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**A Pilot Study To Test Whether Systemic Interferon Gamma Increases Tumor
Class I MHC Expression in Patients with Synovial Sarcoma and Myxoid/
Round Cell Liposarcoma**

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1. INTRODUCTION

This protocol tests whether systemic interferon gamma (IFN γ) will increase class I MHC expression in patients with advanced myxoid/round cell liposarcoma (MRCL) and synovial sarcoma (SS). These patients historically have a very poor prognosis with a median survival of less than one year [1]. Our group has been developing T cell therapy for these patients, however a major barrier is the low MHC expression of these tumors. Identification of a systemic agent that up regulates MHC in these tumors is critical for successful development of T cell therapy. IFN γ increases MHC expression *in vitro* in these tumors and has been observed to increase MHC *in vivo* in melanoma tumors.

Patient taking part in this study will have had tumor biopsy samples taken before entering the study and tissue will be removed after IFN γ administration to determine whether there has been a change in class I MHC expression. In order to obtain post treatment tumor samples, this trial has been designed to enroll patients with metastatic disease undergoing resection, however, certain patients undergoing resection of primary tumors and patients who have disease easily accessible for a core needle biopsy at bedside may also be eligible.

IFN γ is an FDA approved drug with an established safety profile that has been well studied in cancer patients with multiple studies dating back to the early 1980's. However, it is not currently considered standard of care for any cancer type.

1.1 Introduction to the Prevalence, Biology and Natural History of Synovial Sarcoma and MRCL

Soft-tissue sarcomas cause approximately 3900 deaths annually including a disproportionate number of deaths in children, adolescents and young adults. The median survival for patients with advanced soft tissue sarcoma is 8 to 12 months and only 20 to 25% of patients are alive at 2 years [2].

This protocol is focused on 2 soft tissue sarcoma subtypes: synovial sarcoma (SS) and myxoid/ round cell liposarcoma (MRCL). SS has an incidence close to 800 cases annually and represents about 8% of soft tissue sarcomas. It has peak incidence in the 3rd decade of life [3, 4]. SS is by definition associated with a characteristic t(X;18) translocation resulting in one of the SYT-SSX fusion proteins [5]. Liposarcomas account for approximately 10-20% of soft tissue sarcomas and can be classified into three subtypes each with their own distinct clinical behaviors: pleomorphic, well/de-differentiated and myxoid/round cell liposarcoma (MRCL). MRCL accounts for 40-50% of liposarcomas and is almost always associated with a chromosomal translocation, most commonly t(12;16)(q13;p11) though a number of less common translocations have also been described [6]. The resultant fusion proteins have an activity that is not well understood [7].

While chemotherapy sensitive sarcoma subtypes including MRCL and SS may respond to first line chemotherapy in a minority of cases (typically Adriamycin and Ifosfamide), in almost all patients, the disease will ultimately progress. Pazopanib is a small molecule tyrosine kinase inhibitor with potent anti-VEGF activity that has been approved for patients with SS and other metastatic soft tissue sarcomas generally (with the exception of liposarcomas such as MRCL). In a phase III clinical trial pazopanib improved progression free survival benefit (PFS) of sarcoma patients by approximately 3 months but

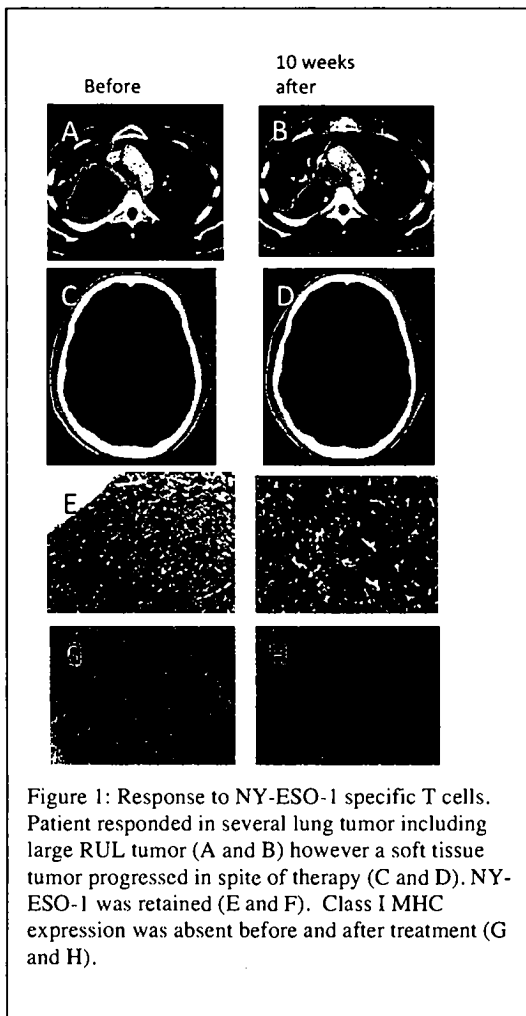
did not demonstrate a statistically significant overall survival benefit (OS). There are no other FDA approved agents for patients with metastatic soft tissue sarcomas [8]. Trabectedin is a drug that has shown promise for liposarcomas and a national phase III trial is currently evaluating its benefit (NCT00210665). However, responses are limited in duration and mortality remains high, suggesting the need for novel approaches [9].

