Effect of microwave ablation treatment against hepatic malignance on serum cytokine level.

CURRENT STATUS: UNDER REVISION

Jing Zhao
First Affiliated Hospital of Soochow University

Qiang Li
Jiangxi Cancer Hospital

Merlin Muktialli
First Affiliated Hospital of Soochow University

Bingjie Ren
First Affiliated Hospital of Soochow University

Yingxi Hu
First Affiliated Hospital of Soochow University

Dapeng Li
First Affiliated Hospital of Soochow University

Zhi Li
First Affiliated Hospital of Soochow University

Daoming Li
First Affiliated Hospital of Soochow University

Yufeng Xie
First Affiliated Hospital of Soochow University

Min Tao
First Affiliated Hospital of Soochow University

Rongrui Liang
First Affiliated Hospital of Soochow University

✉ lengbeng@suda.edu.cn Corresponding Author
ORCiD: https://orcid.org/0000-0001-9879-2808
Abstract

Background
Microwave ablation (MWA) is widely used to treat unresectable primary and secondary malignancies of the liver, and a limited number of studies indicate that ablation can cause not only necrosis at the in situ site but also an immunoreaction of the whole body. This study aimed to investigate the effects of MWA on cytokines in patients who underwent MWA against a hepatic malignancy.

Methods
Patients admitted to the Oncology Department in the First Affiliated Hospital of Soochow University between June 2015 and February 2019 were selected. Peripheral blood was collected from patients with a hepatic malignancy treated with MWA. The levels of cytokines (IL-2, IFN-γ, TNF-α, IL-12 p40, IL-12 p70, IL-4, IL-6, IL-8, IL-10, and vascular endothelial growth factor (VEGF)) were detected with a MILLIPLEX® MAP Kit. The comparison times were as follows: before ablation, 24 hours after ablation, 15 days after ablation, and 30 days after ablation. Data were analyzed using a paired sample t-test and Spearman’s correlation analysis.

Results
A total of 43 patients with hepatic malignancies were recorded. There were significant differences in IL-2, IL-12 p40, IL-12 p70, IL-1β, IL-8, and TNF-α at 24 hours after MWA. Significant increases (>2-fold vs. before ablation) were observed in IL-2, IL-1β, IL-6, IL-8, IL-10, and TNF-α after MWA. Elevated IL-2 and IL-6 levels after ablation were positively correlated with energy output during the MWA procedure.

Conclusions
MWA treatment for hepatic malignancies can alter the serum levels of several cytokines and affect the tumor status.

Introduction
Primary and secondary malignancies of the liver have a substantial impact on morbidity and mortality worldwide. In China, hepatocellular carcinoma (HCC) ranks second in the mortality rate of malignancies[1]. The treatment of primary and secondary hepatic malignancies via interventional
imaging therapy is undertaken by investigators in the field of interventional radiology and possibly by a smaller group of practitioners known as interventional oncologists, whose major focus is cancer care via minimally invasive approaches[2, 3]. Recently, percutaneous ablation therapy has been widely accepted as one of the radical treatment methods for HCC, and its five-year survival rate is similar to that of resection[4]. Microwave ablation (MWA) is widely used to treat unresectable HCC and recurrent HCC with the advantages of minimal invasion, a good curative effect, and no radiation or chemotherapy side effects. Immune checkpoint inhibitors (ICI) such as PD-1/PD-L1 and CTLA4 antibodies were widely applied in several cancers, researches indicated that ICI treatment could enhance the effect of ablation[5]. Evidence indicated that hyperthermia destruction caused release of a large population of heterogenous tumor antigens and inflammatory cytokines may play crucial roles[6]. Cytokines are mediators that regulate a broad range of processes involved in the pathogenesis of cancer. Several cytokines, which can arise from either tumor cells or immunocytes[7], such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, IL-10, and vascular endothelial growth factor (VEGF), have been linked with cancers and can either promote or inhibit tumor development. The serum levels of cytokines differ during cancer development. Although cytokines have found to be altered after anticancer treatment, such as chemotherapy and radiotherapy[8, 9], few investigations have focused on cytokines before and after MWA. It is still unknown whether the above cytokines changed before and/or after MWA in patients with a hepatic malignancy. In this study, we investigated the effects of MWA on the serum levels of cytokines in patients with hepatic malignancies.

