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Case Report

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Preliminary Safety, Effectiveness and Feasibility Analysis of Focused Ultrasound Ablation Surgery Combined with Anti PD-1 in the Treatment of Solid Tumor with Liver Metastasis: 2 Case Reports

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Abstract

Background: FUAS (Ultrasound Ablation Surgery) is a local non-invasive therapy that precisely focuses the ultrasound beam on a specific target, so that high energy can be concentrated on a very small focus inside human body, where the tissue can be ablated. At present, it has been used to treat unresectable primary liver tumors and liver metastases, and it is well tolerated even in patients with poor Child-Pugh score. Compared with TACE (Transcatheter Arterial Chemoembolization) and other methods, the treatment of liver metastases can further block the portal vein blood supply of liver metastases, and may have a more positive impact on the immune microenvironment than radiotherapy. In this article, we describe two patients with liver metastasis of advanced solid tumor who received FUAS combined with anti PD-1 treatment.

Case report: This case report describes two patients with liver metastases from advanced solid tumors, who both used FUAS as a local treatment for liver metastases or primary lesions. One case was treated with gemcitabine based

systemic chemotherapy combined with PD-1 inhibitor; another patient could not tolerate the side effects of chemotherapy, so he chose anti angiogenic drugs combined with PD-1 inhibitors for treatment. FUAS was performed at the beginning of treatment, when the disease progression was identified or systemic drug resistance occurred. The results showed that the tumor growth was effectively suppressed.

Conclusions: During the treatment of these two patients, it was found that FUAS could be used as local treatment measure combining PD-1 inhibitor. The treatment itself did not show obvious side effects, nor did it increase the side effects of other systemic drugs.

Background

Immune checkpoint inhibitor anti PD-1/PD-L1 (Programmed cell death protein-1/Programmed death-Ligand 1) treatment is the current research hotspot, but single drug use is limited to patients with high PD-L1 expression rate (such as cut off value greater than 50%) or MSI-H/dMMR (Microsatellite Instability High/different Mismatch Repair) and the number of these patients is small. How the majority of patients benefit from immunosuppressive agents is the current research interest, and the combination of immunosuppressive agents and other methods is the focus of research. Liver metastasis of advanced solid tumor is very common. For this group of patients, even if the immune agents are combined with other systemic drugs, the efficacy is not ideal. It is often observed in clinical practice that the primary lesion is controllable, but the liver metastasis is still growing, which may be related to the liver being an "immune exempt organ" **[1,2]**. At present, the mainstay treatment strategy for such patients is to combine systemic treatment with the local treatment of liver metastases, such as Radiofrequency Ablation (RFA). FUAS is also one of the means of liver tumor ablation. Its greatest advantages are non-invasive and easy to reapply, and it has the potential to stimulate immune response for several times. The purpose of this study is to analyze the preliminary safety, effectiveness and feasibility of FUAS combined with PD-1 inhibitor in the treatment of patients with solid tumor liver metastasis.

Notes: The following target lesions refer to those that can be measured and calculated according to the evaluation criteria of RECIST1.1 (Response Evaluation Criteria In Solid Tumors) **[3],** while FUAS target lesions refer to those been treated during FUAS. The target lesions are not necessarily FUAS target lesions. And FUAS target lesions may not be target lesions in RECIST evaluation. For example, when FUAS treats multiple metastatic lesions in the liver, and the primary lesion is not treated, the primary lesion is not the FUAS target lesion, but only called the target lesion according to RECIST 1.1 standard. Those treated liver metastatic lesions are called FUAS target lesions, but the diameter of the FUAS target lesions maybe not greater than 1cm, and they are not target lesions according to RECIST 1.1 standard.

Case Presentation

Case I

A 65-year-old male patient developed intermittent upper and middle abdominal pain in March 2022.His NRS (Numerical Rating Scale) scored 4 points and ECOG (Eastern Cooperative Oncology Group) scored 1 point. At that time, the abdomen ultrasonography showed abnormal echo at the body and tail of the pancreas with pancreatic duct

dilatation, and the diagnosis was undetermined: Neoplasm (7.2 * 4.7 cm)? There are multiple abnormal echoes in the liver, and the diagnosis was undetermined: metastasis? (The larger one is located in the left lobe of liver, with a diameter of 1.8 cm). Tumor markers: CEA 86.56 ng/ml, CA19-9>140000 U/ml, CA125 184.6 U/ml, cytokeratin 19 fragment 7.08 ng/ml, CA724 30.92 IU/ml, CA50>500 IU/ml, CA242>200 IU/ml. 2022.4.1 liver neoplasm biopsy results: adenocarcinoma, considering the origin of pancreas in combination with medical history, histological morphology and immune markers. Immunohistochemical results: CKpan (++), TTF-1 (-), NapsinA (-), p40 (-), Arginase-1 (-), Hepatocyte (-), Glypican-3 (-), AFP (-), CK7 (++), CK20 (-), CK19 (++), CK8/18 (++), CDX2 (+), NKX3.1 (-), Villin (++), CA19-9 (+), Ki-67 (++75%) (pathological number: K22-03683). TMB (Tumor Mutational Burden): 2.87 Muts/Mb. MSI result: MSI-L (Microsatellite Instability Low). Diagnostic staging: pancreatic adenocarcinoma stage IV cT4N0M1 (liver). The patients underwent 16 cycles of treatment, in which the PD-1 inhibitor camrelizumab 200 mg day 1 was used in combination with gemcitabine-based chemotherapy for the first 6 cycles, and FUAS was performed within 3 days before the treatment of the first, seventh and tenth cycles (see Table 1-1 for details). The patient's upper abdominal pain completely disappeared after the first cycle FUAS treatment of the primary lesion, NRS 0. The overall efficacy evaluation of the patient's condition in the 10th cycle was PD (Progressive Disease). Abdominal pain with the same nature recurred, and NRS score was 4-6 points. The patient needed to be treated with morphine hydrochloride sustained-release tablets (30 mg q12h) for pain relief. Before administration in the 10th cycle, the pain was relieved again after FUAS treatment of the primary lesion. The NRS score was reduced to about 2-4 points, and the patient stopped taking pain relief drugs by himself. At this time, the chemotherapy plan was changed from the first line (gemcitabine+albumin paclitaxel) to the second line (gemcitabine+tegeol), but camrelizumab was continuously used, and the drug was administered 3 days after the 10th cycle of FUAS treatment. The end time of follow-up was 2023-05. The relevant clinical data of patients before, during and after each cycle of treatment have been collected and preliminary statistics have been made as Table1-1 to **Table 1-4 and Figure1-1 to Figure 1-4.**

Table 1-1: FUAS Parameters.

