Phase I/II Clinical Study of PRaG Regimen Combined With Intraperitoneal Infusion of PD-1 Inhibitor for Advanced Refractory Solid Tumors With Cancerous Ascites (PRaG4.0P Study)

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Abstract

Objective: The prognosis of malignant tumors with peritoneal metastases and cancerous ascites has generally been poor, with limited treatment options. The PRaG regimen, which comprised of hypofractionated radiotherapy, programmed cell death-1 (PD-1) inhibitor, and granulocyte-macrophage colony-stimulating factor (GM-CSF), showed a survival advantage in patients with advanced solid tumors who failed at least the first line of standard systemic treatment. Intraperitoneal infusion of PD-1 inhibitors may be a novel therapeutic strategy for managing malignant ascites. Integrating the PRaG regimen with intraperitoneal perfusion of a PD-1 inhibitor might control malignant ascites and provide further survival benefits in these patients. This proposed study aims to investigate the safety and efficacy of intraperitoneal infusion of serplulimab in combination with the PRaG regimen in patients with simultaneous advanced solid tumors and cancerous ascites who fail at least the first-line treatment. **Methods:** This proposed study is a prospective, single-arm, open-label, multicenter clinical trial. All eligible patients will receive 2 cycles of intensive treatment, a combination of PRaG regimen with an intraperitoneal infusion of PD-1 inhibitor. The patients who are beneficially treated with intensive treatment will receive consolidation treatment every 2 weeks until ascites disappear, disease progression occurs, intolerable toxicity occurs, or for up to 1 year. Phase I of this study will be conducted using a modified 3 + 3 design. The dose of intraperitoneal infusion of PD-1 inhibitor for phase II will be determined according to dose-limiting

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toxicity evaluation in the phase I study. **Conclusion:** This prospective, open-label, multicenter study will potentially lead to intraperitoneal perfusion of a PD-1 inhibitor being a new strategy for malignant ascites patients and provide a meaningful efficacy and safety of the combination of PRaG regimen with an intraperitoneal infusion of PD-1 inhibitor for these patients.

Background

Peritoneal metastatic (PM) carcinoma results from cancer cells metastasizing to the peritoneum via blood circulation or direct peritoneal implantation.[1] PM is mostly secondary to ovarian cancers (46%), followed by gastric cancers (14%), colorectal tumors (7%), gastrointestinal neuroendocrine tumors (6%), and peritoneal cancers of unknown origin (3%–5%).[2] Malignant ascites (MA) is the abnormal accumulation of peritoneal fluid resulting from primary peritoneal tumors or other malignant tumors entering the peritoneum.[3] The life expectancy of patients with refractory MA was poor, with the overall survival (OS) ranging from 1 to 6 months in nonovarian cancers. MA secondary to ovarian cancer had a longer life expectancy than MA originated from other types, and the OS was about 10–24 months.[4,5] In a retrospective study by Mackey et al,[6] 76 patients with MA had a median OS of 11.1 weeks. Significant predictors of poor prognosis were edema, decreased serum albumin, and liver metastasis.[6]

Current Treatment Options for PM Carcinoma

Chemotherapy was recommended for patients with ovarian cancers and gastric cancers, but there were few reports supporting the efficacy of chemotherapy for massive ascites in other cancer types.[7] Due to the presence of a plasma-peritoneal barrier and inadequate blood supply, systemic treatment has limitations in treating PM.[8]

Other treatment options for PM cancer were cytoreductive surgery (CRS), intraperitoneal chemotherapy (IPEC), hyperthermic IPEC (HIPEC), and pressurized intraperitoneal aerosol chemotherapy.[9] Surgery was generally preferred in patients with limited peritoneal metastasis with a peritoneal cancer index (PCI) of <20.[10] IPEC was previously the preferred option for patients with extensive peritoneal metastases, however, its efficacy was limited with severe side effects. The National Particle Component Toxicity study demonstrated that postoperative use of paclitaxel peritoneal infusion compared with the intravenous injection did not significantly increase the efficiency in gastric cancer patients with a high risk of peritoneal recurrence.[11] The PRODIGE 7 study revealed that CRS combined with HIPEC for colorectal peritoneal metastases did not improve the OS compared with CRS alone. Furthermore, CRS combined with HIPEC resulted in an increased rate of late complications.[12]

Compared to other treatments, HIPEC remains the primary option for patients with a high PCI index (>20). However, research indicated that HIPEC did not improve the survival rate in patients with advanced ovarian cancers.[13] In summary, the evidence supporting HIPEC's efficacy in treating peritoneal metastasis is not compelling, and effective treatment for extensive peritoneal metastases remains elusive.

