Cryotherapy for Osteosarcoma

Osteosarcoma (OSA) is the most frequently diagnosed bone cancer in children and dogs with an incidence of roughly 450 cases per year and 10,000 cases per year, respectively.[1] Despite advances in surgery and chemotherapy, local recurrence or pulmonary metastasis of osteosarcoma occurs in about 30-40% of cases often rendering the disease incurable in both pets and people.[2] The goal of our immunotherapy is to improve both local control and prevent/eliminate metastatic disease by generating a potent adaptive immune response – specifically a T cell response - against tumor antigens. Proper activation of tumor-specific T cells can eradicate tumors as evidenced by recent successes in melanoma, lung cancer, and renal cancer utilizing checkpoint blockade antibodies such as anti-programmed death 1 (PD1) targeted agents nivolumab or pembrolizumab.[7,8] However, anti-PD1 agents are showing very little activity as monotherapy in patients with OSA indicating that more extensive immune modulation is required.[3] Our proposal seeks to utilize advances in MRI-guided cryoablation (a technique that uses freezing temperatures to kill cells) to generate a powerful cancer vaccine providing local irradiation as well as a systemic immune response against osteosarcoma.

Cryoablation is a technique that uses freezing temperatures followed by thawing to kill cells and tissues. In addition to directly killing cells in tumors, there is anecdotal evidence that cryotherapy of a primary tumor can activate the patient's own immune system, i.e., immunotherapy, to eradicate tumor cells far from the site of where the primary tumor was frozen. While CT or ultrasound are frequently used to guide cryoablation of masses, only MRI can monitor the development of the iceball in bone during cryotherapy to accurately determine the extent of tissue damage. We hypothesize that the lack of tumor-specific immune activation in dogs and children with OSA results in the ineffectiveness of immunotherapies successfully used for other solid tumors. Thus, we conjecture that cryoblation can prime the immune system to OSA and improve outcomes of subsequent surgical excision, chemotherapy, and immunotherapy.

While cryotherapy has been used in cancer therapy for over 100 years, technological developments, such as systems that allowed delivery of cooled gases to needles, have enabled the treatment of large tumors deep within the body either during surgery or with minimally invasive image-guided techniques. Cryotherapy has been tested to treat a variety of cancers including breast, prostate, kidney, and lung cancers.[4] Cryoablation results in tumor cell death by mechanisms which include intracellular ice formation, resulting in both necrosis and apoptosis, and microvascular thrombosis.[5] The rapid freezing and thawing of cryotherapy causes the release of cellular contents but often leaves intracellular proteins intact, which can act as pro-inflammatory cytokines and antigen source. Initially, leukocytes infiltrate the tumor and reach a maximal concentration within a few days. Macrophages then begin to accumulate with a peak around one week and remain for two weeks. Preclinical evidence shows that antigens released from the tumor by cryoablation are carried to draining lymph nodes and taken up by dendritic cells.[6] The rapidity of freezing and thawing during cryoablation as well as the number of freeze-thaws cycles alters the relative amounts of apoptosis and necrosis.[7] Our team with Dr. Kraitchman and Dr. Fritz has worked to develop MRI-guided procedures including cryoablation for the palliative treatment of vertebral body bone metastasis and pain management. As the Co-Directors of the Center for Image-Guided Animal Therapy (CIGAT), Drs. Kraitchman and Krimins have performed the first MRI-guided bone biopsy and cryoablation in pets.[8, 9] At present, cryoablation therapy is very limited in veterinary medicine, and our team represents the only veterinary hospital performing this procedure under MRI guidance.

Our approach seeks to generate T cell responses to multiple tumor antigens creating an activated polyclonal immune repertoire capable of eradicating or controlling metastatic spread or local recurrence of OSA. Because of the complex genetics of osteosarcomas[10, 11] , we hypothesize that OSA tumors will contain immunogenic mutations that T cells will recognize. Cryoablative therapy of the primary tumor will result in necrosis and apoptosis and the priming of the immune system to these antigens.