1.2 Expression of NY-ESO-1 in Synovial Sarcoma and MRCL

NY-ESO-1, a member of the family of Cancer Testis Antigens, was first discovered through serological analysis in esophageal cancer patients and was subsequently found to induce a strong cytotoxic T-cell response [10, 11]. As their name implies, CT Antigens, are expressed on a protein level in various malignant tumors and germ cells of the testis but not other adult tissues.

Based on its immunogenicity, NY-ESO-1 is considered to be among the most attractive targets for immunotherapy. It has been targeted in a number of clinical studies including several vaccine trials that have induced serologic, CD4+ and CD8+ T cell responses. Delayed type hypersensitivity responses following NY-ESO-1 vaccination have been associated with long-term survival [12, 13]. Objective clinical responses have been observed in melanoma patients following vaccination against NY-ESO-1, including one complete response [14]. T cell therapies targeting NY-ESO-1 have induced both partial and complete responses [15, 16].

A vast majority of synovial sarcomas have been described as expressing NY-ESO-1. In an analysis from Jungbluth et al., 20 of 25 (80%) synovial sarcoma tumors were found to express NY-ESO-1 by IHC. Four of the five tumors not expressing NY-ESO-1 had the biphasic synovial sarcoma subtype suggesting an even higher proportion of NY-ESO-1 expression among the monophasic subtype. Eight of the 20 NY-ESO-1 expressing tumors had homogenous expression [17]. More recently, we have found that MRCL also has a strong prevalence of NY-ESO-1 expression [18]. We tested 25 MRCL tumors and found that all expressed NY-ESO-1 (100%) and that expression was homogenous over 70% of cases. [16].



1.3

1.3 Experience Treating Synovial Sarcoma and MRCL with NY-ESO-1 Specific Effectors

Robbins et al. reported on the clinical outcomes of 6 synovial sarcoma patients [16]. Four of the patients had partial responses however none of the responses lasted longer than 1 year. One of the responding patients was treated with a second infusion after progression following a partial response, when the two responses were combined together the total length of response was 18 months. An intensive regimen was used including pre infusion high dose cyclophosphamide (CY) and fludarabine along with high dose IL-2 post infusion.

Our group has used a strategy of isolating NY-ESO-1 specific T cells from the peripheral blood of SS and MRCL patients. Our first patient treated with antigen specific T cell therapy had significant anti-tumor responses (Figure 1 A and B) however the response was short lived and not all tumors responded despite persistence of NY-ESO-1 specific T cells (Figure 1 C and D). Sampling of a progressing tumor demonstrated homogenous strong NY-ESO-1 expression (E and F) but absent class I MHC (G and H) suggesting that this is a potential mechanism of immune evasion for these tumors.

1.4 MHC expression in sarcoma tumors

We have found that MRCL tumors have decreased expression of both Class I and Class II MHC molecules. Over 50% of tested patients (13/25) had MHC class I expression that was either undetectable or only focally positive (see Appendix A). Another 36% has MHC class I expression that was only "1+" positive meaning that it was in less than 25% of cells examined. None of the tumors examined had over 50% of cells express MHC class I. MHC II data is similarly decreased. We have also looked for MHC expression in SS tumors. On a tissue microarray stained for class I MHC including 12 SS, seven tumors tested had absent MHC and the other 5 had very weak or focal class I MHC expression. Each of the 4 SS patients who have enrolled on our T cell therapy trial have had their tumors tested for class I MHC and all have been negative.

