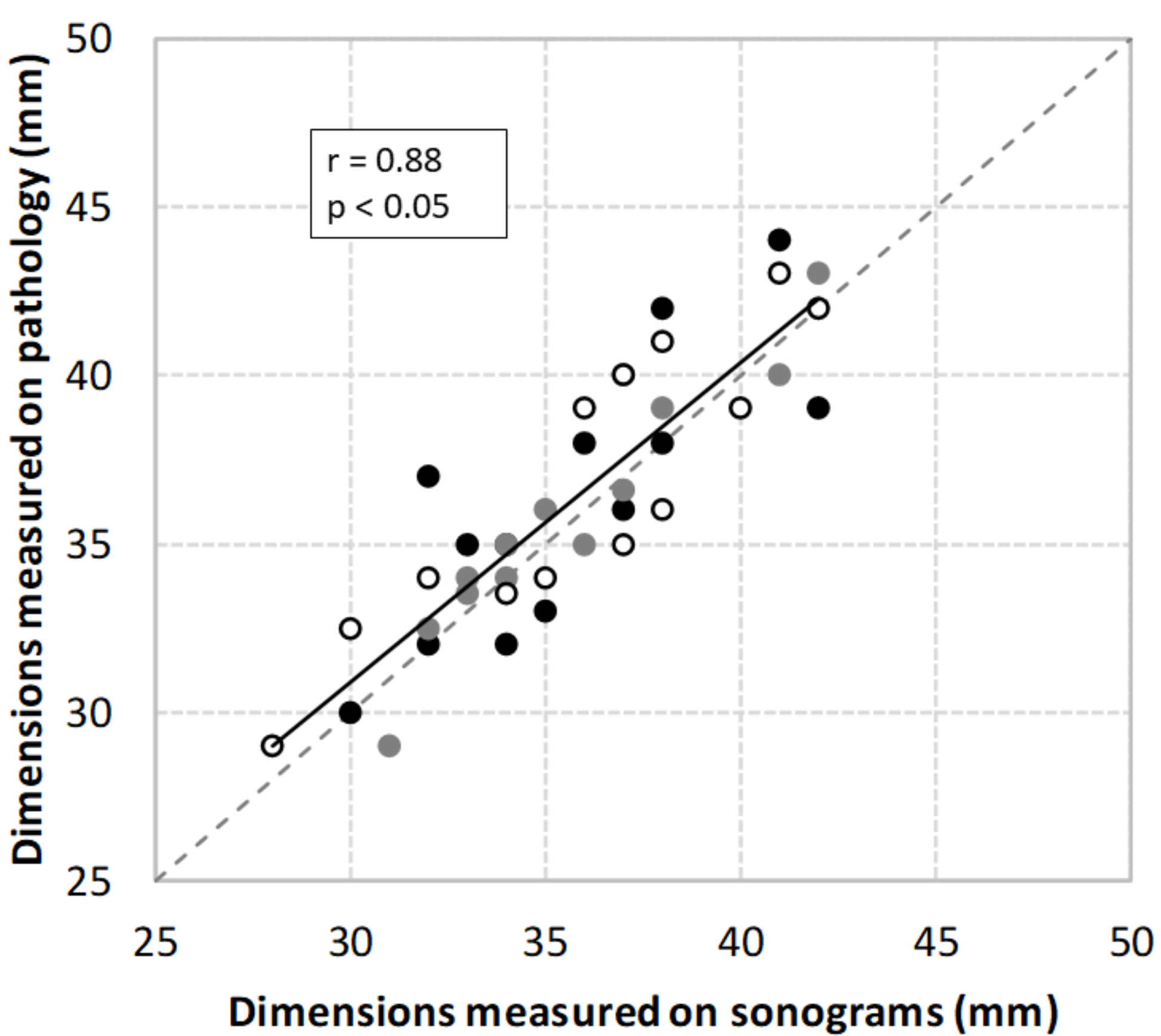
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