Treatment time	04-09-2022	8/20/2022	10/16/2022
Treatment cycle	Cycle 1	Cycle 7	Cycle 10
Methods	Pancreatic cancer primary lesion FUAS treatment	liver metastases FUAS treatment	Pancreatic cancer primary lesion FUAS treatment
The target lesion of FUAS is located in	pancreas	liver	pancreas
Average power (w)	296	400	270
ablation time (s)	647	2139	930
Total time (min)	31	200	83
Energy (J)	1,91,600	74,865	2,51,240

Table 1-2: Target Lesions (primary pancreatic lesions and the largest metastatic lesion in the left lateral lobe of the liver: according to RECIST 1.1).

Treatment cycle	1st	2nd	3rd	4th	6th	7th	9th	10th	11th	13th	15th
Image time	3/31/2022	4/26/2022	5/14/2022	06-10-2022	07-03-2022	8/20/2022	9/19/2022	10/16/2022	11/18/2022	01-04-2023	03-01-2023
Administration time	04-12-2022	4/30/2022	5/24/2022	6/17/2022	07-10-2022	8/23/2022	9/20/2022	10/19/2022	11/19/2022	01-05-2023	03-01-2023
FUAS performance	Yes	No	No	No	No	Yes	No	Yes	No	No	No
Pancreas(cm)	7.2	7.2	5.1	5.9	5.6	6	6.2	7	7	5.6	5.6
Liver(cm)	1.8	1.8	2.3	1.2	1.7	1.8	2	3.5	3.5	2.6	2.4
Total(cm)	9	9	7.4	7.1	7.3	7.8	8.2	10.5	10.5	8.2	8
Efficacy evaluation	/	SD	SD	SD	SD	SD	SD	PD	SD	SD	SD

Table 1-3: Size changes of target and non-target liver lesions in the second FUAS.

Image time	FUAS performance	Target 1(Lower segment of right posterior lobe of liver)	Target 2(Lower segment of right posterior lobe of liver)	Target 3 (Lower segment of right anterior lobe of liver)	Non-target 1 (lower segment of right posterior lobe of liver)	Non-target 2 (lower segment of right posterior lobe of liver)

3/31/2022(1st)	Yes	1.8	1.8	1.1	1.8	1.5
4/26/2022(2nd)	No	1.4	1.6	1.1	1.4	1.3
5/14/2022(3rd)	No	1.4	1.6	1.1	1.4	1.3
6/10/2022(4th)	No	1.1	1.4	1.1	0.9	0.8
7/3/2022(6th)	No	1	1.4	1.4	0.7	0.8
20/8/2022(7th)	Yes	1.2	1.8	1.1	0.7	0.8
9/19/2022(9th)	No	0.9	2	1.9	0.7	0.8
10/16/2022(10th)	Yes	2.2	3	2.4	1.8	2.1
11/18/2022(11th)	No	2.1	2.4	2.5	1.7	1.5
1/4/2023(13th)	No	1.8	1.6	2.6	1.2	1.6
3/1/2023(15th)	no	1.6	2.1	2.4	1.8	1.3

Table 1-4: AE (Adverse Event): According to CTCAE V5.0 (Common Terminology Criteria for Adverse Events Version 5.0) criteria.

	Anemia	thrombocytopenia	liver function impairment	internal jugular vein thrombosis (after deep vein catheterization)	Nausea and vomiting
Number of events (times)	16	2	1	1	16
Grading	1	1	1	2	1
Incidence rate (%)	100	12.5	6.3	6.3	100