1.5 Increasing MHC expression in SS and MRCL cell lines using low dose IFN γ

We have now tested 2 MRCL cell lines and 3 SS cell lines with IFN γ *in vitro* and seen up-regulation of class I MHC in each cell line tested. In all but one of the cell lines, marked increases in class II MHC were also observed. One of the SS cell lines, established in our lab from a potential T cell therapy patient, expressed the HLA type A0201. We were able to increase NY-ESO-1 specific killing by treating this cell line with IFN γ at a physiologic concentration 100 U/mL (Figure 2) readily achievable *in vivo* [19, 20].

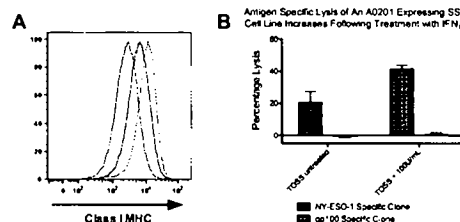


Figure 2: Class I MHC in an A0201+ cell line established in our lab increases from baseline (red) following treatment with IFN γ 100 U/mL (blue). Expression is further increased at the 1000U/mL concentration (green) although this concentration is probably supra physiologic. NY-ESO-1 specific T cell killing is also increased following treatment at the 100U/mL concentration (B). Gp100 (not expressed by this cell line) specific T cells are used as a control for non-antigen specific tumor lysis.

1.6 Increased MHC expression in melanoma tumors using low dose IFN γ

A number of agents have been observed to increase MHC expression *in vitro* in tumor cell lines. For example, both IFN alpha and gamma have both been shown to increase MHC class I and class II molecules *in vitro* in multiple cancer types in numerous studies [21]. We have also seen increased MHC expression in SS and MRCL cell lines following IFN alpha. However, we are not aware of any clinical data demonstrating that systemic administration of IFN alpha increases either class I and II MHC molecules for patients receiving typical doses.

By contrast, IFN γ has been shown to up regulate MHC molecules at low doses (0.1mg/m² once weekly) [22] in as little as 1 week in melanoma tumors. MHC evaluation was based on the presence of positive cells in fine needle aspirates of accessible lesions. Two patients in this study had negative MHC class I expression in their tumor and both developed MHC I expression following therapy. For one patient, class I expression was induced after only 1 week of therapy however for the other patient it took 3 months. In 6 of 14 patients starting without class II expression, MHC class II was induced. To be sure, melanoma and soft tissue sarcoma have different biologies and interact with the immune system in markedly different ways thus the need to test for this effect in SS and MRCL patients specifically.

1.7 Toxicities of IFN γ treatment

IFN γ has been used extensively in clinical trials for patients with melanoma both in the metastatic as well as adjuvant settings. Currently it is FDA approved for patients with chronic granulomatous disease as well as a treatment for severe osteoporosis. Response rates for patients with metastatic melanoma treated with IFN gamma have ranged from 5-15% with including a handful of complete responders [22, 23]. IFN γ has been found to be safe and relatively well tolerated even at doses significantly higher than we are proposing to use in this study.

A large phase I study treated 98 patients with metastatic melanoma at doses up to 0.9 mg/m² administered IV (rather than subcutaneously) three times per week for at least 8 weeks. This study had no fatalities but did see several cases of grade 4 toxicities including anemia, myalgias, renal (proteinuria) and hepatic toxicity at higher doses. There were also 3 cardiac events that occurred in patients on that trial.

A phase III randomized trial was run by SWOG in the early 1990's designed to test the benefit of IFN γ in the adjuvant setting [24]. This trial included 133 patients who completed 1 year of daily IFN gamma and were evaluated for toxicity. There were no fatal complication or grade 4 toxicities, however, 24 patients had grade 3 toxicities including 4 patients with grade 3 myelosuppression, 3 patients with grade 3 neurologic toxicities (confusion, insomnia, personality change) as well as other toxicities including depression, migraine, transaminitis, pruritus, and flu like symptoms. The most common toxicities were fever/chills and headache. It is unclear at what point of the year of therapy patients developed the grade 3 toxicities. Dosing in this trial gave each patient 0.2 mg of IFN γ subcutaneously daily, similar to the 0.1mg/m² considered to be low dose. Although the

authors of the study concluded that single agent IFN gamma had no clinical benefit for melanoma patients in the adjuvant setting, it was safe and generally well tolerated.