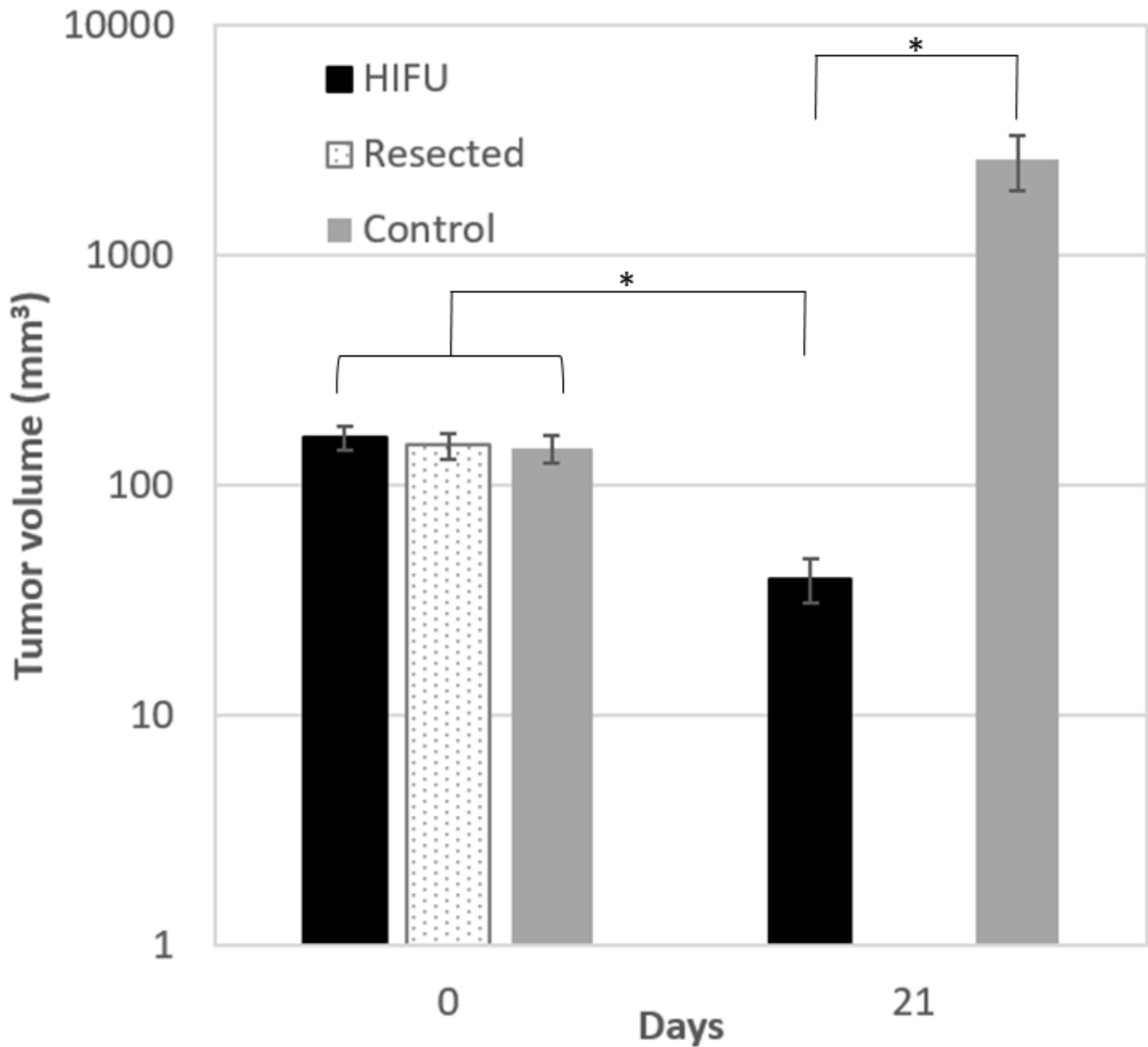