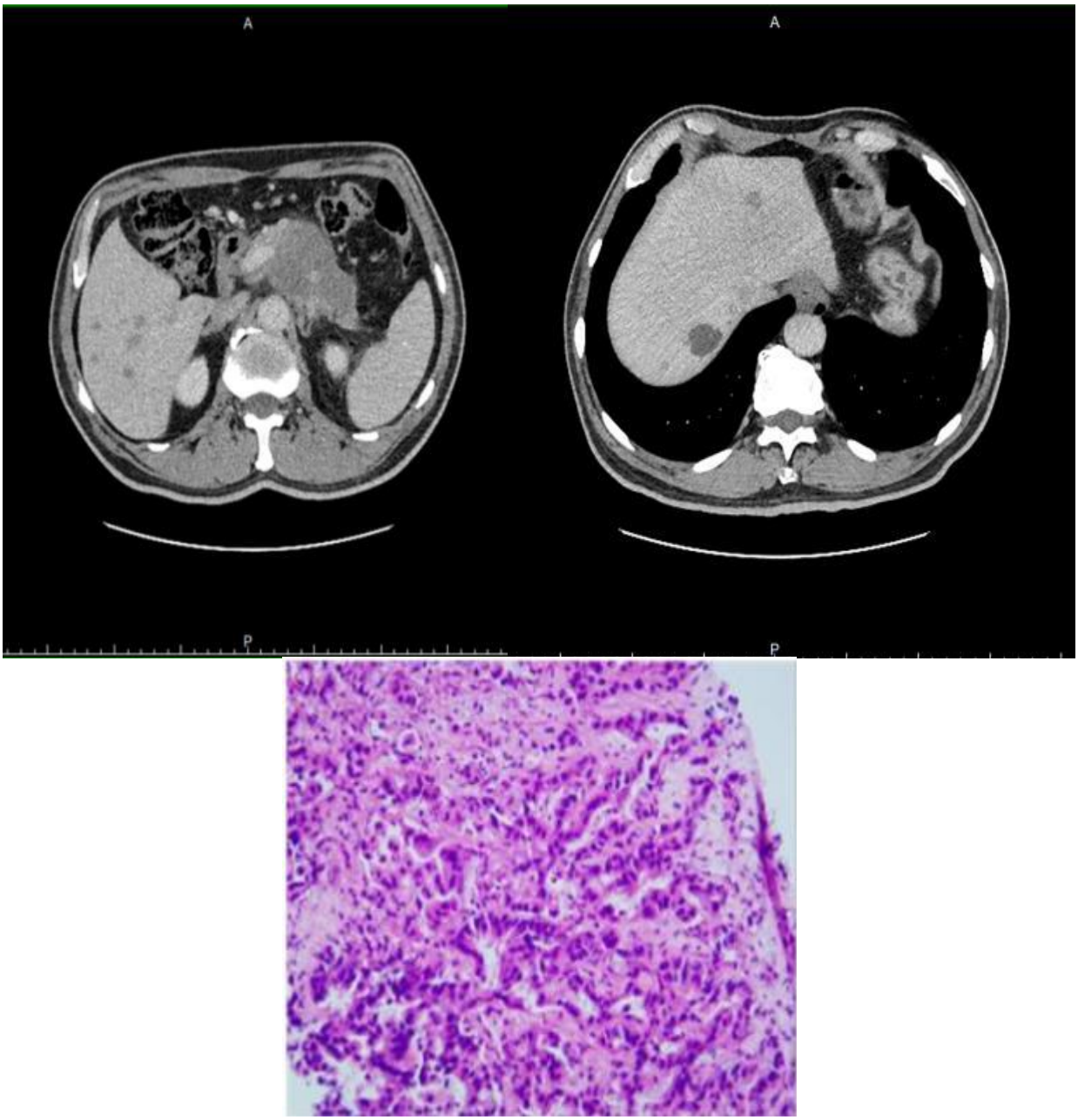

Figure 1-1: A: Primary lesion of pancreas; B: The largest metastatic lesion of left lateral lobe of liver; C: Pathological image of liver lesions.

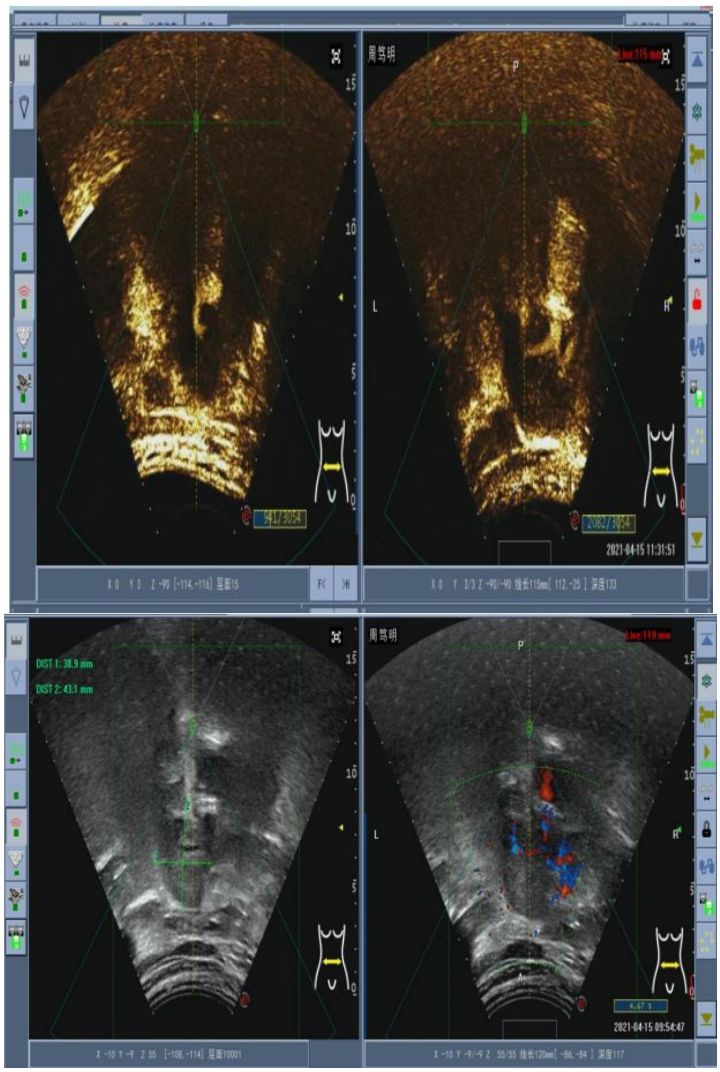

Figure 1-2: Images of FUAS treatment on April 15, 2022. A-B: The ultrasound CDFI (A) shows the blood flow of the lesion by comparing with the gray-scale ultrasound image (B); C-D: Contrast-enhanced ultrasound before (C) and after treatment (D).

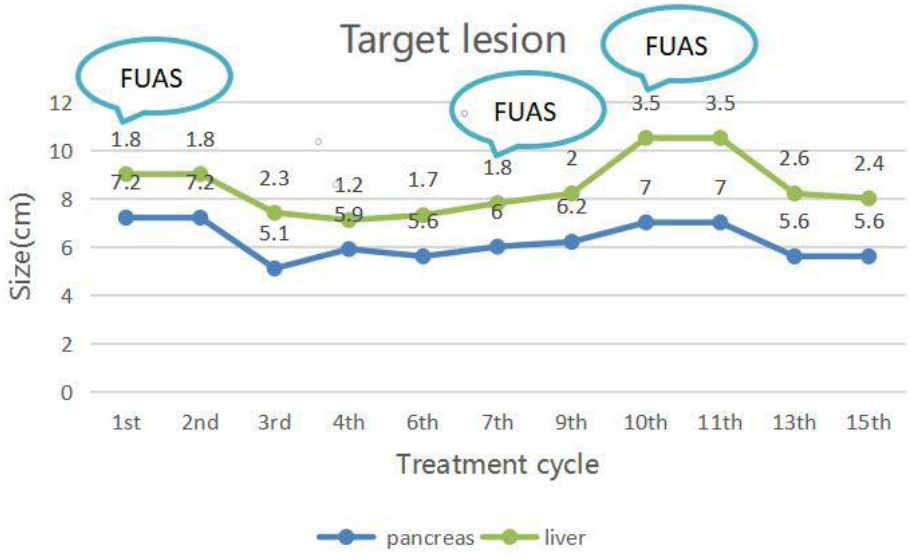

Figure 1-3: Target lesion.