The Potential Rationale of Intraperitoneal Infusion of Immunotherapy for Peritoneal Metastasis Patients

T-cell activation depends on effective antigen processing and presentation by antigen-presenting cells in lymph nodes, as well as in the peritoneal fluid. The immune cells can enter the underlying terminal lymphatic lacunas, which further can drain into the main lymphatic channels through the mediastinal lymph nodes.[14] In the tumor microenvironment of ovarian cancers with MA, aggregates containing activated $CD4^+T$ cells and $CD8^+T$ cells were present.[15] Programmed cell death-1 (PD-1) inhibitors can interact with activated cytolytic lymphocytes, killing tumor cells, which provides a theoretical basis for the intraperitoneal infusion of PD-1 inhibitors. Previous studies considered the peritoneum to be a lymphoid

organ, with the double-layered greater omentum possessing peritoneal composites. This structure contained clusters of immune cells, known as milky spots, comprising 67.9% macrophages, 10.1% B cells, 10.2% T cells, and a few dendritic cells (DCs).[16] The research studies found that PM tumor cells were more prone to infiltration into these milky spots. Among macrophages, tumor-associated macrophages (TAMs) exhibited plasticity and polarized toward M1/M2 phenotypes when stimulated by corresponding cytokines. In animal models of colon cancer, more than 50% of TAMs within tumors expressed PD-1 on their surface. These PD-1-positive TAMs were M2 types, known to promote tumor growth. Furthermore, the function of PD-1^{+} TAMs was reversed by blocking the PD-1 pathway.[17] This finding provided additional theoretical support for the function of intraperitoneal injection of PD-1 inhibitors.

The New Treatment Model of Combining PD-1 Inhibitors, Radiotherapy, and Granulocyte-Macrophage Colony-Stimulating Factor (PRaG Therapy)

Hypofractionated radiotherapy (HFRT) or stereotactic body radiotherapy (SBRT) is routinely performed in clinical practice, which induces immunogenic cell death, creating the "in situ tumor vaccine." Radiotherapy also alters the tumor microenvironment, promoting DC and T-cell functions and activating immune effects. This response can be enhanced by combining radiotherapy with systemic immunotherapy, such as PD-1 inhibitor or granulocyte-macrophage colony-stimulating factor (GM-CSF).[18]

GM-CSF promotes the differentiation of monocytes/M1-type macrophages and DCs, enhances their activity, amplifies antigen presentation, and boosts the immune effects.[19] The GM-CSF improved the efficacy of PD-1 inhibitors and boosted the radiotherapy's immune effects in a clinical study.[20]

We combined a PD-1 inhibitor, radiotherapy, and GM-CSF (PRaG therapy)[21] and designed a prospective clinical trial (ChiCTR1900026175) that utilized the PRaG regimen to rescue patients with advanced solid tumors who failed at least the first-line chemotherapy. Preliminary results revealed that this triple therapy improved the efficacy of PD-1 inhibitors and broadened the spectrum of PD-1 immunotherapy against cancers.[22–25] Prior research suggested that PRaG treatment may be a promising salvageable treatment for advanced multiple metastatic solid tumors.

Based on recent research findings, we propose that intraperitoneal infusion of PD-1 inhibitor can produce antitumor effects and hypothesize that the combination of PD-1 inhibitor intraperitoneal infusion treatment and the PRaG therapy may offer survival benefits to patients with advanced malignant PM and simultaneous MA.

Methods

Objectives of the Proposed Study

This proposed study is a prospective, single-arm, open-label, multicenter clinical trial and was registered on ClinicalTrials.gov under the trial number NCT05501340. Our study was approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University (approval number JD-LK-2022-033-01).

This study will investigate the safety of combined PRaG therapy and intraperitoneal infusion of a PD-1 inhibitor through a phase I clinical trial. Furthermore, the effectiveness of this combined therapy will be verified through a phase II clinical trial.

The main objective of phase I of the PRaG 4.0P study is to assess the safety of combined PRaG therapy and intraperitoneal infusion of a PD-1 inhibitor in the treatment of patients with advanced solid tumors and simultaneous cancerous ascites. Safety will be evaluated from dose-limiting toxicity (DLT) response, adverse events (AEs), and serious AEs (SAEs).

The main objective of phase II (single-arm prospective trial) of the PRaG 4.0P study is to evaluate the safety and efficacy of combined PRaG therapy and intraperitoneal infusion of a PD-1 inhibitor in the treatment of advanced solid tumors and simultaneous cancerous ascites.

Furthermore, this proposed research will analyze the mechanism of combined therapy and will screen potential biomolecular markers for predicting the efficacy. This will be achieved by detecting lymphocyte subsets, cytokines, and metabolite levels in peripheral blood and malignant ascitic fluid.