The low doses of IFN γ proposed in this study (0.1mg/m² weekly) were examined in 19 patients who received up to six months of therapy. No patients on this study had grade 3 or grade 4 toxicity. The most common toxicity were flu-like symptoms (grade 1). Grade 2 toxicities included fever (2 patients) as well as chills, myalgias, flu-like symptoms and leukopenia (1 patient each). IFN γ has also been used in prior trials safely in the perioperative period both right before surgery [25] and after surgery [26].

1.8 Fatality in a patient receiving NY-ESO-1 specific T cells with IFN γ

The first two SS patient treated at the FHCRC with NY-ESO-1 specific T cells received low dose weekly IFN γ . The first patients results are shown in Figure 1. The second synovial sarcoma patient treated with autologous NY-ESO-1 specific T cells in combination with IFN γ died of sudden cardiac death. This patient also received Cyclophosphamide and IL-2 concurrently with the IFN γ s conditioning. This patient had uncontrolled diabetes with a hemoglobin A1C >10%, mild renal dysfunction at baseline and had recently been started on metformin.

Autopsy ruled out a myocardial infarction or a pulmonary embolism. Analysis of the heart showed cardiomegaly (500 g), as well as selective myocyte necrosis and myocarditis suggesting both a metabolic derangement and myocardial inflammation, which may or may not have been related to each other. The cause of the potential metabolic abnormality is unclear as the only evaluation of his electrolytes was after the patient had been undergoing resuscitation for well over an hour. However, this may be a result of the patient's treatment with metformin and/or his underlying renal insufficiency.

The myocardial infiltrate was overwhelmingly CD68+ and CD4+ cells. Since, the T cell product was >98% CD8+ T cells and NY-ESO-1 was not expressed by the myocardial tissue at autopsy the death was thought most likely related to the patients co-morbidities and conditioning, and unlikely to be directly related to the autologous T cell product itself. Interferon gamma and IL-2 have been removed in order to focus more closely on the safety of the T cell product. 3 patients have now safely been treated with the NY-ESO-1 specific T cells, though it is too soon to determine their clinical out comes. We believe that NY-ESO-1 specific T cells can likely be safely combined with IFN γ but this requires further study. For this reason, patients who have received NY-ESO-1 specific T cells are excluded from this study. If we find in this study that IFN γ does indeed increase MHC expression in SS and MRCL tumors, a follow up study will be specifically designed to address the safety considerations of combining T cell therapy with IFN γ .

1.9 Surgery in patients with metastatic sarcoma

In order to test whether IFN γ increases class I MHC expression, we have chosen to include patients in a number of different clinical scenarios. Primary tumors where there is a recent biopsy specimen available can participate (so long as the patient has not received systemic therapy or radiation since the biopsy was performed). Patients with metastatic disease who have a painful untreated primary tumor they would like remove may participate. Patients with lesions accessible to bed side core needle biopsy will be allowed

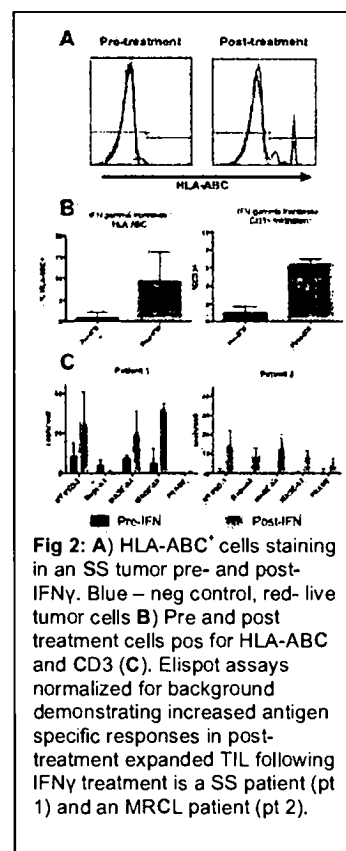
to participate. However, our expectation is that one of the most common situations for patients considering this trial are patients with oligometastatic lung metastasis considering resection.