Material And Methods
2.1 Patients and samples
The patient population examined in this study was derived from the First Affiliated Hospital of Soochow University. Patients were admitted to the Oncology Department between June 2015 and February 2019. The total number of patients was 43, with 37 liver metastases and 6 primary liver cancers. The inclusion criterion was a tumor located at a hepatic site (either primary or metastases). All patients with metastastic hepatic malignances should be underwent systemetic treatments
(chemotherapy or target therapy) and get at least stable disease (SD) or partial partial response (PR) for more than 45 days. Informed consent for blood draw and the relevant therapy was obtained from all patients. The protocol was approved by the Human Ethics Committee of the First Affiliated Hospital of Soochow University and was conducted in accordance with the Declaration of Helsinki. Whole blood (4 mL) was drawn into EDTA anticoagulant tubes on days -3 to 0 before and 24 hours, 15 days, and 30 days after ablation, mostly on the last day of the course, for cytometry and cytokine analyses.

2.2 Ablation procedure
The ablation procedure used in this research was MWA. The puncture site and pathway were determined under the guidance of a computed tomography (CT) scan. Local infiltration anesthesia was achieved by using 0.5% lidocaine. The placement of microwave ablation probes was guided by CT scan or ultrasonic device, all probes were placed at the maximum diameter layer. Double probes were employed when the maximum diameter of tumor was up to 3cm. The power and time of ablation were designed for each patient in the range of 40−70 W and 5−20 min, respectively, based on the size, number, and position of the tumor. The boundaries of ablation zones were designed as extended 1cm upon tumor site.

2.3 Cytokine detection
A MILLIPLEX MAP Kit with 10 human cytokine/chemokine panels that measured IFN-γ, IL-2, IL-6, IL-8, IL-10, IL-12 p40, IL-12 p70, IL-1β, TNFα, and VEGF was utilized according to the manufacturer's instructions. Briefly, chemically dyed antibody-bound beads were mixed with standard or sample, incubated overnight at 4 °C, washed, and then incubated with a biotinylated detection antibody. After washing, beads were incubated with a streptavidin phycoerythrin complex, and the mean fluorescent intensities were quantified on a Luminex 200 analyzer (Luminex Corporation). All samples were measured in duplicate. Standard curves of known concentrations of recombinant human cytokines/chemokines were used to convert fluorescence units to cytokine concentration units (pg/mL). The minimum detectable concentrations were as follows: IFN-γ: 2.6 pg/mL, IL-2: 2.77 pg/mL; IL-12 p40: 3.94 pg/mL, IL-12 p70: 2.84 pg/mL, IL-1β: 2.99 pg/mL, IL-6: 2.79 pg/mL, IL-10: 2.42 pg/mL,
TNF-α: 3.3 pg/mL, and VEGF: 1.5 pg/mL. All results below the minimum concentrations were processed as the minimum concentrations.

2.4 Statistical analysis
IBM SPSS Statistics 20.0 software was used for the statistical analysis, along with GraphPad Prism 8 for figure creations. Normally distributed numerical data are expressed as the mean ± standard deviation (SD), and nonnormally distributed numerical data are expressed as the median and 95% confidence interval (95% CI). Cytokines at different times were compared using a one-tailed paired t-test. Spearman’s correlation analysis was executed to analyze the correlation between clinical indexes and cytokine levels. p<0.05 indicated a significant difference.

Results
3.1 Clinical characteristics of the patients enrolled
As shown in table 1, a total of 43 patients with tumors located on the liver (37 liver metastases, 6 primary liver cancers) were analyzed. The patients’ cytokine levels were compared according to time: before treatment, 24 hours after treatment, 15 days after treatment, and 30 days after treatment.