Overview of Study Design

Phase I Clinical Trial: Sample Size and Safe Dose Decision. A data review panel, comprising investigators, medical supervisors, physicians, clinical representatives, and statisticians, will determine whether phase I dose should be reduced, based on the occurrence of DLT. The data review panel will assess all data related to AEs, including non-DLT, laboratory results, and other safety parameters. Any other stipulations specified in the dose reduction plan will be examined. Quality control measures for critical safety data, including ongoing study monitoring parameters, clinical database review reports, and data accuracy and completeness by site investigators will be detailed. Decisions and rationale on dose reduction will be finalized by study personnel and documented, and copies will be maintained at each study site.

In phase I trial, serplulimab will be administered as intraperitoneal infusions from a starting dose of 100 mg. If no one among the first 3 patients develops DLT, the original dose will be continued, and 3 more cases will be enrolled. If there is still no one or one develops DLT, the dose of 100 mg intraperitoneal infusion will be used as the recommended phase 2 dose (RP2D) trial. If 1 of the first 3 patients, experiences DLT, then 3 additional patients will be treated with the same dose; if 2 or more cases in all 6 patients experience DLT, then the dose will be considered to exceed the maximum tolerated dose (MTD), then the dose will be reduced to 80 mg intraperitoneal infusion. Likewise, if DLT occurs in 2 or more patients, then the dose will be reduced to 60 mg. Accordingly, with each subsequent reduction being 20 mg a

minimum dose will be 20 mg. If a trial dose surpasses the MTD, and 6 patients are administered with the next lower dose, this dose will be considered MTD. If the dose is reduced to 20 mg and 2 or more patients still experience DLT, the trial will be terminated.

The overview of the treatment design is shown in Figure 1.

Definition of DLT Reaction by Intraperitoneal Infusion of PD-1 Inhibitor. Severe diarrhea, abdominal infection/peritoneal infection, intestinal obstruction and perforation, gastrointestinal and abdominopelvic organ hemorrhage, acute kidney injury (AKI), liver failure, allergic reactions, and other hematologic abnormalities within 2 weeks of intraperitoneal infusion will be considered PD-1 inhibitor-related toxic reactions. The definition of DLT reaction related to intraperitoneal infusion of PD-1 inhibitor is outlined in Table 1.

Phase II Clinical Trial Dose Selection. Upon completion of the phase I trial, all available data, including information on the subjects who discontinued treatment will be compiled and summarized. The determination of dose for the phase II trial will be determined by a comprehensive analysis of all data from the phase I trial, including assessing safety, tolerability, and efficacy.

The Sample Size for Phase II Clinical Trial. According to retrospective reports, the OS of a substantial number of patients with cancerous ascites varies from 1 to 6 months. Some literature reports a median OS of 5.7 months from the time of diagnosis.[26] In the phase II study, the doses found to be safe in the phase I study will be utilized. The sample size of the enrolled patients will be calculated based on OS data from the analysis of the phase I study.

Treatment Protocol

The treatment program consists of 2 distinct stages, the intensive treatment phase and the consolidation treatment phase (Figure 2).

Intensive Treatment Phase. Patients will be enrolled and treated with HFRT or SBRT (8Gy × 3f) to particular cancer lesions (appropriate peritoneal metastases which can be localized or other organ metastatic lesions), and serplulimab at a dose of 3 mg/kg will be administered intravenously within 1 week of radiotherapy onset, and a GM-CSF (Molgramostim; Xiamen Amoytop Biotech Co., Ltd, Xiamen, China) at a dose of 200 μg will be administered by subcutaneous injection daily for 5 days, and serplulimab (HLX10; Shanghai Henlius Biotech, China) will be administered by intraperitoneal infusion after the end of radiotherapy. This treatment constitutes a 2-week cycle, and every cycle will be performed on different target lesions. A total of 2 cycles of triple therapy will be completed.

