Weill Cornell Medicine

Enhancing Anti-Tumor Immunity in Peritoneal Metastatic Cancer

Proposal Prepared for ReMission Foundation

January 2025

Background

Metastatic cancer of the peritoneum is more common than primary peritoneal malignancy and typically occurs in advanced-stage locoregionally involved gastrointestinal (GI) or gynecological cancers. One in three patients with metastatic GI cancer develops peritoneal metastases due to the high propensity for early metastatic progression of the primary tumor. The incidence of peritoneal metastases varies between the primary tumor sites of GI cancers; the most prevalent GI malignancies to acquire peritoneal metastases are gastric and colon cancers, accounting for 14% of all gastric and 7% of all colon cancer cases, respectively.

Depending on the underlying tumor site, patients with peritoneal metastasis have a poorer prognosis when compared to other sites of metastases. Patients with peritoneal metastases due to pancreatic cancer have a mean survival of 2.4–2.9 months, those with gastric cancer 2.2–6.5 months, and those with CRC 6.9–8.5 months. Unlike other sites of metastases, patients with peritoneal disease remain undetected on radiographic imaging and present only when symptomatic disease develops. A lower quality of life is experienced by individuals with peritoneal disease, which is brought on by the development of ascites, severe abdominal pain, bowel obstruction, and malnourishment. In addition, systemic therapies for GI metastases have limited efficacy in peritoneal disease. Current literature supports the hypothesis of a peritoneal plasma barrier impairing drug distribution and perfusion into the peritoneum. Therefore, treatment methods include the administration of hyperthermic intraperitoneal chemotherapy (HIPEC) or pressurized intraperitoneal aerosol chemotherapy (PIPAC), which provides direct local treatment within the peritoneum and is associated with significant pharmacokinetic and pharmacodynamic benefits.

Numerous immune cells are present in the peritoneal cavity, and recent studies have demonstrated that the immune system is crucial in regulating tumor growth in the peritoneum. However, peritoneal tumors frequently evolve defense mechanisms to evade the immune system. Consequently, significant efforts have been made to create novel immunotherapeutic strategies that can improve anti-tumor immunogenicity. In colon cancer resistant to conventional immunotherapy, the combination of Botensilimab; an Fc-enhanced next-generation anti-CTLA-4 antibody, and

Balstilimab; an anti- programmed death ligand 1 (Anti–PDL-1) inhibitors, as a neoadjuvant therapy, has demonstrated favorable results in mismatch repair proficient colon cancer. The modified FC-enhanced structure of Botensilimab leads to a downstream immune memory response, depletion of T regulatory cells, and amplification of immune response by activation of NK cells. Balstilimab - a monoclonal IgG4 antibody that binds to PD-1, restores inactivated tumor-reactive T cells due to chronic stimulation.

A separate but related challenge with peritoneal metastases is the trafficking of anti-tumor immune cells into the peritoneum. In humanized mouse model of microsatellite high colorectal cancer, it was found that peritoneal metastases were devoid of B cells and TLS (tertiary lymphoid structures), while the T cells in these lesions displayed a dysfunctional phenotype. Complete lack of functional T-cells could explain why immunotherapy does not work in most cases. In these cases, we believe that intra- peritoneal introduction of functional T-cells could be the answer.

We hypothesize that intra-peritoneal administration of immunotherapies and T-cells are safe and effective in treating peritoneal carcinomatosis from GI malignancies. The advantage of peritoneal disease model is the analysis of ascitic fluid developed secondary to peritoneal disease, which can help monitor the disease burden and immune cell activities in real time. Given this important unmet need, we believe it is important to develop novel therapies that are tolerable and effective for this population of patients.

SPECIFIC AIMS

Aim 1: Evaluate the role of intraperitoneal administration of dual checkpoint inhibitors in GI malignancies with peritoneal carcinomatosis.