3.2 IFN-γ, IL-12 p40, and IL-12 p70 were slightly increased after MWA treatment
As shown in table 2 and figure 1, the median level of IFN-γ before MWA treatment was 3.25 pg/mL (95% CI 2.72-6.12 pg/mL); at 15 days and 30 days after MWA treatment, there was slight increase compared to that pre-MWA, with a median level of 5.71 pg/mL (95% CI 5.15-7.51 pg/mL) and 5.65 pg/mL (95% CI 4.47-6.71 pg/mL), respectively. The median level of IL-12 p40 before MWA treatment was 4.16 pg/mL (95% CI 3.94-5.56 pg/mL). There was a slight increase to 6.81 pg/mL (95% CI 6.17-7.90 pg/mL) 30 days post-MWA. The median IL-12 p70 level before MWA treatment was 3.00 pg/mL (95% CI 2.84-4.01 pg/mL) and increased to 5.49 pg/mL (95% CI 4.90-6.79 pg/mL) 15 days after MWA treatment and to 4.61 pg/mL (95% CI 4.07-5.75 pg/mL) 30 days post-MWA. No significant alteration in the VEGF median level was detected after MWA treatment.

3.3 IL-2, IL-1β, IL-6, IL-8 and IL-10 were elevated over 2-fold after MWA treatment
As shown in table 2, figure 1 and figure 2, the median level of IL-2 before MWA treatment was 2.77 pg/mL (95% CI 2.77-3.38 pg/mL). There was a significant increase at 24 hours post-MWA, with a
median level of 6.69 pg/mL (95% CI 3.31-11.56 pg/mL). The median level of IL-1β before MWA treatment was 3.58 pg/mL (95% CI 2.99-7.36 pg/mL), and a significant increase was noted 15 days after MWA treatment (16.30 pg/mL) (95% CI 9.67-19.79 pg/mL). The median level of IL-6 before MWA treatment was 4.63 pg/mL (95% CI 3.00-6.72 pg/mL) and significantly increased 15 days after MWA treatment (15.68 pg/mL) (95% CI 13.9-26.99 pg/mL). The median level of IL-8 before MWA treatment was 2.92 pg/mL (95% CI 2.92-4.19 pg/mL) and increased significantly to 10.07 pg/mL (95% CI 6.38-19.91 pg/mL) 15 days after MWA treatment. The median level of IL-10 before MWA treatment was 5.42 pg/mL (95% CI 3.66-9.01 pg/mL) and increased significantly 15 days after MWA treatment (18.32 pg/mL) (95% CI 12.71-26.22 pg/mL). The median level of TNF-α before MWA treatment was 7.19 pg/mL (95% CI 5.53-10.97 pg/mL) and increased significantly to 20.77 pg/mL (95% CI 7.87-37.85 pg/mL) 15 days after MWA treatment.

3.4 Elevated IL-2 and IL-6 levels after ablation were positively correlated with energy output during MWA

To further evaluate the relationship between increased cytokine levels and MWA treatment, we employed a concept of “energy” (time 1×power 1 + time 2×power 2, time 1/2 and power 1/2 indicated the time and power of different probes used in the operation) to reflect total hyperthermia damage on hepatic tissues during the MWA procedure. As shown in table 3 and figure 3, IL-2 levels at 24 h post-MWA and IL-6 levels at 15 d post-MWA illustrated significant correlations with energy; the relative indexes were 0.35 and 0.29, respectively.

Discussion

Local therapies such as surgical resection play a critical role in liver cancer. However, surgical operations are limited by an insufficient patient condition or tumor metastasis. As technology continues to develop, other types of local therapy, such as radiotherapy, chemical ablation and hyperthermal ablation, for primary and metastatic liver cancer are increasingly being used. MWA for liver malignances is reserved for patients who are not suitable for surgical removal or whose other treatments have failed[10]. A consensus guideline was recently developed to address indications for MWA in these patients. Thermal ablation is a process that heats the target tissue to a temperature that causes immediate coagulative necrosis (usually over 100°C). Terminal treatment requires that a
necrotic area surrounds the target site with an additional 5-10-mm margins[11]. However, in the liver, high tissue perfusion and large blood vessels can cause a "heat sink effect" around the ablation zone, making it difficult to achieve terminal ablation[12]. The heat sink effect can lead to sublethal temperatures and the retention of malignant cells, thereby increasing the likelihood of local tumor progression (LTP)[13]; however, an incompletely ablated zone containing immune cells and cancer cells, as well as functional vessels, could establish a serious inflammatory site that may provide tumor-specific antigens, cytokines, and activated immune cells.