Consolidation Treatment Phase. For patients who are effective after the intensive treatment, serplulimab at a dose of 3 mg/kg IV every 2 weeks and a GM-CSF at a dose of 200 μg subcutaneously for a total of 5 days, every 2 weeks will be administered
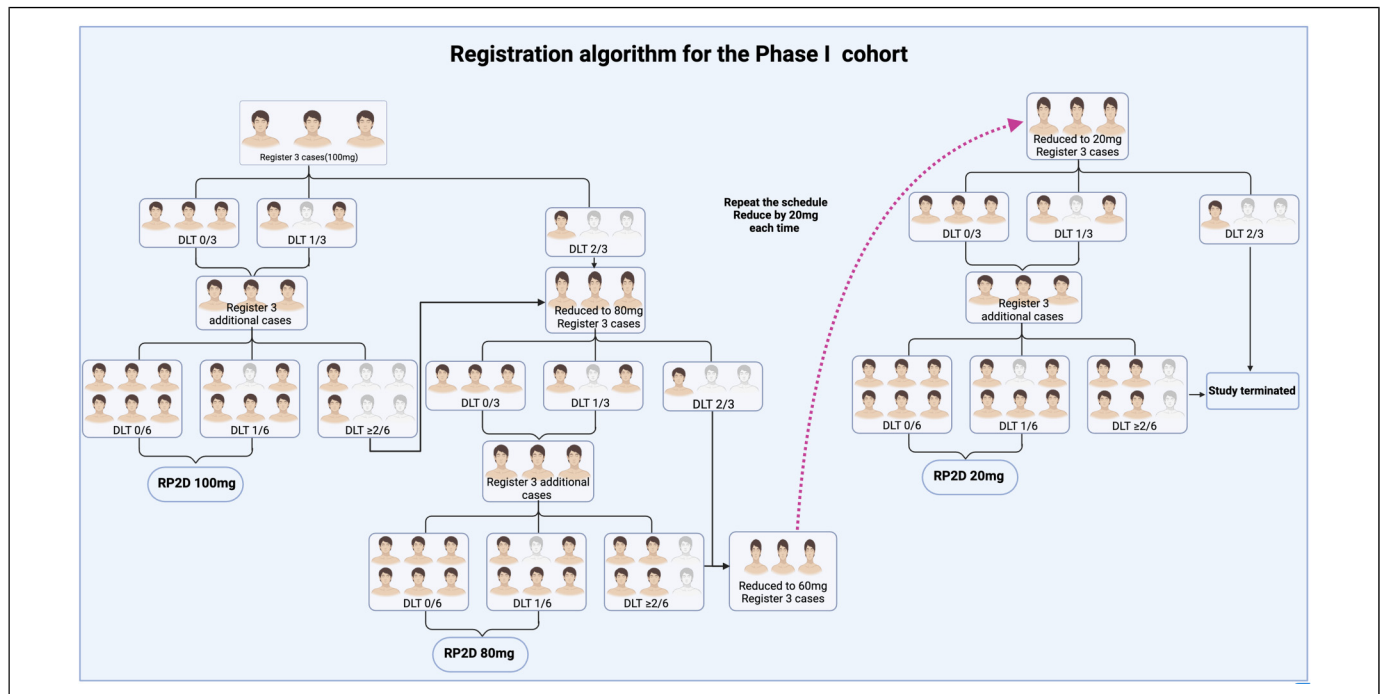

Figure 1. Registration model for the proposed study. Phase I of this study will be conducted using a modified 3 + 3 design. RP2D of intraperitoneal infusion of PD-1 inhibitor will be verified according to the DLT evaluation in the phase I study.
Abbreviations: DLT, dose-limiting toxicity; RP2D, recommended phase 2 dose.

Table 1. Definition of DLT Reactions.

PD-1-related toxic events within 2 weeks of intraperitoneal infusion	Diarrhea	Increased number of defecations compared to baseline, ≥7 per day; requiring hospitalization;
	Abdominal/peritoneal infection	Post-treatment procalcitonin (PCT) level of >2.0 ng/mL; exclusion of spontaneous peritonitis and preexisting peritoneal infections; positive post-treatment ascites pathogenic microbial culture, requiring intravenous antibiotics, antifungal or antiviral agents; requiring invasive therapy
	Intestinal obstruction	Gastrointestinal decompression with no relief from conventional noninvasive treatment and requirement of surgical intervention.
	Gastrointestinal, hepatobiliary, or pancreatic perforation	Requiring invasive intervention or hospitalization
	Gastrointestinal and abdominopelvic organ hemorrhage	Requiring blood transfusion; requiring invasive intervention or hospitalization.
	AKI	Requiring hospitalization, with occurrence of AKI within 3 days of infusion
	Liver failure	Fluttering tremor; mild hepatic encephalopathy; presence of drug-induced liver damage
	Allergic reactions	Symptomatic bronchospasm with or without urticaria; requiring parenteral therapy; allergic reaction-related edema/angioedema; hypotension.
	Hematologic adverse events	Grade ≥3 neutropenia and grade ≥4 anemia and thrombocytopenia
	Other systemic toxic reactions	≥Grade 3 adverse events according to the CTCAE 5.0

