Title: The effect of intraperitoneal immune checkpoint inhibitor on malignant ascites of patients with gastric, pancreatic or biliary tract cancer

Keywords: immune checkpoint inhibitor, malignant ascites, gastric cancer, pancreatic cancer, biliary tract cancer

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Abstract:

Malignant ascites appear when cancer cells metastasize to peritoneal cavity and interfere the circulation of lymph and blood. Patients with malignant ascites suffer from abdominal fullness, abdominal pain, poor intake, decreased nutrition, disability, and eventually inability to further anticancer treatment. Malignant ascites put a heavy burden on patient, their family, society and health care system.

Malignant ascites from cancers of stomach, pancreas and biliary tract are more refractory to intravenous chemotherapy or intraperitoneal chemotherapy than ascites from ovarian or chemo-naïve colorectal cancers because of the inherent nature of chemoresistance. Paracentesis is a treatment with immediate effect but the ascites regrow rapidly and repetitive paracentesis puts patients on the risk of intraabdominal infection. Intraperitoneal infusion of OK-432 can reduce malignant ascites but with intolerable adverse effects of fever, chills, pain, vomiting and septation of ascites which limit the further paracentesis.

Cumulating clinical experience suggests a tolerable safety profile of immune checkpoint inhibitors compared to chemotherapy for patients with malignancy. One theoretical advantage of intraperitoneal administration of immune checkpoint inhibitors is the existence of abundant inflammatory cells, immune cells and mesothelial cells dispersed in the malignant ascites. In addition, both pembrolizumab and nivolumab have been administrated intraperitoneally without obvious toxicities in murine tumor models

At China Medical University Hospital, one hundred gastric cancer patients, 50 pancreatic cancer patients and 50 biliary tract cancer patients are registered and treated each year. Among them, more than 20 patients suffer from malignant ascites which will ultimately be managed by repeated paracentesis with increasing frequency. Till now, there is no reported or ongoing clinical trial to investigate the efficacy of intraperitoneal checkpoint inhibitor on the malignant ascites. In this project, we propose to evaluate the efficacy of immune checkpoint inhibitor (pembrolizumab or nivolumab) on the malignant ascites of patients with advanced gastric, pancreatic and biliary tract cancers.

The major works in the 3-year-project are summarized as:

1. Screening patients, executing intraperitoneal administration of immune checkpoint inhibitor (pembrolizumab or nivolumab), and evaluating the clinical response.
2. Ascites cells and malignant cells in ascites are to be analyzed using flow cytometry, immunohistochemical staining, exon sequencing and total mutational burden determination.
3. Correlation study using clinical characteristic, ascites data and clinical response.

Background:

Malignant ascites are often noted in patients with ovarian, colorectal, gastric, pancreatic and biliary tract malignancy. Among them, malignant ascites from cancers of stomach, pancreas and biliary tract are more refractory to chemotherapy because of the inherent nature of chemoresistance which remains a major challenge to physicians. The incidence of malignant ascites varies according to the condition of patients when they are evaluated (Maeda H, World J Gastroenterol 2015). A report from Norway identified 6.2% of 356 patients with gastric cancer had malignant ascites at their initial diagnosis (Lello E, Acta Oncol 2007). A large retrospective study from China found ascites in 2.6% of 5542 patients at the initial diagnosis of gastric cancer, and in 3.7% of patients thereafter (Fang N, Tumour Biol 2014). For patients with gastric cancer in T2-3 stage at diagnosis, the incidence of malignant ascites reached 15.0% of 293 patients (Yajima K, Am J Surg 2006). It is estimated that 3%-6% of patients with gastric cancer have malignant ascites at initial presentation, and 10%-15% of patients treated with operation develop peritoneal recurrence with half of them to have malignant ascites. Therefore, 8%-13.5% of patients with gastric cancer suffer from malignant ascites (Table 1; Maeda H, World J Gastroenterol 2015).