In recent years, ablation-induced systemic effects, such as the tumor-associated immune response, have attracted wide attention[14]. de Baere T first reported two cases of the spontaneous regression of multiple pulmonary metastases occurring after the radiofrequency ablation of a single lung metastasis[15]. Although growing evidence suggests that thermal ablation can induce spontaneous regression of the so-called “abscopal effect” on distant tumors, the incident rate of such an effect is rare, probably due to uncontested immunological activation caused by one ablation treatment and the lack of immuno-amplification management. In 2004, it was described that in situ tumor destruction can provide a useful antigen source for the induction of antitumor immunity[16]; however, clinical studies could not sufficiently utilize such an effect until the development of immune checkpoint blockade reagents[17, 18].

Ablation therapy can mediate antitumor immunity, as tumor tissue necrosis caused by ablation may release a scale of antigens that eventually form a kind of “in situ vaccination”[19]. Moreover, ablative therapy cannot only directly kill cancer cells in situ but also regulate immune cells and promote the immune function of patients with liver cancer[20, 21]. Many immunoregulatory cytokines are released or expressed after thermal ablation. It is important to note that the cytokines released after thermal ablation can regulate the positive and negative aspects of the cancer immune cycle. Previously, researchers demonstrated that proinflammatory cytokines such as IL-1, IL-6, IL-8, IL-18, and TNF-α were increased several hours or days after thermal ablation[22-24]. In our investigation, we employed a concept of “energy” to evaluate the relationship between the increased levels of cytokines and MWA treatment. Our findings indicated that IL-2 and IL-6 were significantly evaluated after the
ablation procedure and positively correlated with MWA energy. IL-2 is commonly derived from activated T cells, primarily Th1 cells. IL-2 can stimulate T cells to proliferate and differentiate, activate natural killer (NK) cells and macrophages, and enhance the functions of cytotoxic T lymphocytes (CTLs)[25]. Our findings suggest that IL-2 is significantly increased at 24 h after MWA, indicating that IL-2 may induce a nonspecific immune response after MWA. The increase IL-2 did not last as long, but it decreased 24 h post-MWA in our study, suggesting that the IL-2-induced immune response may not last as long.

Immune checkpoint inhibitors (ICI) such as PD-1/PD-L1 and CTLA4 antibodies were widely applied in several cancers, researches indicated that ICI treatment could enhance the effect of ablation[5]. Evidence indicated that hyperthermia destruction caused release of a large population of heterogenous tumor antigens and inflammatory cytokines may play crucial roles[6]. However, opposite evidence indicated that by incomplete radiofrequency ablation-induced inflammation could accelerates tumor progression and hinders PD-1 immunotherapy[26], suggesting there could be a mechanism that ablation treatment may promote tumor progression. Our data demonstrated that IL-6 was significantly increased after MWA treatment, IL-6 is derived from monocytes, macrophages, Th2 cells and sometimes cancer cells, and it plays a key role in T cell proliferation and survival[27]. Evidence indicates that IL-6 plays an indispensable role in T cell infiltration to the tumor site, which could benefit immunomodulatory therapy. However, IL-6 can increase myeloid-derived suppressor cells (MDSCs)[28], inhibit the development and maturation of dendritic cells (DCs)[29], and inhibit the polarization of Th1 cells[30], eventually illustrating a negative immunomodulatory function. According to Muneeb Ahmed’s work, the adjuvant use of a nanoparticle small interfering RNA (siRNA) can be successfully used to target the IL-6-mediated locoregional and systemic effects of thermal ablation. IL-6 knockout via a nanoparticle anti-IL-6 siRNA in mice could decrease the local VEGF level at the ablation site[31]. Therefore, how to utilize the positive effect of IL-6 while avoiding the negative effect after MWA needs further investigation. Preclinical research indicated that IL-6 and PD-L1 blockade combination therapy reduced tumour progression in animal models[32, 33]. Thus, anti-IL-6 strategy after ablation should be considerable while combined with ICI therapy. Previous studies and ours
demonstrate that most cytokine levels returned to pretreatment levels 30 days after ablation. This result suggests that 24 hours to 15 days after ablation may be optimal timing for additional immunomodulatory therapy.

Declarations

Acknowledgment

This work was supported by the National Natural Science Foundation of China (81402477; 81501563; 81602802), the Natural Science Foundation of Jiangsu Province of China (BK20140295), the Jiangsu Government Scholarship for Oversea Studies (JS-2018-179), and the "Six one projects" for high-level health personnel in Jiangsu Province (LGY2018077).