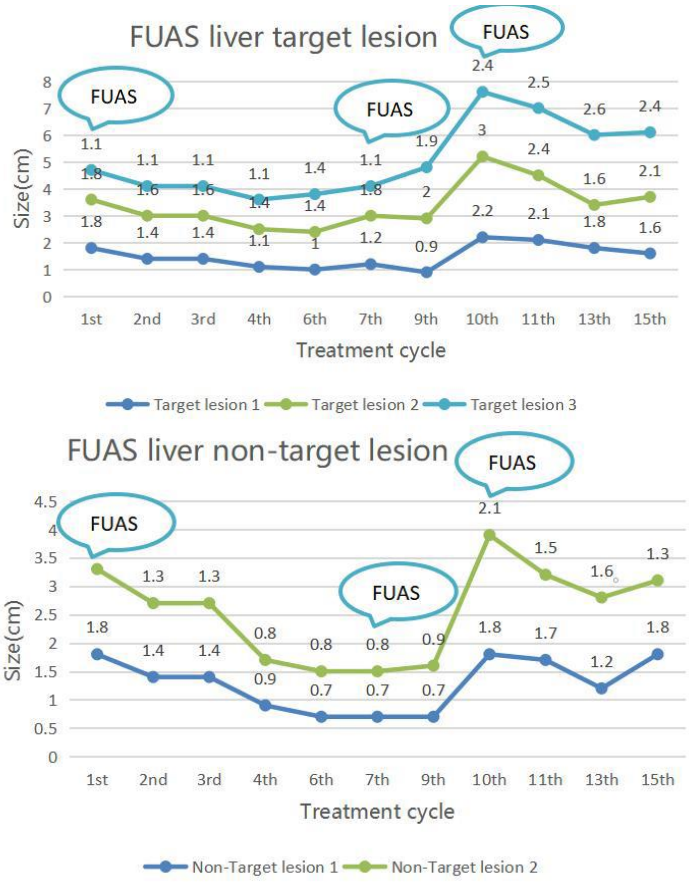

Figure 1-4: A: Changes of liver target lesion size in the second FUAS; B: Changes in the size of non-target liver lesions in the second FUAS.

Case II

A 68-year-old male patient developed right upper quadrant abdomen distension and pain in 2022.1, NRS 2-3 points, ECOG 1 point. At that time, the chest and abdomen CT showed: 1. The ileocecal intestinal wall was thickened (the range was about 4.2 * 2.1 cm), and the peripheral lymph nodes were displayed, and some of them were swollen (the shorter diameter was about 1.1cm of the largest lymph node). Considering the possibility of malignant tumor, there was a little exudation around them. 2. Multiple metastatic tumors of liver (the largest one is about 3.3 cm in diameter). The tumor markers CEA, CA125 and CA19-9 were all at normal levels. 2022.1 Pathologic result of colonoscopic biopsy: (ileocecal region) poorly differentiated carcinoma, which consistent with poorly differentiated adenocarcinoma. Immunohistochemical results: CK pan (++), CK5/6 (-), CK7 (-), CK20 (++), CK8/18 (++), CDX2 (+), CEA (weak+), p63 (-), P40 (-), p16 (-), Arginase-1 (-), Hepatocyte (-), Glypican-3 (-), Syn (-), CD56 (-), CgA (-), Ki-67 (++65%) (pathological number:). Detection of colorectal cancer targeting gene: V600E/D/K/R of exon 15 of BRAF gene was found to be mutant. No mutation was detected in KRAS, NRAS and PIK3CA genes. PD-L1 immunohistochemistry (22C3): TPS 70%, CPS 70. MSI result: MSS. Diagnosis stage: colon adenocarcinoma cT3N1M1 stage IV liver. After 11 cycles of treatment, the patient used the first-line chemotherapy regimen FOLFOX6 in cycles 1-3. The patient could not tolerate the side effects of chemotherapy, mainly gastrointestinal reactions, IV degree, and refused to receive chemotherapy again. Therefore, in cycle 4, the patient began to use the PD-1 inhibitor Tereprimab 200 mg day 1 combined with bevacizumab (Avastin) 7.5 mg/kg day 1 for every 21 days, and continued to the 11th cycle. The patient underwent FUAS treatment of liver metastases within 3 days before the first and sixth cycles of treatment (see Table 2-1 for details). The patient's right upper abdominal distension and pain relieved after 2 cycles of treatment; now sporadic symptoms remain, NRS 1-2 points. The end time of follow-up was 2023-05. The relevant clinical data of patients before, during and after each cycle of treatment have been collected and preliminary statistics have been made as **Table 2-1 to Table 2-4 and Figure 2-1 to Figure 2-4.**

Table 2-1: FUAS Parameters.

FUAS treatment time	2/19/2022	07-09-2022
Treatment cycle	Cycle 1	Cycle 6
Treatment	liver metastatic tumor FUAS treatment	liver metastatic tumor FUAS treatment
FUAS target lesions located in	liver	liver
Anesthesia mode	general anesthesia	sedation and analgesia
Average power (w)	397	400
abalation time (s)	1906	2540
Total time (min)	166	150
Energy (J)	757400	88900

Table 2-2: Target Lesions (primary colon lesion and the largest metastatic lesion in the left medial lobe of liver: according to RECIST 1.1).

Treatment cycle	1st	2nd	4th	6th	7th	9th	10th	11th
Image time	1/27/2022	03-01-2022	4/19/2022	6/26/2022	8/30/2022	11-08-2022	1/29/2023	4/26/2023
Administration time	2/16/2022	03-05-2022	4/23/2022	07-06-2022	09-09-2022	11-09-2022	1/30/2023	4/27/2023
FUAS performance	Yes	No	No	Yes	No	No	No	No
Colon(cm)	4.2	2.6	1.2	1.8	1.8	0	0	0
Liver (cm)	3.3	4	4.5	6.6	5.4	6.6	6.3	5.3
Total(cm)	7.5	6.6	5.7	8.4	7.2	6.6	6.3	5.3
Efficacy evaluation	/	SD	SD	PD	SD	SD	SD	SD

Table 2-3: Size changes of target and non-target lesions of FUAS liver.