Table 1. Incidence of peritoneal dissemination and ascites development due to gastric cancer

Ref.	No. of patients	Period	Country	Status of primary disease	Incidence
Development of peritoneal dissemination					
Nakajima *et al*[10]	7060	1960-1988	Japan	After gastrectomy	14.2%
Nashimoto *et al*[12]	13002	2002	Japan	After gastrectomy	9.9% (related to death)
Development of ascites					
Lello *et al*[25]	356	1980-2004	Norway	At initial diagnosis	6.2%
Yajima *et al*[31]	293	1988-2002	Japan	GC with T2-3 at diagnosis	15.0%
Fang *et al*[26]	5542	2007-2012	China	At initial diagnosis	2.6%[1]
				During the course of disease	3.7%[1]
Kitayama *et al*[14]	83	2006-2008	Japan	Peritoneal recurrence	40.0%
Tahara *et al*[13]	56	1993-1999	Japan	Peritoneal recurrence	46.4%

(Maeda H et al., World J Gastroenterol 2015;21:10936)

Patients with advanced pancreatic cancer are also prone to have malignant ascites. Once diagnosed, most pancreatic cancer patients with malignant ascites died within 2 months (Zervos EE, World J Surg Oncol 2006). Ascites is seen in 20% of pancreatic cancer patients (Adam RA, J Am Coll Surg 2004), with cancer cells identified in less than half of patients (Zervos EE, World J Surg Oncol 2006). Takahara et al., retrospectively reported 15% of patients (73/494) with advanced pancreatic cancer and malignant ascites, with better prognosis for patients with synchromous ascites compared with patients with metachronous ascites (Takahara N, Pancreas 2015). As same as pancreatic cancer, biliary tract cancer is also a cause of primary tumor for patients with malignant ascites (Fig. 1; Ayantunde AA, Ann Oncol 2007; Ayantunde AA, Clin Med Diag 2012).

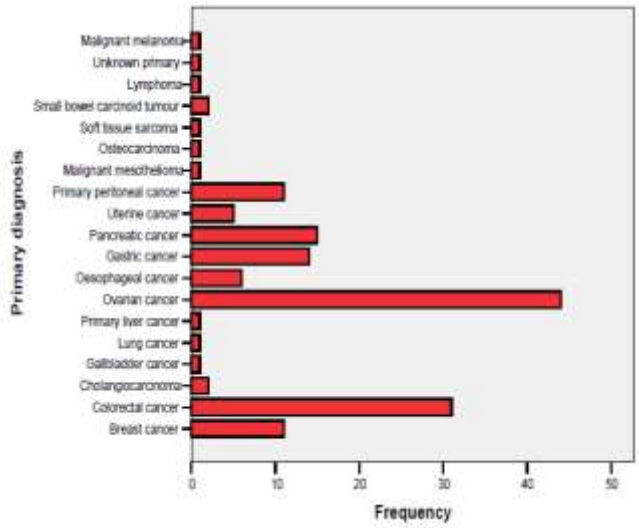

Fig. 1. Primary cancer types causing malignant ascites (Ayantunde AA, Clin Med Diag 2012)

Malignant ascites is usually associated with disease in late stage. Patients with malignant ascites have more morbidity than patients without ascites. The distended abdomen will limit and reduce patients' daily activity. Furthermore, patients with large amount of malignant ascites suffer from back soreness and abdominal pain which render them to take more pain killer and frequent paracentesis. Overall, malignant ascites put a heavy burden on patient, their family, society and health care system.

Treatment for malignant ascites depends on multiple factors including the general condition of patients, the nature of their malignancies, the amount and growing rate of ascites, and the availability of certain compounds. There are several choices for treating malignant ascites: systemic chemotherapy or antitumor therapy, intraperitoneal chemotherapy, intraperitoneal targeting therapy, and paracentesis. The response of malignant ascites to intravenous chemotherapy depends on the inherent chemosensitivity of the primary tumor, therefore, malignant ascites in patients with gastric, pancreatic and biliary tract are more resistant to intravenous chemotherapy, especially for patients refractory to lines of previous chemotherapy. Paracentesis is a treatment with immediate effect but the ascites grow rapidly, besides, it puts patients on the risk of intraabdominal infection. Chemotherapy administrated intraperitoneally theoretically kills the tumor cells and penetrates the tumor nodules by diffusion. Most commonly used agents for intraperitoneal chemotherapy are taxane, mitomycin-C, mechlorethamine, thiotepa, cisplatin and 5-fluorouracil. Generally, a temporary control can be achieved with ascites control rate 33%-85% depending on the chemotherapeutic agent and the tumor type (Cavazzoni E, Int J Clin Oncol 2013). Intraperitoneal chemotherapy yields better ascites control in patients with ovarian cancer than patients with GI tract cancers.