Immunotherapy:

The immune system is designed to detect and attack foreign agents that are harmful to the body to help prevent infections and protect it from cancer and other diseases. However, cancer can evade an immune response by disguising itself as normal cells or by producing signals that suppress an immune response.[a] When a tumor initially forms, the body's immune system recruits and activates a host of immune cells to fight the invading tumor. However, in cases where cancer progresses, the cancer cells send out signals that promote tumor growth and suppress an immune response. Immunotherapy is designed to override the tumor defense mechanisms. In the current clinical trial, we will be using a Stimulator of Interferon Genes (STING) pathway activator derived synthetically which is called a STING agonist. The STING agonist is a small molecule that when injected directly into a tumor can stimulate an immune response against the components of the tumor. The process is expected to use the tumor itself as a "vaccine,"[12] enabling the induction of a tumor-specific immune response that is unique to each dog with osteosarcoma. The STING agonist was developed for human patients with soft tissue tumors and is currently in clinical trials in people. This STING agonist has been shown to cause an immune response in normal dogs similar to people. As osteosarcoma has very little native immune response in dogs, we hypothesis that giving STING agonist directly in the tumor will stimulate an immune response against OSA whether the cells are in the main tumor or in cells that may have spread to other parts of the body.

Combination Cryoablation/STING agonist:

Some dogs will receive both cryoablation and STING agonist. While either therapy may kill tumor cells directly or upregulate the dog's own immune system against the cancer cells, we hypothesize that the combined treatment with cryoablation followed by two doses of STING agonist may be more synergistic and effective. Thus, some dogs will receive both therapies. All dogs in this study will receive free treatment with cryoablation, STING agonist, or both cryoablation and STING agonist.

Cryoablation Procedure:

Typically in veterinary practice, dogs with metastatic disease (based on chest radiographs) would receive no further treatment and have a poor prognosis. Standard of care (SOC) for dogs without metastatic disease is amputation or limb sparing surgical excision followed by chemotherapy.[6]

We are recruiting dogs with OSA for MRI-guided biopsy and cryoablation and/or STING agonist treatment. A chest CT scan will be obtained to assess the presence of macroscopic metastatic disease. We anticipate that CT will detect metastatic disease in some of the recruited dogs. Immediately prior to cryotherapy or STING agonist treatment, peripheral blood and an MR-guided

[a] National Cancer Institute. Website accessed February 25, 2016: http://www.cancer.gov/research/areas/treatment/immunotherapy-using-immune-system

or CT-guided tumor biopsy will be performed to obtain research specimens to measure the native T cell response prior to treatment. MR-guided cryoablation will be performed on a 1.5T MRI scanner (Espree, Siemens) equipped with an MR-compatible cryoablation system (Seednet, Galil Medical) using two freeze-thaw cycles with the goal of freezing to the extent of the MR-visible tumor margins using a protocol developed by Drs. Kraitchman for dogs. Dogs receiving STING agonist alone or in combination with cryotherapy will received one dose of STING agonist in the tumor at the time of biopsy. A second dose of STING agonist will be given approximately one week later. All treatments will be performed under general anesthesia or sedation. We anticipate that some swelling may occur after either treatment. STING agonist my cause some redness and increased warmth as well.

Dogs will undergo SOC amputation (or limb sparing surgical excision) at two weeks post-cryoablation followed by chemotherapy. Fresh tumor tissue and peripheral blood will be obtained at amputation (or limb sparing procedure) for standard pathological analysis and both peripheral blood and tumor tissue saved for immune response analysis. Serial MR and CT imaging and blood sampling will be performed at intervals every 3 months after cryoablation. Dr. Kraitchman will perform quantitative image analysis of tumor burden based on MR and CT imaging. More frequent reimaging will be performed on dogs with clinical signs of progression at the referring veterinarian. Necropsies will be performed on all dogs that die at any time after treatment with assessment of gross and histological presence of metastatic disease. Survival data will also be collected for all treated dogs.

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