		First FUAS liver target lesions (cm)			Second FUAS liver target lesions (cm)			First and second FUAS liver non-target lesions	
Image time	FUAS performance	Target 1(Lower segment of right posterior lobe of liver)	Target 2(Lower segment of right posterior lobe of liver)	Target 3(Lower segment of right posterior lobe of liver)	Target 4(Left medial lobe of liver)	Target 5(Right anterior lobe of liver)	Target 6(Caudate lobe of liver)	Non-target 1(Upper segment of right posterior lobe of liver)	Non-target 2(Lower segment of right posterior lobe of liver)
1/27/2022(1st)	Yes	1.6	2.3	2.5	3.1	1.9	2.3	3.2	2.7
3/1/2022(2nd)	No	2.4	2.6	3	3.7	2.5	2.3	2.8	2.1
4/19/2022(4th)	No	2.7	3.1	3.4	4.3	2.7	2.4	2.8	2.2
6/26/2022(6th)	Yes	3.3	3.4	3.4	5.7	3.5	2.9	3	2.8
30/8/2022(7th)	No	4	4.2	3.2	5.8	3.5	2.7	2.3	2.7
11/8/2022(9th)	No	2.9	2.9	2.1	4.9	2.9	1.9	1.7	2.1
1/29/2023(10th)	No	2.9	2.8	2	5	2.8	1.6	1.4	1.6
4/26/2023(11th)	No	2.7	2.7	1.6	4.6	2.6	1.8	1.5	1.7

Table 2-4: AE.

	Anemia	liver function impairment	Nausea and vomiting
Number of events (times)	5	1	3
Grading	1	1	2
Incidence rate (%)	55.56	11.11	33.33

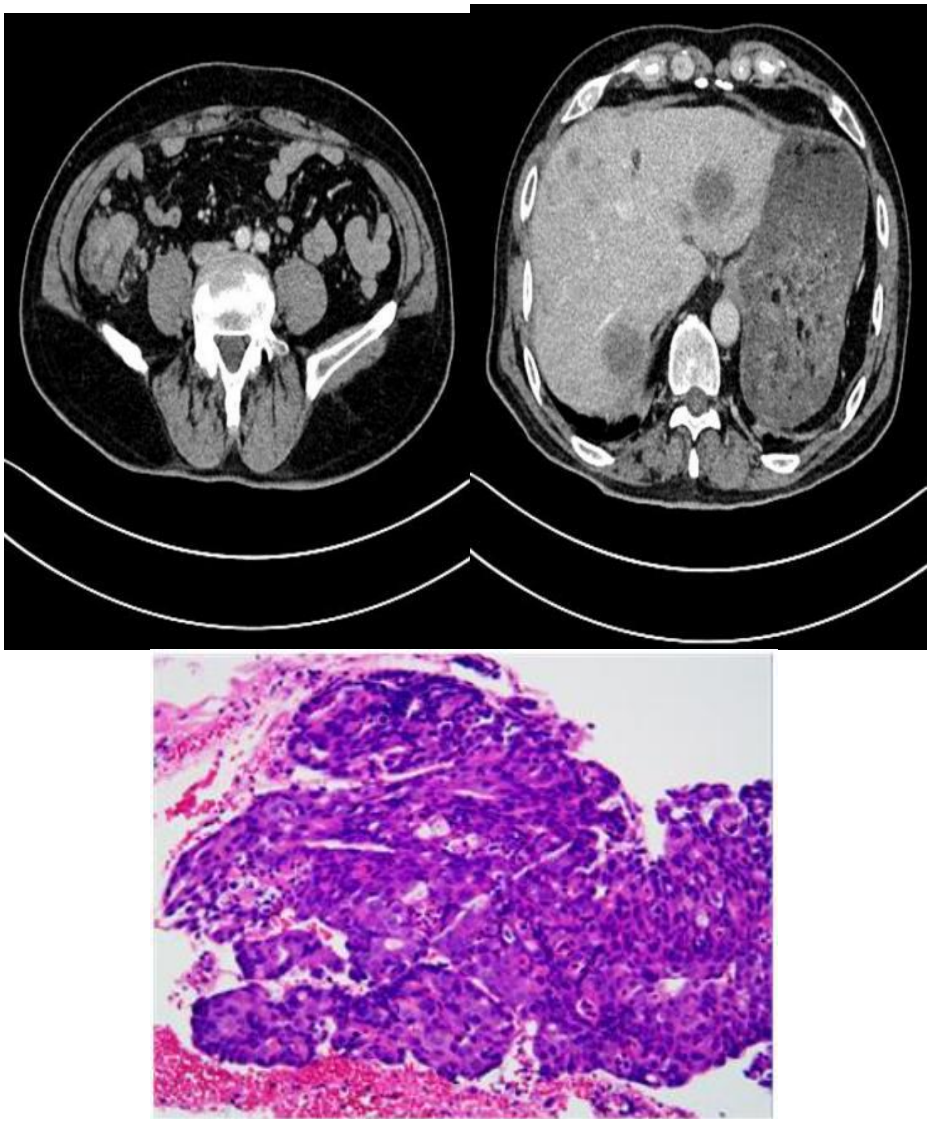

Figure 2-1: A: Primary lesion of colon; B: The largest metastatic lesion of left medial lobe of liver; C: Pathological image of ileocecal lesions.