Intraperitoneal immunotherapies using tumor necrosis factor (TNF), interferon or OK-432 (picibanil) have been utilized clinically since early 1980s. However, administration of TNF or

interferon intraperitoneally has been proposed to improve the function of killer cells in the peritoneal space but their activity in human is still controversial (Gebbia V, In Vivo 1991; Cavazzoni E, Int J Clin Oncol 2013). OK-432, a penicillin-heat-treated powder of Streptococcus pyogenes A3, is instilled into the peritoneal cavity to control malignant ascites through its effect to activate cytotoxic T cells (Torisu M, Surgery 1983; Katano M, Anticancer Res 1998). Intraperitoneal administration of OK-432 results in a reduction of ascites in 50%-70% of patients. The major challenge of intraperitoneal OK-432 is the adverse effects which sometimes are intolerable to patients. More than 90% of patients receiving intraperitoneal OK-432 administration suffered from abdominal cramping pain, fever, chills, bowel distention, nausea and vomiting.

Intraperitoneal administration of catumaxomab, a chimeric trifunctional antibody against EpCAM, CD-3 and Fc Rs, obtained a significant and persistent reduction of ascites in patients with ovarian cancer (Burges A, Clin Cancer Res 2007). Although a survival benefit was not demonstrated in a phase II/III trial, catumaxomab prolonged the puncture-free survival time (52 vs. 11 days in ovarian cancer; 37 vs. 14 in non-ovarian cancer) (Heiss MM, Int J Cancer 2010). However, patients receiving catumaxomab suffered from pyrexia, nausea and vomiting, and the preliminary data needs further confirmation.

Immune checkpoint inhibitors have demonstrated efficacy for several types of tumor in recent years (Fig. 2). The advantage of immune check-point inhibitors compared with conventional chemotherapy includes the avoidance of non-specific killing of cells, the absence of chemotherapy-induced adverse effects and the generalization of activity across several types of malignancies. Cumulating clinical experience suggests the more tolerable safety profiles of immune checkpoint inhibitors compared to chemotherapy. Recent data suggest the immune checkpoint inhibitors are effective against cancers of gastrointestinal tract. Nivolumab is superior to best supportive care for patients with gastric cancer who failed at least two lines of systemic anticancer therapies (Kang YK, Lancet 2017). The combination of nivolumab with chemotherapy showed promising efficacy in first-line therapy of patients with gastric cancer (ESMO 2017) from the preliminary data of a large phase III clinical trial. Based on the available data, nivolumab has been proved by Japan Ministry of Health, Labor and Welfare for the treatment of patients with unresectable advanced or recurrent gastric cancer which has progressed after chemotherapy in September 2017. As well, another PD-1 inhibitor, pembrolizumab, received American Food Drug Agent (FDA)’s approval for the treatment of patients with PD-L1-positive recurrent or advanced gastric or gastroesophageal junction adenocarcinoma who have received 2 or more lines of chemotherapy in September 2017.

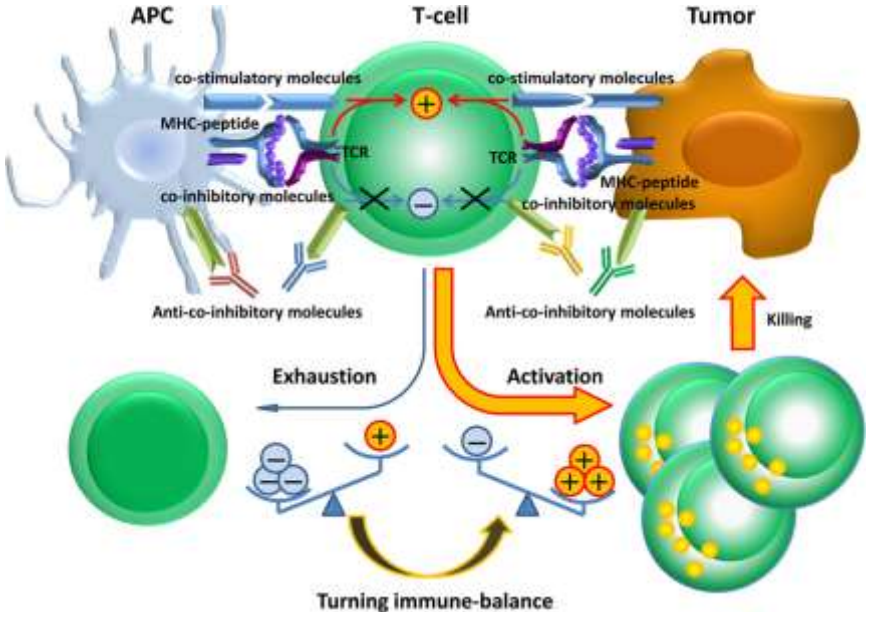

Fig. 2. Monoclonal antibodies against negative checkpoints enhance the immune response and turn immune-balance. (Suzuki S et al., JJCO 2016;46:191)