Conflict of Interest Statement

There is no financial or personal relationship with other people or organizations that could inappropriately influence (bias) this work.

References

1. Fu J, Wang H: **Precision diagnosis and treatment of liver cancer in China.** *Cancer Lett* 2018, **412**:283-288.

2. Bruix J, Han KH, Gores G, Llovet JM, Mazzaferro V: **Liver cancer: Approaching a personalized care.** *J Hepatol* 2015, **62**(1 Suppl):S144-156.

3. Rognoni C, Ciani O, Sommariva S, Bargellini I, Bhoori S, Cioni R, Facciorusso A, Golfieri R, Gramenzi A, Mazzaferro V et al: **Trans-arterial radioembolization for intermediate-advanced hepatocellular carcinoma: a budget impact analysis.** *BMC Cancer* 2018, **18**(1):715.

4. Nault JC, Sutter O, Nahon P, Ganne-Carrie N, Seror O: **Percutaneous treatment of hepatocellular carcinoma: State of the art and innovations.** *J Hepatol* 2018, **68**(4):783-797.

5. Yin J, Dong J, Gao W, Wang Y: **A case report of remarkable response to association of radiofrequency ablation with subsequent Atezolizumab in**
stage IV nonsmall cell lung cancer. *Medicine (Baltimore)* 2018, **97**(44):e13112.

6. Shi L, Chen L, Wu C, Zhu Y, Xu B, Zheng X, Sun M, Wen W, Dai X, Yang M et al: **PD-1 Blockade Boosts Radiofrequency Ablation-Elicited Adaptive Immune Responses against Tumor.** *Clin Cancer Res* 2016, **22**(5):1173-1184.

7. Lippitz BE: **Cytokine patterns in patients with cancer: a systematic review.** *Lancet Oncol* 2013, **14**(6):e218-228.

8. Jin YB, Zhang GY, Lin KR, Chen XP, Cui JH, Wang YJ, Luo W: **Changes of plasma cytokines and chemokines expression level in nasopharyngeal carcinoma patients after treatment with definitive intensity-modulated radiotherapy (IMRT).** *PLoS One* 2017, **12**(2):e0172264.

9. Kim MJ, Jang JW, Oh BS, Kwon JH, Chung KW, Jung HS, Jekarl DW, Lee S: **Change in inflammatory cytokine profiles after transarterial chemotherapy in patients with hepatocellular carcinoma.** *Cytokine* 2013, **64**(2):516-522.

10. Gillams A, Goldberg N, Ahmed M, Bale R, Breen D, Callstrom M, Chen MH, Choi BI, de Baere T, Dupuy D et al: **Thermal ablation of colorectal liver metastases: a position paper by an international panel of ablation experts, The Interventional Oncology Sans Frontieres meeting 2013.** *Eur Radiol* 2015, **25**(12):3438-3454.

11. Ahmed M, Solbiati L, Brace CL, Breen DJ, Callstrom MR, Charboneau JW, Chen MH, Choi BI, de Baere T, Dodd GD, 3rd et al: **Image-guided tumor ablation: standardization of terminology and reporting criteria--a 10-year update.** *Radiology* 2014, **273**(1):241-260.

12. Chiang J, Hynes K, Brace CL: **Flow-dependent vascular heat transfer during microwave thermal ablation.** *Conf Proc IEEE Eng Med Biol Soc* 2012, **2012**:5582-5585.
13. Huang HW: Influence of blood vessel on the thermal lesion formation during radiofrequency ablation for liver tumors. Med Phys 2013, 40(7):073303.

14. Mehta A, Oklu R, Sheth RA: Thermal Ablative Therapies and Immune Checkpoint Modulation: Can Locoregional Approaches Effect a Systemic Response? Gastroenterol Res Pract 2016, 2016:9251375.

15. Rao P, Escudier B, de Baere T: Spontaneous regression of multiple pulmonary metastases after radiofrequency ablation of a single metastasis. Cardiovasc Intervent Radiol 2011, 34(2):424-430.

16. Chu KF, Dupuy DE: Thermal ablation of tumours: biological mechanisms and advances in therapy. Nat Rev Cancer 2014, 14(3):199-208.