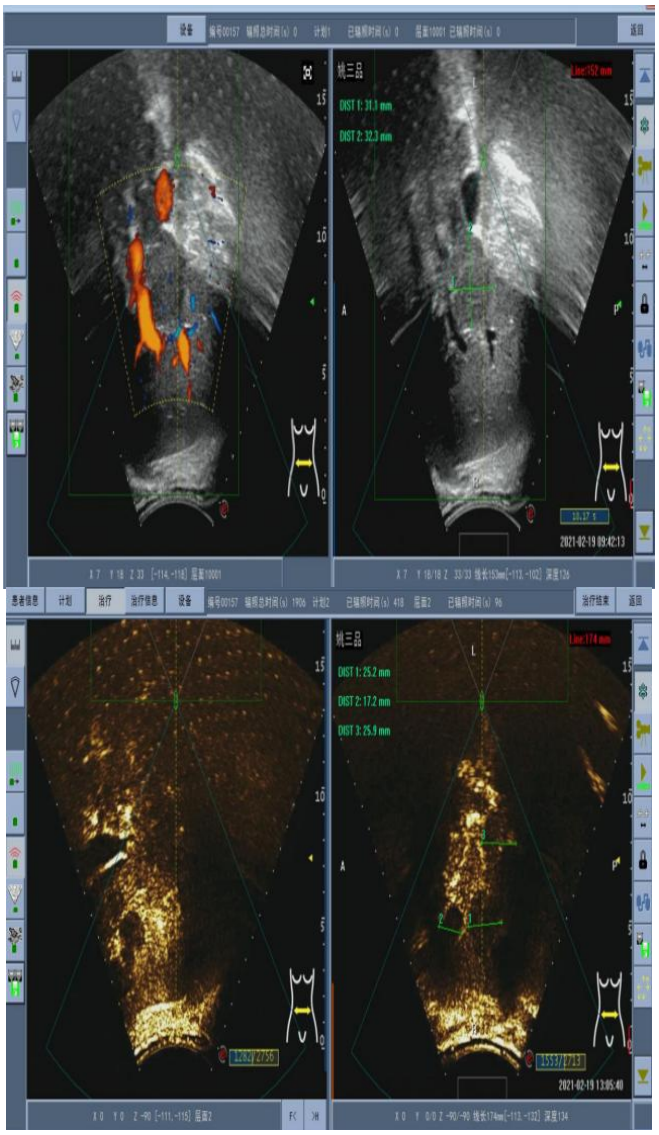

Figure 2-2: 2022.2.19 images of FUAS treatment A-B: The ultrasound CDFI (A) shows the blood flow of the lesion by comparing with the gray-scale ultrasound image (B); C-D: Contrast-enhanced ultrasound before (C) and after treatment (D).

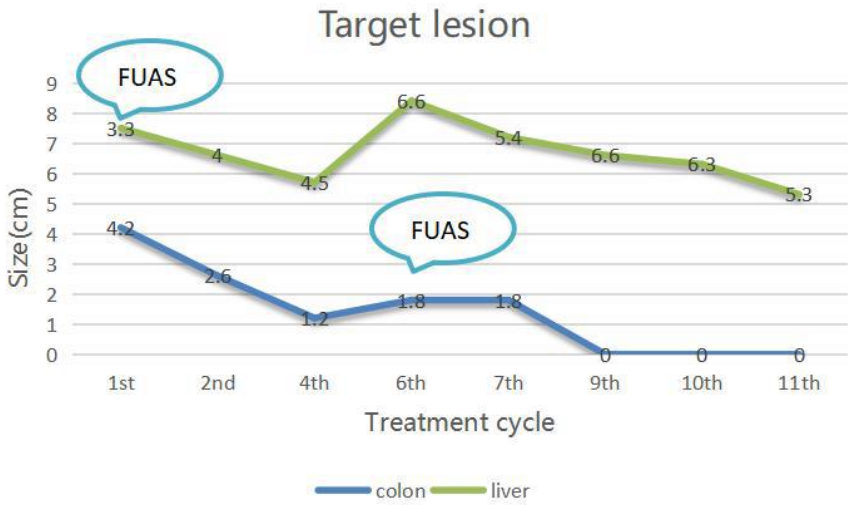

Figure 2-3: Target lesion.

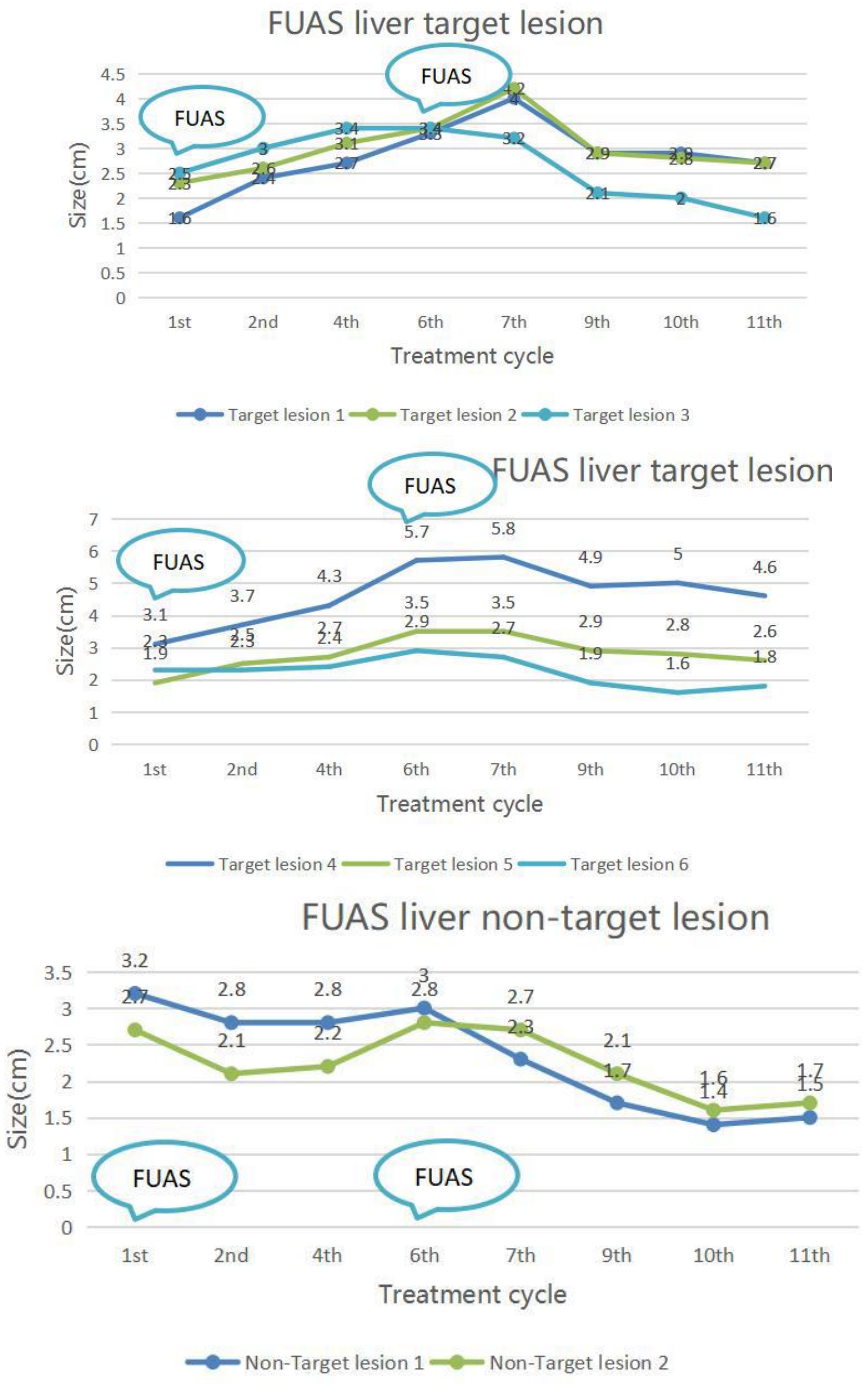

Figure 2-4: A: Changes in the size of the liver target lesion of the first FUAS; B: Changes in the size of the liver target lesion of the second FUAS; C: Changes in the size of non-target liver lesions of the first and second FUAS.