Abbreviations: DLT, dose-limiting toxicity; PD-1, programmed cell death-1; AKI, acute kidney injury;
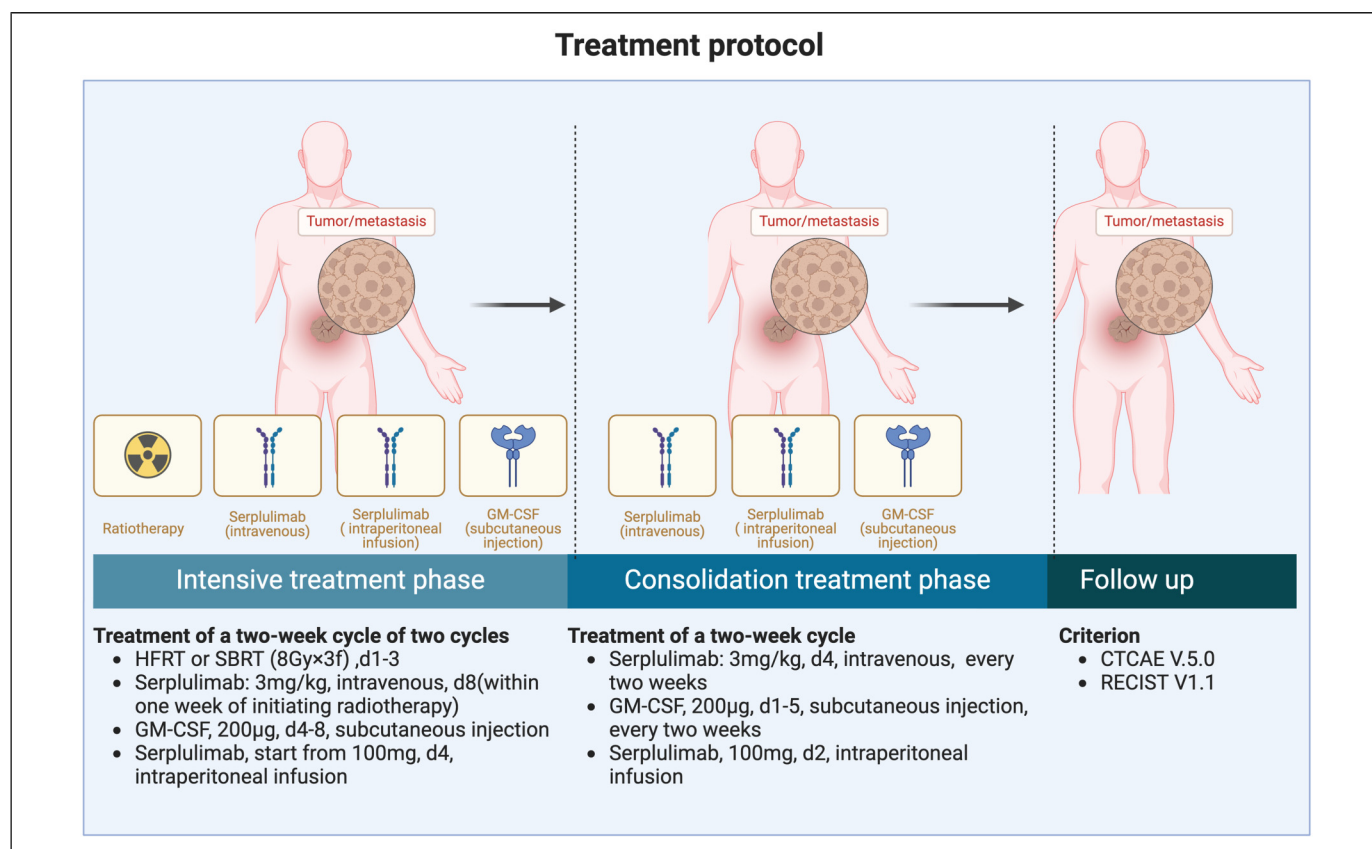
Figure 2. Treatment plan of this proposed study. The treatment includes an intensive treatment phase and a consolidat ion treatment phase.

Table 2. Inclusion and Exclusion Criteria.