17. Greten TF, Mauda-Havakuk M, Heinrich B, Korangy F, Wood BJ: Combined locoregional-immunotherapy for liver cancer. J Hepatol 2019, 70(5):999-1007.

18. Slovak R, Ludwig JM, Gettinger SN, Herbst RS, Kim HS: Immuno-thermal ablations - boosting the anticancer immune response. J Immunother Cancer 2017, 5(1):78.

19. den Brok MH, Sutmuller RP, van der Voort R, Bennink EJ, Figdor CG, Ruers TJ, Adema GJ: In situ tumor ablation creates an antigen source for the generation of antitumor immunity. Cancer Res 2004, 64(11):4024-4029.

20. Zerbini A, Pilli M, Laccabue D, Pelosi G, Molinari A, Negri E, Cerioni S, Fagnoni F, Soliani P, Ferrari C et al: Radiofrequency thermal ablation for hepatocellular carcinoma stimulates autologous NK-cell response. Gastroenterology 2010, 138(5):1931-1942.

21. Zhang H, Hou X, Cai H, Zhuang X: Effects of microwave ablation on T-cell subsets and cytokines of patients with hepatocellular carcinoma. Minim Invasive Ther Allied Technol 2017, 26(4):207-211.

22. Ahmad F, Gravante G, Bhardwaj N, Strickland A, Basit R, West K, Sorge R, Dennison
AR, Lloyd DM: Changes in interleukin-1beta and 6 after hepatic microwave tissue ablation compared with radiofrequency, cryotheraphy and surgical resections. Am J Surg 2010, 200(4):500-506.

23. Erinjeri JP, Thomas CT, Samoilia A, Fleisher M, Gonen M, Sofocleous CT, Thornton RH, Siegelbaum RH, Covey AM, Brody LA et al: Image-guided thermal ablation of tumors increases the plasma level of interleukin-6 and interleukin-10. J Vasc Interv Radiol 2013, 24(8):1105-1112.

24. Fietta AM, Morosini M, Passadore I, Cascina A, Draghi P, Dore R, Rossi S, Pozzi E, Meloni F: Systemic inflammatory response and downmodulation of peripheral CD25+Foxp3+ T-regulatory cells in patients undergoing radiofrequency thermal ablation for lung cancer. Hum Immunol 2009, 70(7):477-486.

25. Volko J, Kenesei A, Zhang M, Varnai P, Mocsar G, Petrus MN, Jambrovics K, Balajthy Z, Muller G, Bodnar A et al: IL-2 receptors preassemble and signal in the ER/Golgi causing resistance to antiproliferative anti-IL-2Ralpha therapies. Proc Natl Acad Sci U S A 2019.

26. Shi L, Wang J, Ding N, Zhang Y, Zhu Y, Dong S, Wang X, Peng C, Zhou C, Zhou L et al: Inflammation induced by incomplete radiofrequency ablation accelerates tumor progression and hinders PD-1 immunotherapy. Nat Commun 2019, 10(1):5421.

27. Fisher DT, Appenheimer MM, Evans SS: The two faces of IL-6 in the tumor microenvironment. Semin Immunol 2014, 26(1):38-47.

28. Mantovani A, Sica A, Allavena P, Garlanda C, Locati M: Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. Hum Immunol 2009, 70(5):325-330.
29. Chomarat P, Banchereau J, Davoust J, Palucka AK: **IL-6 switches the differentiation of monocytes from dendritic cells to macrophages.** *Nat Immunol* 2000, 1(6):510-514.

30. Diehl S, Rincon M: **The two faces of IL-6 on Th1/Th2 differentiation.** *Mol Immunol* 2002, 39(9):531-536.

31. Ahmed M, Kumar G, Navarro G, Wang Y, Gourevitch S, Moussa MH, Rozenblum N, Levchenko T, Galun E, Torchilin VP et al: **Systemic siRNA Nanoparticle-Based Drugs Combined with Radiofrequency Ablation for Cancer Therapy.** *PLoS One* 2015, 10(7):e0128910.

32. Mace TA, Shakya R, Pitarresi JR, Swanson B, McQuinn CW, Loftus S, Nordquist E, Cruz-Monserrate Z, Yu L, Young G et al: **IL-6 and PD-L1 antibody blockade combination therapy reduces tumour progression in murine models of pancreatic cancer.** *Gut* 2018, 67(2):320-332.