- Sub-Aim 1a: Assess the anti-tumor activities of intraperitoneal BOT/BAL in mouse models of peritoneal metastases.
- Sub-Aim 1b: Evaluate the cytological composition of pre- and post-BOT/BAL ascitic fluid as well as peritoneal tumor implants to understand the differences between tumors that respond vs tumors that do not respond to treatment.

Aim 2: Safety and efficacy of administering T-cell therapies intraperitoneally in murine models of peritoneal metastasis.

- Sub-Aim 2a: Characterize T-cell cell subsets from human ascitic fluid associated with peritoneal carcinomatosis.
- Sub-Aim 2b: Assess safety and efficacy of intraperitoneal administration of tumor-primed T cells in murine model of peritoneal carcinomatosis.

PROPOSED METHODOLOGY

Aim 1

Evaluate the role of intraperitoneal administration of dual checkpoint inhibitors in GI malignancies with peritoneal carcinomatosis. In this aim, we propose to evaluate the anti-tumor activities of dual checkpoint inhibitors when administered intraperitoneally. This approach has been used in early phase trials for patients with ovarian cancer but has not been extensively explored with patients with gastrointestinal malignancies. Recently, we had evidence that Fc-modified anti-CTLA4 BOT in combination with anti-PD1 inhibitor BAL has led to anti-tumor activities in cancer not known to respond to immunotherapies such as microsatellite stable colorectal cancer.

Sub-aim 1a: Assess the anti-tumor activities of intraperitoneal BOT/BAL in mouse models of peritoneal metastases. We will use 6 cohorts of 6 immunocompetent C57BL/6 mice per cohort. These mice will be injected with their respective neoplastic cell suspension intraperitoneally in the abdominal cavity directly.

These pre-treatment mice will then be evaluated weekly for up to 4 weeks for the development of peritoneal disease and disease burden by using an ultrasound to assess the presence of ascites and sampling of ascitic fluid, if present. The experimental cohorts A, B, and C will be treated with subsequent doses of intraperitoneal botensilimab and balstilmab. Whereas the control cohorts A, B, and C will be treated with vehicle only – InvivoPure dilution buffer.

Sub-aim 1b: Evaluate the cytological composition of pre- and post-BOT/BAL ascitic fluid as well as peritoneal tumor implants to understand the differences between tumors that respond vs tumors that do not respond to treatment. Cytological examination of ascitic fluid pre- and post- treatment including flow cytometry to detect cancer cells, T-cell subsets, myeloid cells, macrophages, and NK cells. In addition, we will use multiplexed immunofluorescence on paraffin embedded tissue sections to understand the spatial relationship of different cell types in implanted tumors. Markers we believe are important for the formation of peritoneal implants include TGF-B, VEGF and IL-10, which we will evaluate with ELISA and IHC.

Expected Results: If our hypothesis is correct, we will see a synergistic effect of BOT/BLA combination in peritoneal metastases models in reducing disease burden and ascites compared to untreated control. Further analyses of ascitic fluid will demonstrate cytological clearance of cancer cells and upregulation of T cells. The positive results from this pre-clinical investigation could be the basis for subsequent phase 1/2 testing in patients with peritoneal disease of GI origin.

Aim 2

Safety and efficacy of administering T-cell therapies intraperitoneally in murine models of peritoneal metastasis. Cytotoxic T-cells kill tumors by producing cytotoxic granules and enzymes that initiate the lysis of target cells. For patients whose tumors do not contain tumor infiltrating lymphocytes (TILs), there are no T-cells for the immunotherapy agents to work on. Cellular therapy is the

introduction of ex vivo generated T cells where there are none. Cell therapies have been shown to be effective in certain cancers that are refractory to immunotherapies, but their safety and efficacy via intraperitoneal administration is unknown. In this aim, we will first characterize the T-cells in human peritoneal carcinomatosis and evaluate the safety of introducing ex vivo tumor-primed T-cells into this peritoneal space.

Sub-Aim 2a: Characterize T cell subsets from human ascitic fluid associated with peritoneal carcinomatosis. In this sub-aim, we propose to use ascitic fluid from patients with peritoneal carcinomatosis to distinguish which GI tumor have active T-cells and which ones lack these tumor-fighting cells. We will collaborate with our interventional radiology colleagues to obtain peritoneal fluid from patients with malignant ascites.