Inclusion criteria	Exclusion criteria
Patients must fulfill all the following criteria	Patients should not be included if one of the following exclusion criteria is found.
▶ Patients aged >18 years.	▶ Pregnant or breastfeeding women.
▶ Patients who have histopathologically confirmed advanced malignant solid tumors.	▶ Patients with a history of other malignant diseases within the last 2 years, except cured skin cancer and carcinoma in situ.
▶ Patients with pathologically confirmed peritoneal lesions or positive exfoliated tumor cells in ascitic fluid.	▶ Patients with a history of uncontrolled epilepsy, central nervous system disease, or psychiatric disorders.
▶ Patients who have been newly diagnosed with malignant ascites for no more than 1 month.	▶ Patients with clinically significant heart disease, including NYHA class II or worse congestive heart failure, severe arrhythmias requiring pharmacologic intervention, or a history of myocardial infarction within the last 12 months.
▶ Patients with the progression after at least the first-line standard therapy or who are unsuitable for standard systemic therapy.	
▶ Patients with no previous congestive heart failure, unstable angina, or unstable arrhythmia within the last 6 months.	▶ Patients who had organ transplantation requiring immunosuppressive therapy.
▶ Patients with an activity status score of 0–3 on the ECOG scale with a life expectancy assessment of ≥2 months.	▶ Patients with known active infection, or the presence of significant hematologic, renal, metabolic, gastrointestinal, endocrine dysfunction, or other serious uncontrolled concomitant disease.
▶ Patients with no previous serious abnormalities in hematopoietic, cardiac, pulmonary, hepatic, or renal function and immunodeficiency.	▶ Patients who show hypersensitivity to any investigational drug component.
▶ One week prior to enrollment, patients with absolute peripheral blood total T lymphocytes of ≥0.5 the lower limit of normal, absolute CD8$^+$T cells of ≥200/μL, neutrophils of ≥1.0 × 10^9/L; AST and ALT of ≤3.0, the upper limit of normal; creatinine of ≤1.5, the upper limit of normal or creatinine clearance of ≥50 mL/min, serum albumin of ≥30 g/L. Patients will be allowed after indicators meet the above criteria for 2 weeks after treatment. Transfusion therapy or granulocyte-stimulating factor therapy within 2 weeks prior to treatment will not be allowed.	▶ Patients with a history of immunodeficiency, including positive HIV test or other acquired or congenital immunodeficiency disease, history of organ transplantation, or other related conditions requiring long-term oral hormone therapy (>10 mg/day prednisone).
	▶ Patients with acute or chronic tuberculosis infection (positive T-spot test, suspected tuberculosis foci on chest x-ray), acute hepatitis infection, or chronic hepatitis B virus abnormalities.
	▶ Contraindications to laparotomy include severe thrombocytopenia, severe intestinal dilatation, bowel paralysis, and peritoneal adhesions.
▶ Patients with the ability to understand and voluntarily sign an informed consent form.	▶ Patients who had prior receipt of immune checkpoint inhibitor therapy were discontinued due to drug-related toxicity. Other conditions deemed unsuitable for enrollment by the investigator.
	▶ Other conditions deemed unsuitable for enrollment by the investigator.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NYHA, New York Heart Association.

until ascites disappears, disease progression occurs, intolerable toxicity arises, or for up to 1 year.

Furthermore, intraperitoneal infusion of serplulimab every 2 weeks will be given, until the disappearance of ascites, disease progression, intolerable toxicity occurrence, or for up to 1 year.

Patient Eligibility

This study will enroll patients diagnosed with malignant metastases and simultaneous MA. Participants will be pathologically confirmed for a malignant solid tumor, as well as validated by either peritoneal lesions or the presence of positive exfoliated tumor cells in ascitic fluid. The specific inclusion and exclusion criteria are detailed in Table 2. Written informed consent will be obtained for the enrolled patients.

Endpoints of this Proposed Study

The tolerability of intraperitoneal infusion of serplulimab remains unexplored. Consequently, this phase I study will assess the

safety and tolerability of this combination therapy. The primary endpoints will be the incidence rates of AEs and SAEs, including DLTs, and will be evaluated according to the Common Terminology Criteria for AE (CTCAE) V.5. In the phase II study, the primary endpoint will be l OS, which will be calculated from the enrollment date to the date of death or last known alive. Secondary endpoints will include objective response rates (ORRs), which will be based on the Response Evaluation Criteria in Solid Tumors, version 1.1, progression-free survival (PFS), disease control rate (DCR), and ascites control rate. ORR will be defined as the proportion of patients with complete response (CR) or partial response (PR). DCR will be the percentage of patients with CR, PR, or stable disease (SD) from enrollment. PFS will be calculated from enrollment to disease progression, death, or censored at the last clinical follow-up.

Ascites will be assessed using abdominal computed tomography (CT) or ultrasound. The immediate outcome of MA will be assessed through ultrasound. Complete remission (CR) will be defined as the complete disappearance of ascites, lasting more than 4 weeks, and partial remission (PR) as a >50%

reduction of ascites lasting over 4 weeks. SD will be defined as a decrease in ascitic fluid volume of <50% or an increase of ≤25%, lasting over 4 weeks, and progression disease (PD) will be defined as an increase of >25% in ascitic fluid volume on ultrasound. The recent outcome of MA will be assessed based on CT and will be categorized as none (grade I), mild (grade II, limited to the pelvis), moderate (grade III, beyond the pelvis), and massive (grade IV, entire abdomen).[10,27,28] Furthermore, peripheral blood and ascitic fluid will be sampled before and after the treatment. As an exploratory objective, these samples will be evaluated to determine correlations between individual immune responses and prognosis-related biomarkers. The endpoints are summarized in Table 3.