Discussion

The presence of liver metastasis is often an independent factor in the PD-1 inhibitor treatment efficacy scoring system [4-7]. It is reported that the presence of liver metastasis in gastrointestinal tumor patients receiving immune

checkpoints inhibitor treatment is worse than patients without liver metastasis in PFS and OS [8]. After the liver metastatic lesions are treated by local treatment methods such as radiotherapy and RFA, their related immunosuppressive microenvironment may be improved, thus changing the poor efficacy of PD-1 inhibitors [9,10]. However, as an immune stimulation method for targeted treatment of liver local metastasis, the above methods still have limitations, including: 1. Liver metastasis lesions are often multiple and in different sizes, RFA or radiotherapy are not suitable for treatment of multiple, wide range and large diameter lesions, and the number of lesions that can be treated at one time is limited; 2. RFA, which requires direct tumor puncture, is an invasive treatment, and its repeated use is very limited. Meanwhile, RFA is considered as a relative contraindication in patients with advanced cirrhosis or ascites. However, the liver is relatively radiosensitive but has low tolerance to radiotherapy. Reducing liver toxicity is a challenge for radiotherapy [11]. 3. RFA, radiotherapy and other methods may not be able to induce the full release of tumor molecules of immunogenic or inflammatory ubcellular components, making the relevant T lymphocytes less aggressive to tumors [12,13]. At present, as one of the targeted therapies for unresectable liver cancer (hepatocellular carcinoma) and liver metastases, focused ultrasound ablation surgery has the advantages of non-invasive, accurate and repeatable approach [14,15], good efficacy and tolerance even in patients with advanced liver cirrhosis and liver cirrhosis with poor Child-Pugh score [16], and may have a more positive impact on the immune microenvironment than radiotherapy. The possible mechanisms of FUAS leading to immune response [17-20] include: ①tumor antigen: residual tumor fragments and TAA (Tumor Associated Antigen) after focused ultrasound treatment release and cause over expression of dangerous signals, which together act as tumor vaccine to improve tumor immunogenicity. ②Release of danger signals: induce the expression of DAMPs, HSP-70 (Heat Shock 70 kDa Protein) and HSP-27. ③Trigger Th (helper T cell) type 1 response, leading to significant changes in "cell-mediated immunity". And ④play a role by balancing tumor induced immunosuppression in the tumor microenvironment: such as VEGF (Vascular Endothelial Growth Factor), TGF- β 1 and β 2(Transforming Growth Factor- β), etc. Therefore, this study plans to use FUAS combined with PD-1 inhibitor to treat patients with solid tumor liver metastasis, and preliminarily understand the safety, clinical effectiveness and feasibility of this combined treatment. First, the safety of FUAS combination therapy: From the analysis of the treatment safety of case 1 and case 2, whether FUAS is combined with PD-1 inhibitor and chemotherapy or angiogenesis inhibitor, its main related AE is hematological events: including anemia, thrombocytopenia and liver function impairment, mainly anemia. The above AE events are all Level 1. The non-hematological events were gastrointestinal toxicity and side effects: nausea and vomiting. Considering that they were related to chemotherapy drugs, the second patient did not have this AE after he could not tolerate this reaction and actively stopped chemotherapy. It should be noted: 1. During the treatment of FUAS combined with PD-1 inhibitor and other multiple methods, the patient did not have irAE (immune related adverse event) [21], and did not aggravate the classification of other treatment related AEs. 2. The above AE may be related to FUAS and other drug treatment, but may not be directly related to FUAS, but in any case, it is not ≥ level 2, and no medication treatment is required. 3. With the extension of treatment and observation time, the severity and types of AE did not increase and become complex, and it is still relatively safe to use FUAS in combination with other methods of treatment.