Both pembrolizumab and nivolumab have been administrated intraperitoneally without obvious toxicities in murine tumor models (Fessas P, Semin Oncol 2017; EMA report: pembrolizumab 2015; EMA report: nivolumab 2015). Pembrolizumab at a dose of 10 mg/kg given intraperitoneally on days 6, 10, 13, 16 and 20 after implantation led to tumor growth inhibition of 92.5% at day 20 in murine. Similarly, nivolumab at a dose of 10 mg/kg given intraperitoneally on days 7, 10 and 13 after implantation inhibited tumor growth by 76% at day 20.

At our hospital (China Medical University Hospital), about one hundred gastric cancer patients, 50 pancreatic cancer patients and 50 biliary tract cancer patients are registered and treated each year (Chiu CF, Gastroenterol Res Pract 2016; Chiu CF, Springerplus 2016, Report of Cancer Registration in China Medical University Hospital). Patients with advanced or metastatatic cancer are ultimately refractory to chemotherapy with or without targeted therapy. Among them, more than 20 patients suffer from intractable malignant ascites which is managed by repeated paracentesis with increasing frequency. Till now, there is no reported or ongoing clinical trial to investigate the efficacy of intraperitoneal checkpoint inhibitor on the malignant ascites. Based on the previous findings and the literature review, we propose to evaluate the efficacy of immune checkpoint inhibitor (pembrolizumab or nivolumab) on the malignant ascites of patients with advanced gastric, pancreatic and biliary tract cancers.

The major works in the 3-year-project are summarized as:

1. Screening the candidate patients. Patients who fulfill the inclusion and exclusion criteria will be

screened. The study purpose and protocol will be explained thoroughly by physicians. Study starts only if patient read, understand and sign the inform consent.

2. Intraperitoneal administration of immune checkpoint inhibitor (pembrolizumab or nivolumab) per protocol followed by scheduled evaluation.
3. Ascites studies: Ascites cells and malignant cells in ascites are to be analyzed using flow cytometry, immunohistochemical staining, exon sequencing and total mutational burden determination.
4. Correlation study using clinical characteristic, ascites data and clinical response.

Materials and Methods:

1. **Study design**：A pilot study to evaluate the effect of intraperitoneal immune checkpoint inhibitor on malignant ascites of patients with gastric, pancreatic or biliary tract cancer
2. **Inclusion criteria**
   (1) Patients have cyto-/histologically confirmed malignant ascites from gastric, pancreatic or biliary tract adenocarcinoma
   (2) Patients have malignant ascites more than 1000ml
   (3) Patients have no history of prior intraperitoneal therapy for malignant ascites
   (4) Patients have life expectancy of at least 4 weeks
   (5) Patients have adequate platelet count ≥ 50,000/ul
   (6) Women or men of reproductive potential should agree to use an effective contraceptive method
   (7) All patients must be informed of the investigational nature of this study and must sign written informed consents.
3. **Exclusion criteria**
   (1) Patients have ascites which is related to causes other than the malignancies
   (2) Patients who are receiving intraperitoneal treatment for their malignant ascites including therapeutic paracentesis
   (3) Patients with active infection
   (4) Patients with bleeding disorders
   (5) Patient with active cardiopulmonary disease or history of ischemic heart disease
   (6) Patients have intolerant abdominal pain
   (7) Patients who have serious concomitant systemic disorders incompatible with the study, i.e. poorly controlled diabetes mellitus, auto-immune disorders, cirrhosis of the liver, and the rest will be at the discretion of in-charged investigator
4. **Procedure**

(1) Prior to the administration of first dose immune checkpoint inhibitor (pembrolizumab or nivolumab), ascites at least 200 ml will be collected by paracentesis. The ascites will be sent for examinations listed in "ascites study". The same procedure is repeated before each administration of immune checkpoint inhibitor.

(2) At the first 3 patients, immune checkpoint inhibitor (pembrolizumab or nivolumab) will be administered intraperitoneally at a dose of 20 mg diluted in 100 ml normal saline through a temporary catheter in 10 min (reason seen below). If there is no grade 3 or higher toxicity, the dose of pembrolizumab / nivolumab will be escalated to 50 mg for subsequent dose.