When metastatic disease is operable, removal is frequently considered the optimal choice. While a randomized control trial comparing a surgical approach to a non-surgical approach would be impossible, retrospective analysis and historical controls seem to suggest a benefit. For example, in a large analysis performed by the MSKCC [27], overall survival of sarcoma patients undergoing resection for metastatic disease was significantly increased relative to a similar historical group of patients (15 vs. 11 months). Isolated cases of patients with long-term disease free survival following metastasectomy have been reported [28]. In patients where complete resection can be achieved, survival at 3 years has been reported above 30%, particularly when the burden of disease is low [29]. Patients with a significant disease free interval or who relapse with only a small volume of disease after an initial metastasectomy may benefit from a second surgery [30]. Radiotherapy is another option for patients with a few isolated sarcoma metastasis [31].

Despite the potential benefits of surgery for some patients with metastatic sarcoma, there are patients who will not benefit. Some patients will rapidly progress despite surgery and undergo a major operation without any clinical benefit from it. Standard practice is to wait and observe the patient either with or without systemic therapy in order to assess the pace of disease before bringing the patient to surgery in order to avoid performing an unnecessary procedure on a patient with rapidly progressing disease [32-34]. It is during this waiting period that we expect to treat the majority of patients on this study.

1.10 Results of the study to date

To date we treated six patients; all evaluable biopsy specimens showed dramatically increased MHC expression; by flow cytometry, HLA-ABC expression rose from a mean of 3.3% to 17.0% (**Fig. 2A and B**). Markedly increased CD3+ cell infiltration was also observed (**Fig. 2B**). In order to interrogate the specificity of these T cells, TIL were expanded from each 1-2 mm core samples and using an ELISPOT assay consisting of overlapping 15 amino acid peptides (**Fig. 2C**) from the most immunogenic regions of cancer testis antigens (including NY-ESO-1), we found that post-treatment T cells had improved recognition of these antigens. Thus, a single inflammatory cytokine can dramatically alter each component of the TME state. We are very pleased that based on our results a Cancer Immunotherapy Trials Network Study is being planned combining IFN γ with pembrolizumab. However, even though we believe that the study has met its primary end point and demonstrated increased MHC expression following IFN γ treatment in these sarcoma tumors, we are seeking to treat up to four additional patients in order to have better data regarding our secondary objectives, specifically to study the PD-L1+ macrophages, which we have observed increased in the tumors of three of the patients treated on the study to date.



2. OBJECTIVES

2.1 Primary Objectives

- To determine whether systemic administration of IFN γ will increase class I MHC expression in SS and MRCL tumors.

2.2 Secondary Objective

- To determine whether systemic administration of IFN γ will increase class II MHC expression in SS and MRCL tumors.
- To examine changes in the immune response to MRCL and SS by examining changes in the immune infiltrates, antibody response and antigen specific T cell response before and after IFN γ treatment.

3. STUDY DESIGN

This protocol will test whether systemic IFN γ will increase class I MHC expression in patients with advanced myxoid/round cell liposarcoma (MRCL) and synovial sarcoma (SS).

Patients with available prior tissue may use this as their pre-treatment sample. Patients may also have a research biopsy prior to receiving IFN γ . For their post IFN γ sample, patients may either have surgery or biopsy as part of their standard care or have a research biopsy.

Ten patients will receive 4 weeks of low dose weekly IFN γ at a dose of 100 mcg/m². If there are any issues regarding data from any of these patients, additional patients may be enrolled as replacements as the discretion of the PI. Patients will be given a patient log marked with the scheduled dates for IFN γ administration. They will be instructed to record the day and time of IFN γ administration in their logs. Deviations of up to 48 hours in administration of IFN γ will be permitted while on the weekly schedule and up to 24 hours while on the three times weekly schedule. The final tumor sample should be taken 24-48 hours after the last dose of IFN γ and not more than 72 hours.

While not ideal, the length of time that a patient is on IFN γ with either regimen could be extended to up to 8 weeks to accommodate the scheduling changes sometimes necessary in the course of patient care. The patient may also stop IFN γ and restart to accommodate a scheduling delay. Any such change in IFN γ must be approved by the PI.

If there is a patient who would be a good study candidate except that surgery is scheduled (or is being scheduled) too soon for them to receive 4 weekly doses of IFN γ , but they could receive at least 2 doses of IFN γ , they may participate and receive only 2 doses.