(e)



(f)

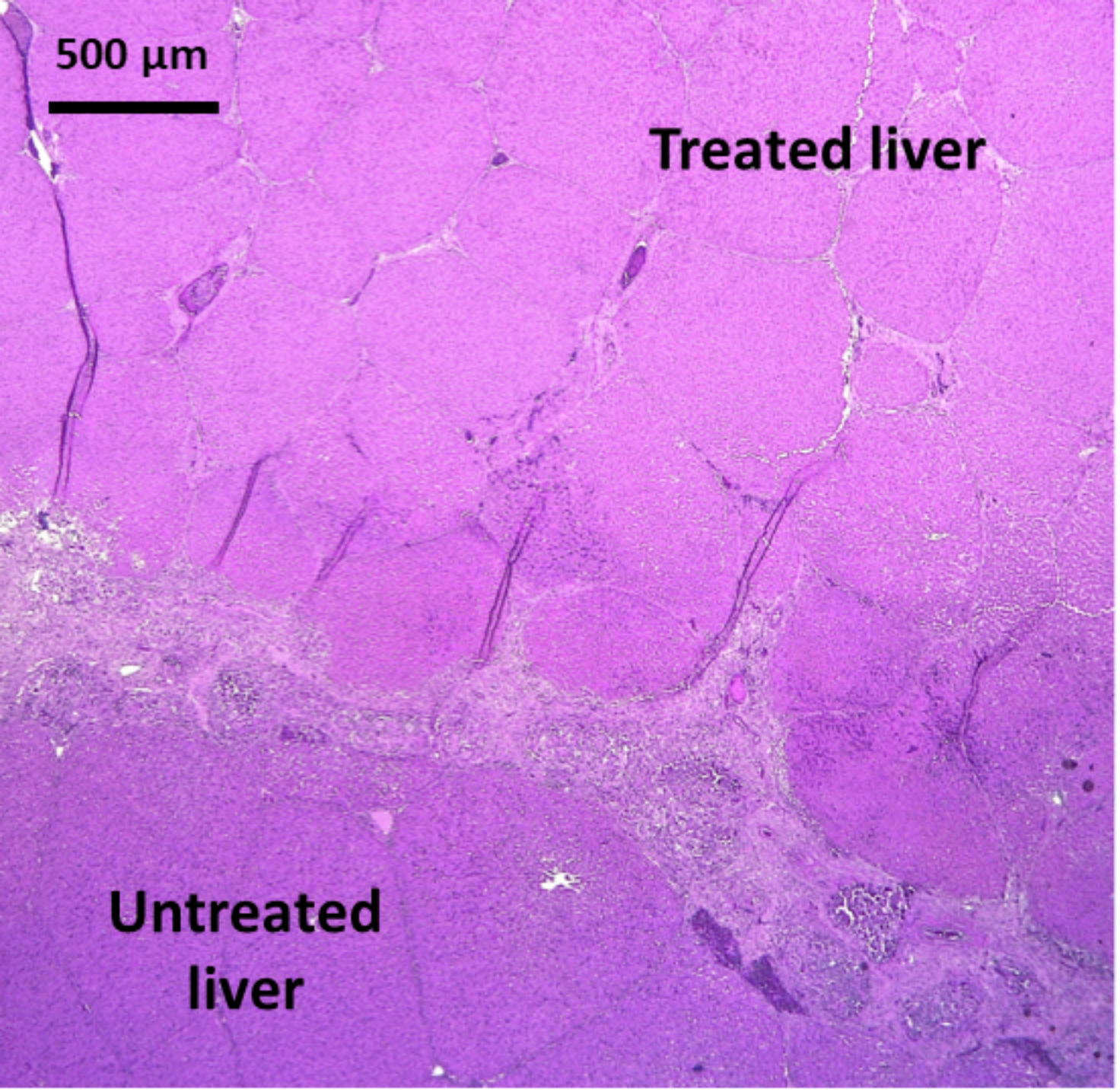




500 μm

Treated liver

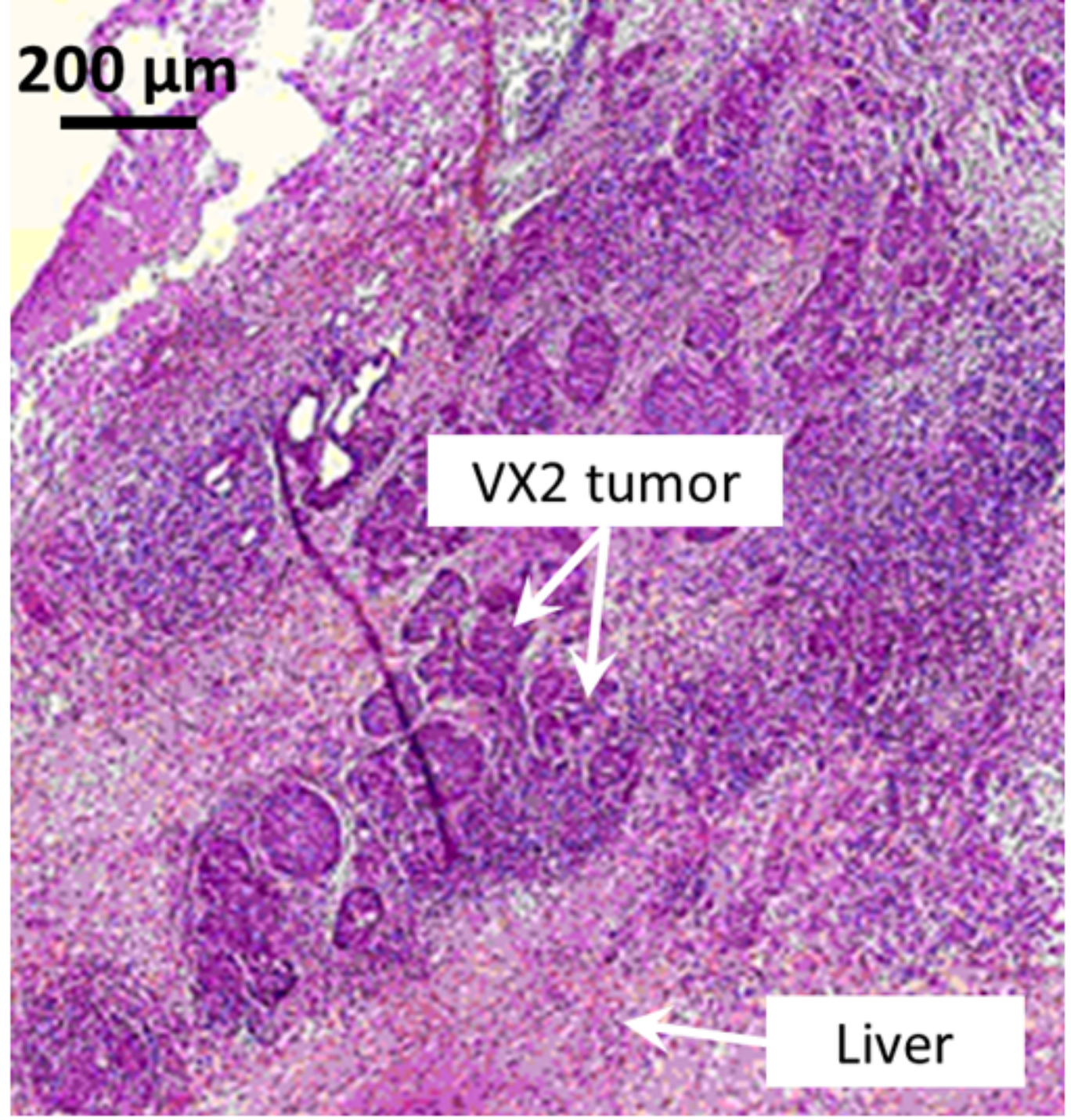
**Untreated
liver**



200 μ m

VX2 tumor

Liver



Group	Grade 0	Grade 1	Grade 2	Grade 3	Total
1	4	0	0	0	4
2	2	2	1	0	5
3	0	3	5	2	10

Table 1. Tumor metastasis and grade in group 1 (HIFU), 2 (Resection) and 3 (Control).