(3) If there is no grade 3 or higher toxicity for the initial 3 patients receiving first dose 20mg of immune checkpoint inhibitor, the first dose of pembrolizumab / nivolumab will be escalated to 50 mg for subsequent patient.

(4) The estimated initial dose for pembrolizumab / nivolumab is based on the report that both compounds were given safely to murine at a dose of 10 mg/kg. Human Equivalent Dose (HED) is 10 divided by Km ratio 12.3 = 0.8 mg/kg (Nair AB, J Basic Clin Pharm 2016). For a man with a weight 60kg, the dose will be 48 mg. Although intravenous injection of either pembrolizumab or nivolumab is well tolerated at a dose up to 10 mg/kg in previous clinical study, we reduce the initial dose of intraperitoneal infusion to 20 mg for either pembrolizumab or nivolumab.

(5) Remove the temporary catheter after infusion.

(6) Repeat the above procedure every 2 weeks for 1-4 times depending on the response of ascites clinically.

5. Ascites study

(1) Ascites clinical study

a. Cell count, ascites culture, tuberculosis culture, protein, albumin, lactate dehydrogenase (LDH)

(2) Ascites exploratory study

a. Flow cytometric analysis of ascites cells: the cells in ascites will be analyzed the expression percentage of CD3, CD4, CD8, CD 11, CD14, CD33, CD56, CD57 using specific antibodies.

b. Cell block: the cells in ascites will be prepared to be cell block and for HE staining, PD-L1 staining, tumor proportion score, combined positive score.

c. Perspective study: in order to examine which genetic trait is related to the response of malignant ascites to immune checkpoint inhibitor, we will sequence the exons of 341 cancer-associated genes and determine the deleterious mutation status. The total mutational burden (Teo MY, Clin Cancer Res 2017) will be executed using cell blocks from 4 patients (2 with good ascites response and 2 with ascites refractory to pembrolizumab / nivolumab).

6. Endpoints

Primary endpoint: reduction of malignant ascites. The ascites amount will be estimated by *a five-point method* developed by Oriuchi et al. (Oriuchi N, Jpn J Clin Oncol 2005; Maeda H, World J Gastroenterol 2015).

Secondary endpoints: ascites signs, ascites symptoms, safety and overall survival.

(1) Ascites symptoms (anorexia, nausea, early satiety, vomiting, abdominal pain, abdominal swelling, dyspnea, fatigue, swollen ankles and heartburn) were assessed subjectively using a patient questionnaire with a four-point Likert scale (none, mild, moderate and severe) (Likert R, Arch Psychol 1932).

(2) Ascites signs (abdominal distension dull to percussion, shifting dullness, fluid thrill and bulging flanks) were assessed objectively after abdominal examination by the investigator.

(3) Overall survival of patients is defined as the period from the first dose of intraabdominal immune checkpoint inhibitor to the time of lost-of-follow or death, whichever occurs first.

7. Data Safety and Monitoring Plan

Data Safety and Monitoring Plan is set for ensuring the safety of patients. The plan is composed of three phases.

(1) Screening and recruitment period
   a. Screening patient by pre-set inclusion and exclusion criteria
   b. Explain the current medical situation to patients, and alternative treatment methods
   c. Obtain signed inform consent

(2) Trial execution period
   a. Conduction of study during hospitalization
   b. Bedside monitoring by a physician or nurse from beginning till 10 min after administration of medication
   c. Overnight monitoring

(3) Follow-up period
   a. Outpatient clinic every week till 2 weeks after the last dose of medication; then every one month for 6 m, then
   b. Follow the patient every 2 m till end of life

8. Assessment of response and toxicity

(1) Prior to treatment, a medical history, physical examination, ascites symptoms, ascites signs, laboratory studies (blood cell count, electrolytes, liver and renal function tests, and urinalysis), chest radiography, and abdominal CT are performed.

(2) Physical examination, ascites symptoms and ascites signs are assessed every week.

(3) The amount of ascites is assessed by radiologists using CT which will be performed at 4^{th} and 8^{th} week after treatment.

(4) Patients safety is assessed by adverse event reporting throughout the study. Adverse events

are graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTCAE version 4.0).

(5) For the analysis of the secondary endpoint, any patient lost to follow-up is censored at the time of last visit.

9. **Correlation study of predictive factors:** the association of ascites response and the characteristic of ascites, clinical features of patients and type of tumors will be analyzed.

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