Statistical Analysis

The statistical analysis will be performed using SPSS version 25.0 software (IBM Corp, Armonk, NY, USA). The frequencies of DLT will be calculated in all patients who receive at least 1 cycle of treatment. The period for evaluating DLTs will extend from the start of treatment until 92 days thereafter. DLTs will be summarized at 30 and 92 days after the initiation of the carbon-ion radiotherapy treatment. The AEs/SAEs will also be summarized and listed according to their severity and frequency. The PFS and OS endpoints in the intention-to-treat patients will be estimated using the Kaplan–Meier method. A p-value of <.05 (two-sided) will be considered statistically significant. The study will compare changes in the white blood cell, granulocyte, and lymphocyte counts, as well as differential cells ($CD45^+CD3^+$, $CD45^+CD3^+CD4^+CD8^-$, $CD45^+CD3^+CD8^+CD4^-$, $CD3^+CD8^-CD45RA^-CD197^+CD38^+HLA-DR^+$, $CD3^+CD8^+CD39^+PD-1^+$, $CD3^+CD8^-CD45RA^-CD197^-$, $CD3^+CD8^-CD45RA^-D197^+$, $CD3^+CD8^+CD45RA^-CD197^-$, $CD3^+CD8^+CD45RA^-CD197^+$, $CD3^+CD8^-CD45RA^-CD197^-CD38^+HLA-DR^+$, $CD3^+CD8^+CD45RA^-CD197^-CD38^+HLA-DR^+$, $CD3^+CD8^+CD45RA^-CD197^+CD38^+HLA-DR^+$, $CD3^+CD4^+CD25^{high}CD127^\pm$, $CD3^-CD19^-CD14^+CD16^-HLA-DR^{high}CD40^+$, $CD3^-CD19^-CD14^-HLA-DR^+CD11c^+$

$CD123^-$, $CD3^+CD8^+OX40^+CD40L^+$, $CD8^+PD-1^+$, $CD8^+CD122^+$, $CD8^+CD27^+$, $CD8^+CD39^+$, $CD4^+CD38^+HLA-DR^+$, and $CD8^+CD38^+HLA-DR^+$) and cytokines (interleukin [IL]-2, IL-4, IL-6, IL-10, IL-17A, tumor necrosis factor, interferon-γ) before and after radiotherapy both in peripheral blood and ascitic fluid, and will determine if any of the differences are statistically significant. Cox regression analysis will be performed, combining changes in these counts with the patient's survival time to assess their influence on the survival rate. Additionally, the study will employ the Kaplan–Meier method to analyze survival, focusing on the relationship between survival rate and changes in cytokines and lymphocyte counts in both peripheral blood and ascitic fluid.

The reporting of this study conforms to SPIRIT guidelines.[29]

Ethics and Dissemination

Our study was approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University (Approval No. JD-LK-2022-033-01). Findings will be disseminated through national and international conferences. We also plan to publish our findings in a high-impact peer-reviewed journal.

Consent to Participate

All patients provided written informed consent prior to enrollment in the study. This consent form must be signed by the subject and the investigator-designated research professional obtaining the consent. Consent will be obtained by the study coordinator or investigator of the study at each site. Consent will include provisions for collection and use of participant data and biological specimens. Consent will be documented in the medical record.

Discussion

As of now, several intraperitoneal immunotherapy strategies are under investigation. For example, Catumaxomab, a bispecific

Table 3. The Endpoints of the Study.

	Phase I	Phase II
Main endpoints	DLT	OS
	AEs	
	SAEs	
Secondary endpoints	OS	PFS
	PFS	DCR
	DCR	ORR
	ORR	Ascites improvement rate
	Ascites improvement rate	
Observational parameters	T-cell activation and cytokines change in ascitic fluid	
	QOL score	
	FACIT-AI	
	Biomarkers in ascitic fluid and peripheral blood	

Abbreviations: DLT, dose-limiting toxicity; AE, adverse event; SAE, serious adverse event; OS, overall survival; PFS, progression-free survival; DCR, disease control rate; ORR, objective response rate; QOL, quality of life; FACIT-AI, functional assessment of chronic illness therapy-ascites index.