33. Tsukamoto H, Fujieda K, Miyashita A, Fukushima S, Ikeda T, Kubo Y, Senju S, Ihn H, Nishimura Y, Oshiumi H: **Combined Blockade of IL6 and PD-1/PD-L1 Signaling Abrogates Mutual Regulation of Their Immunosuppressive Effects in the Tumor Microenvironment.** *Cancer Res* 2018, 78(17):5011-5022.

Tables
Table 1. Clinical characteristics of the patients enrolled. (n=43)
| Characteristic                                                                 |   |
|-------------------------------------------------------------------------------|---|
| **Sex**                                                                        |   |
| male                                                                          | 28 |
| female                                                                        | 15 |
| **Age**                                                                        |   |
|                                                                               | 62.81±8.14 |
| **Pathogenesis**                                                              |   |
| primary                                                                       | 6  |
| secondary                                                                     | 37 |
| **Primary site (For metastatic hepatic malignances)**                         |   |
| Colon & rectal                                                                 | 18 |
| Pancreas                                                                      | 7  |
| Stomache                                                                      | 3  |
| Breast                                                                        | 3  |
| Others                                                                        | 9  |
| **Maximum tumor length (mm)**                                                 |   |
|                                                                               | 33.58±13.29 |
| **Ablation probe used**                                                       |   |
| 1                                                                             | 31 |
| 2                                                                             | 12 |
| **Ablation time (min)**                                                       |   |
|                                                                               | 27.09±14.36 |
| **Average power per probe (W)**                                               |   |
|                                                                               | 51.98±5.13 |
| **Average energy (time 1×power 1 + time 2×power 2)**                          |   |
|                                                                               | 1426±806 |

\[\n, \text{Time } 1/2 \text{ and power } 1/2 \text{ indicate the time and power, respectively, of different probes used during the operation.}\]

Table 2. Median levels of cytokines before and after MWA.

|                      | 15 days post-MWA (pg/mL) |
|----------------------|--------------------------|
|                      | 3                        |
Table 1. \( \text{MW} (\text{pg/mL}) \)

|                  | Mean        | 95% CI       |
|------------------|-------------|--------------|
| TKIN - MW        | 5.71        | 5.15-7.51    |
| TKIN - MW        | 5.65        | 4.81-6.50    |
| IL - MW          | 4.82        | 4.41-5.53    |
| IL - MW          | 5.69        | 5.32-6.07    |
6.86 (95% CI 5.90-7.63)

5.49 (95% CI 4.90-6.79) *

16.30 (95% CI 9.67-19.79) *▼
15.68 (95% CI 13.90-26.99) ▼

10.07 (95% CI 6.38-19.91) ▼

18.32 (95% CI 12.71-26.22) ▼

20.77 (95% CI 7.87-37.85) ▼
Table 3. Correlation between the ablation energy and significantly elevated cytokines.

|                      | Energy vs. IL-2 24 h post-MWA | Energy vs. IL-1β 15 d post-MWA | Energy vs. IL-6 15 d post-MWA |
|----------------------|-------------------------------|--------------------------------|-------------------------------|
| Spearman’s r         | 0.3501                        | -0.06957                       | 0.2911                        |
| P value (one-tailed) | 0.0107*                       | 0.3288                         | 0.0291*                       |

*, p<0.05

Figures

\[\text{IFN-γ}\]  \[\text{IL-2}\]
Levels of cytokines before and after MWA treatment. Slightly increased IFN-γ, IL-12 p40, and IL-12 p70 levels after MWA treatment. Over 2-fold enhancement of IL-2 24 h post-MWA and of IL-1β, IL-6, IL-8, IL-10 and TNF-α 15 d post-MWA. *, p<0.05.
Trends in cytokines significantly altered after MWA treatment. The levels of IL-2 at 24 h post-MWA, IL-1β at 15 d post-MWA, IL-6 at 15 d post-MWA, IL-8 at 15 d post-MWA and IL-10 at 15 d post-MWA were elevated over 2-fold compared to the levels pre-MWA.

Correlation between the ablation energy and the serum levels of IL-2 and IL-6. The serum levels of IL-2 at 24 h post-MWA and IL-6 at 15 d post-MWA were positively correlated with energy output during the MWA procedure.