Patients identified as having malignant ascites who undergo standard-of-care paracentesis will be consented to our GI tissue acquisition protocol. Approximately 1L of ascitic fluid will be collected and brought to the lab for processing and analysis. The fluid will be centrifuged at 300 rpm (rotations per minute) for 10 min at 4□C to isolate the cellular components from the ascitic fluid. Some of the liquid will be cryopreserved for later analysis. We will then lyse red blood cells from the mixture using the Ficoll method and wash the remaining cells. After the cells are counted, approximately 1 million cells each will be used to make cell pellet for immunohistochemistry and cell suspension for flow cytometry. The remaining cells will be cryopreserved in single cell suspension using 90% FBS and 10% DMSO.

For analysis of T-cell subsets, we will be evaluating for the number of CD4+ vs CD8+ T-cells as percentage of the total cell components as well as other cell subsets including tumor cells, macrophages, myeloid cells, B-cells, NK cells, and dendritic cells. Within the CD4 and CD8 subset, we will be looking at markers of memory T cells and exhausted T-cells. From prior research, tumors with higher amounts of central memory T-cells are more likely to respond to immunotherapy. Of the approximately 24 patient samples we collect, we will quantify how many samples have memory T-cells. For tumor types with less than 25% of samples containing memory T-cells, this will be the basis for a grant application to evaluate the generation tumor-primed T-cells for the treatment of these patients.

Sub-Aim 2b: Assess safety and efficacy of intraperitoneal administration of tumor-primed T cells in murine model of peritoneal carcinomatosis. We have been successful at making tumor-primed T-cells from mouse spleens by exposing and activating them in the presence of the target tumor.

Similar technique has also been replicated using human peripheral blood leukocytes in our lab. T-cells will be isolated using CD3 antibody embedded magnetic beads. The tumor and T-cells will be cultured with CD3-CD28 beads in combination with interleukins 2, 15, and 21. After 1 week of co-culture the T- cells will be isolated and injected into the peritoneal space of mice carrying peritoneal tumors.

We will observe the mice for any signs of distress for up to 4 weeks, at which time we will humanely euthanize the mice and evaluate the extent of peritoneal disease and the effect of cellular therapy on the peritoneal cavity. Safety will be assessed by looking for evidence of blood or bowel contents in the ascitic fluid.

We will also fix and analyze the protective layers of major organs such as the aorta, inferior vena cava, liver capsule, the stomach wall, small bowel walls, large bowel walls, the ureters, and kidney capsules as they compared to non- treated mice.

Expected Results: we expect to see differences in T-cell subsets in different GI malignancies. For example, we expect to see more functional T-cells in tumors that have been known to respond to immunotherapy such as gastric cancer, and less functional T-cells in colorectal cancer and pancreatic cancer.

If we can demonstrate that intraperitoneal cellular therapy is safe and efficacious, this could be the basis for a trial evaluating tumor-primed T-cells in patients with peritoneal carcinomatosis from tumors devoid of T-cells.

BUDGET

Program Needs	**Total**
Research technician	$33,125
Cell counter, culture media, reagents, facility equipment, cytometry agents, cell analysis expenses	$39,235
Biostatical costs, computational support, animals (mice) and project specific expenses	$19,000
Travel to scientific meeting	$2,000
Facilities and administration	$20,000
Total	**$113,360**

GRATITUDE

A generous gift of $113,360 will provide resources critical to accelerating Dr. Alana Nguyen's research efforts in metastatic cancer of the peritoneum at Weill Cornell Medicine. Your gift will strengthen and grow our expertise in this disease, which in turn will elevate our ability to provide novel treatments to patients and change the paradigm of care.

We are deeply grateful for your support, and for your consideration of a new gift to advance our cancer research efforts. Thank you for changing medicine with us!

We're Changing Medicine.