(anti-epithelial cell adhesion molecule and anti-CD3) trifunctional antibody, was approved by the European Union in 2009 for intraperitoneal treatment of patients with MA.[8] Other immunotherapy, such as immune stimulators, cytokines, and cancer vaccines are considered potential strategies for intraperitoneal immunotherapy.[30]

Given the lack of a previous dose reference for intraperitoneal infusion of PD-1 inhibitors, we referred to data from intravenous infusion when selecting the perfusion dose. The dose–exposure–effect relationship (D–E–R) was outlined for the safety and efficacy of serplulimab (HLX10). The probability of various AEs such as immune-related AE, ≥grade 3 AE, ≥grade 2 AE, and ≥grade 3 immune-related AE were used as safety indices. The maximum serum concentration at steady state, the minimum serum concentration at steady state and the area under the curve normalized to a week (WAUC) served as drug exposure indices. This suggested that the D–E–R curves were consistent for 3 mg/kg once every 2 weeks, 3 mg/kg once every 3 weeks (Q3W), 4.5 mg/kg Q3W, and 6 mg/kg Q3W administration of HLX10 dosing regimens. The correlation curves between HLX10 exposure and the probability of immune-related AE were found to be horizontal, aligning with the results of pembrolizumab and nivolumab studies. It was suggested that the likelihood of immune-related AE occurrence remained unchanged under any of the four HLX10 dosing regimens, indicating that all four regimens had a favorable safety profile. Therefore, we utilized a dose-decreasing model to swiftly determine the feasible intraperitoneal infusion dose. A prior case report highlighted that a patient with peritoneal metastasis from pancreatic cancer, accompanied by massive ascites, experienced a notable reduction in ascites after bi-weekly intraperitoneal infusions of 20 mg nivolumab.[31] As such, PD-1 inhibitor intraperitoneal infusion may emerge as a novel therapy, but more evidence from prospective clinical trials is requisite.

The therapeutic efficacy of PD-1 inhibitors as monotherapy is limited. Based on the cancer-immunity cycle, certain treatments, such as GM-CSF and radiotherapy, might augment the effectiveness of PD-1 inhibitors. Various phase I/II clinical trials have confirmed the safety and efficacy of PD-1 inhibitors when combined with HFRT or SBRT for advanced metastatic tumors. GM-CSF, which can enhance antigen presentation by amplifying dendritic cell function, was safe and tolerable with a dose of 150 $\mu g/m^2$ per day or 200 μg per day for 14 consecutive days.[24,32] Furthermore, prior phase II trials have demonstrated that 12% of the patients experienced grade 3 or above treatment-related adverse effects of the triple combination (PRaG) therapy,[22–25] which was acceptable and tolerable.

Peripheral lymphocytes, based on blood circulation, can partially reflect a patient's immune status and predict the efficacy of immunotherapy. In this trial, we explored the subsets of lymphocyte subsets and cytokines biomarkers in both peripheral blood and ascitic fluid. T cells can be differentiated into naïve T cells (T_N), effector T cells (T_{Eff}), and memory T cells (T_M).[33] Further, T_M can be divided into stem cell memory T cells (T_{SCM}), central memory T cells (T_{CM}), and effector

memory T cells (T_{EM}) according to the different effector functions, proliferation, and migration abilities.[34] T_N cells can respond to immune mobilization reserve capacity. The T_{CM}/T_{Eff} ratio and the increase of T_{CM} and T_{EM} after immune checkpoint therapy were correlated with outcomes. We tested the $CD8^+T_N(CD45RA^+CD3^+CD8^+CD4^-)$, $CD4^+T_N(CD45RA^+CD3^+CD4^+CD8^-)$, $CD4^+T_{CM}(CD3^+CD8^-CD45RA-D197^+)$, $CD4^+T_{EM}(CD3^+CD8^-CD45RA^-CD197^-)$, $CD8^+T_{EM}(CD3^+CD8^+CD45RA^-CD197^-)$, and $CD8^+T_{CM}(CD3^+CD8^+CD45RA^-CD197^+)$ in each cycle. The T-cell activation phenotype, which responds to the activated proliferation and functional status of T cells, positively predicts the efficacy of immune checkpoint inhibitor treatment. T-cell activation phenotype often expresses costimulatory molecule receptors, and chemokine receptors, such as CD39, HLA-DR, CD38, and OX40.[35–40] So we also sought to explore the different phenotypes within treatment. Myeloid-derived cells ($CD3^-CD19^-CD14^+CD16^-HLA-DR^{high}CD40^+$ and $CD3^-CD19^-CD14^-HLA^-DR^+CD11c^+CD123^-$) were also explored in this study. As we know, this is the first prospective study to combine systemic anti-PD-1 therapy with intraperitoneal anti-PD-1 therapy. We will evaluate the safety and efficacy of combining intraperitoneal infusion of a PD-1 inhibitor with the PRaG therapy model.

Conclusion

This prospective, open-label, single-arm, multicenter study will potentially lead to intraperitoneal perfusion of a PD-1 inhibitor being a new strategy for MA. And will provide a safe reference dose for intraperitoneal infusion of serplulimab in the Phase I study. Furthermore, we aim to evaluate the efficacy and safety of a combination of PRaG regimen with an intraperitoneal infusion of PD-1 inhibitor for MA patients. Experimental biomarkers of cytokines and lymphocyte subsets will be explored in this study.

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