In terms of the preliminary clinical efficacy of FUAS, from Case 1, it is well known that the prognosis and long-term survival rate of pancreatic cancer are not satisfactory. For patients with advanced metastatic pancreatic cancer, FOLFIRINOX or gemcitabine combined with albumin paclitaxel is the most effective chemotherapy regimen, and the efficacy of other second-line regimens is not very satisfactory [22,23]. The former extended mPFS (Progression Free Survival) to 6.4 months and mOS (Overall Survival) to 11.1 months, but it was extremely toxic; In the latter case, the mPFS was extended to 5.5 months, and the mOS was extended to 8.5 months [24-26]. The PFS of case 1 is up to 6.5 months, and the current OS is 14 months. Considering that the patient has a good ECOG score by the time of follow-up, and the estimated survival time is more than 3 months, the OS may be more than 17 months. It can be seen from the table of case 1 that: 1) The first and third FUAS target focus of the patient was selected on the primary lesion of the pancreas, and both the pancreas itself and other multiple liver metastases rapidly shrunk after FUAS treatment. Considering that there may be a synergistic effect on chemotherapy combined with immunotherapy in addition to FUAS itself, which can reduce the local tumor load. That is to say, after FUAS stimulates anti-tumor immune reaction in local areas, it further amplifies the whole body through chemotherapy or the use of immunosuppressive agents at immune checkpoints, resulting in beneficial clinical changes, leading to rapid shrinkage of other multiple lesions in the whole body, and significantly prolonging the PFS and OS of patients. According to the literature, this immune microenvironment change conducive to treatment usually occurs about 72h after focused ultrasound treatment [27], so we choose focused ultrasound treatment time to complete within 3 days before immunotherapy as far as possible. 2) The patient's tumor progressed in the 10th cycle, and the systemic treatment plan was still immune therapy combined with chemotherapy. However, the patient is MSI-L, which is not the type with good efficacy of using ICIs (Immune checkpoint inhibitors). At present, there is no clinical trial related to ICIs that has obtained clear positive results for pancreatic cancer; At the same time, although the chemotherapy plan has been changed, it is a second-line palliative chemotherapy with poor prognosis. Therefore, we believe that the more important reason for obtaining the reduction of systemic and local lesions is that the third Focused Ultrasound Ablation Surgery (FUAS) of pancreatic cancer treatment (2022.11.12) was carried out. It can be seen that in the later efficacy evaluation, the target lesions (according to RECIST1.1), whether the primary pancreatic lesions or liver metastases, were significantly reduced. The clinical data collected before the 15th treatment showed that their reduction rates were 20% and 31.4% respectively, and the overall reduction rate was 25%. Although it did not meet the PR (partial response) evaluation criteria (according to RECIST1.1), it was close, further confirming the synergistic effect of FUAS on chemotherapy combined with immune therapy, which is also consistent with the literature [28]. 3) Long term use of PD-1 inhibitor will lead to immune resistance in patients. There are many reasons for drug resistance and the mechanism is very complex [29], so there is no effective solution. In order to expand the scope and effectiveness of immunotherapy, the combination therapy strategy is mostly used in clinical practice. It can be seen that before the second FUAS treatment, multiple lesions in the whole body had a tendency to increase, but the PD criteria had not been reached. Considering that immune resistance may occur at this time, the chemotherapy regimen has not been changed according to the diagnosis and treatment guideline, we chose FUAS treatment at this time, hoping to salvage the occurrence of immune resistance. However, the target lesion selected by FUAS this time is the liver. It is undeniable that, although FUAS treatment, both pancreatic and liver multiple

lesions have an increasing tendency until the 10th cycle of disease progression. According to the cause analysis, the total number of liver target lesions selected by FUAS is only 3, and none of them exceeds 2cm, which may not stimulate enough antigen released by the tumor to cause effective immune microenvironment changes. It further suggests that we should consider tumor size, number, speed of progress and other factors. Therefore, after discovering this problem, we selected FUAS liver target lesions greater than 2cm and multiple different lesions for fractional FUAS as far as possible in case 2, so as to fully release tumor antigen. Case 2 also achieved good clinical efficacy, which proved that our consideration could be correct, but it still needs further confirmation. Let's take a look at the efficacy of case 2: this case is a patient with advanced liver metastasis from MSS colorectal cancer. Systemic chemotherapy was used in the 1-3 cycles, followed by target immunotherapy with anti-angiogenesis inhibitor bevacizumab combined with PD-1 camrelizumab in the 4-9 cycles. Liver metastasis of colorectal cancer is one of the cruxes in the treatment of colorectal cancer, and it is also the main cause of death of colorectal cancer patients **[30].** The effects of various treatments are poor. The median total survival period of untreated patients with liver metastasis is only 6.9 months, and the 5-year survival rate of patients whose lesions cannot be resected is less than 5% **[31,32].** By 2023-05, PFS1 of patients had reached 5.5 months, and OS had reached 15 months. In case 2: 1, In multiple liver metastatic lesions, whether or not it is the target lesion selected by FUAS, most of the lesions still grow during the 1-3 cycles of chemotherapy. After performing FUAS, the growth rate may be slowed down due to the local tumor load reduction from FUAS treatment and the synergistic effect of chemotherapy. However, FUAS itself has not been proven to cause a lasting ablation reaction. For example, it is reported that the NK, CD3, CD4, CD8 and other cell levels of primary liver cancer significantly rise and fall back three months after ablation **[33].** Indeed, in the fourth to sixth cycles after the first FUAS treatment, since there was no continuous effect of FUAS and the treatment regimen was changed to target-immune combination therapy, multiple liver metastatic lesions increased rapidly, which proved that target-immune combination therapy was ineffective for liver metastatic lesions. This is also consistent with the report: in many trials **[34,35]** on the treatment of refractory MSS colorectal cancer with anti-angiogenesis inhibitors and immune-checkpoint inhibitors, it is described that patients only have 27.8% - 53.5% DCR (Disease Control Rate) without objective response. Especially for patients with liver metastasis, such as the Japanese REGONIVO (Regorafenib+Nivolumab treatment in patients with advanced gastric cancer or colorectal cancer) trial **[36]** reported that among 24 patients with MSS type metastatic colorectal cancer, the response rate of patients with liver metastasis was only 8.3% (1/12). Similarly, the response rate of patients with MSS colorectal cancer without liver metastasis was 30.0%, while the response rate of patients with liver metastasis was only 8.7% **[34].** The above data indicate that although the combination of target immune therapy has potential clinical benefits, there is a poor response or even no clinical response in patients with liver metastasis. However, these multiple metastatic liver lesions were rapidly and significantly reduced after the sixth cycle of FUAS treatment, and the overall reduction rate was 36.9% in the 11th cycle of efficacy evaluation compared with the sixth cycle, reaching PR. It is confirmed that FUAS can have synergistic effect on the combination of target and immune drugs, so that the ineffective combination of target and immune drugs can have exact disease control and objective response. This is a very important signal, suggesting that it may open up a new therapeutic modality for clinical treatment to eliminate traditional chemotherapy and only use the combination of target and immune drugs. 2) It is worth noting

that the two target lesions of FUAS in case 2 were on the liver metastasis lesions. As a non-target lesion, the colon target lesion was rapidly reduced with the use of chemotherapy drugs after the first FUAS, and subsequently increased with the cessation of chemotherapy during the period of target immunotherapy. It was confirmed that the colon target lesion may be more sensitive to chemotherapy, but not sensitive to target immunotherapy. Until the second time FUAS was performed along with target immunotherapy, the lesion shrank rapidly again, which does not rule out that FUAS may have the same "distant effect" as radiotherapy [37], but further research is needed.

Conclusion

During the treatment of these two patients, it was found that FUAS could be used as local treatment measure combining PD-1 inhibitor. The treatment itself did not show obvious side effects, nor did it increase the side effects of other systemic drugs.

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