While response to IFN γ is not an endpoint of the study and no imaging is planned to evaluate response, should a clinical response to IFN γ be noted through the normal course of clinical care, it is conceivable that a situation would develop where it was determined that continuation of IFN γ will clinically benefit the patient (e.g. if the patient's tumor appears to be responding). In this case, IFN γ may be continued at the discretion of the PI and the treating physician.

For research biopsies, ideally 4-6 cores will be collected. Unless otherwise specified by the PI, one will be placed in formalin for IHC. The other samples should be placed in

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RPMI for analysis by the Pollack Lab. Biopsies may be done in the clinic at the SCCA if deemed safe and appropriate by the treating clinician. Alternatively, biopsies may be done under ultrasound guided at the University of Washington.

Data from the study including patient outcomes and biomarker related information learned from analysis of patient samples may be shared with Horizon Pharma who will now be providing drug for the study.

4. PATIENT ELIGIBILITY

4.1 Inclusion Criteria

- 4.1.1** A diagnosis of synovial sarcoma and myxoid/ round cell liposarcoma
- 4.1.2** Male or female subject, 18 or older
- 4.1.3** A superficial tumor easily and safely accessible for a research biopsy or are being considered for resection or biopsy of their tumor as part of standard of care and have recent pathology.
- 4.1.4** Zubrod performance status of '0-2' or Karnofsky score >60%.
(Appendix A)
- 4.1.5** No treatment with systemic anti-cancer treatment (chemotherapy or biologics) within 2 weeks of starting interferon gamma.
- 4.1.6** Patients with a history of coronary artery disease must have had a normal stress test within 180 days of starting IFN γ .
- 4.1.7** Must have been off metformin for at least 2 weeks prior to starting IFN γ .
- 4.1.8** No use of full dose, therapeutic anti-coagulation. However, low dose warfarin for catheter prophylaxis or acetylsalicylic acid are acceptable.
- 4.1.9** No thrombotic event within 6 months (deep vein thrombosis, pulmonary embolism) of starting IFN γ .

4.2 Exclusion Criteria

- 4.2.1 Active infection requiring oral or intravenous antibiotics
- 4.2.2 Pregnant women, nursing mothers, men or women of reproductive ability who are unwilling to use effective contraception or abstinence. Women of childbearing potential must have a negative pregnancy test within two weeks prior to entry.
- 4.2.3 Serum creatinine > 1.5 mg/dL or Glomerular Filtration Rate <50.
- 4.2.4 Significant hepatic dysfunction (SGOT > 150 IU or > 3x upper limit of normal; bilirubin > 1.6 mg/dL; prothrombin time > 1.5 x control).
- 4.2.5 Known CNS metastasis. Once CNS metastasis have been treated these patients may participate if they are otherwise good trial candidates.
- 4.2.6 Current treatment with steroids (must be discontinued 1 week before starting IFN γ).
- 4.2.7 Hemoglobin A1C >8.5%.
- 4.2.8 Uncontrolled hypertension, BP >150/100mmHg. Patients with elevated BP may enroll once BP is corrected.
- 4.2.9 NY-ESO-1 specific T cell therapy within 1 year of starting on the trial.
- 4.2.10 New (<6 months) cardiac arrhythmia (EKG should be performed within 2 weeks of starting IFN γ).
- 4.2.11 History of clinically significant congestive heart failure.

5. STUDY AGENT

Low Dose Weekly IFN γ

Interferon gamma 1b (Actimmune) is FDA approved for the treatment of Chronic Granulomatous Disease and Osteopetrosis. Although it has been extensively studied for the treatment of cancer, it is not FDA approved for the treatment of cancer and is not considered a standard treatment for patients with sarcoma.

IFN γ will be given by sub-cutaneous administration at a dose of 0.1mg/m² on one of the two schedules described in the study design section prior to surgery. Patients or their caregiver will be instructed on subcutaneous self-administration.

6. INVESTIGATIONAL PLAN

All patients will have available tissue prior to study entry. The first two patients will receive low dose IFN γ (0.1mg/m²) by sub-cutaneous administration at a dose of 0.1mg/m² for four weeks prior to surgery. Injection should be given every 7 days though an allowance of 2 days before or after will be acceptable to accommodate specific circumstance. Any deviation from the recommended schedule of IFN γ dosing must be discussed with the PI. Patients or their caregiver will be instructed on subcutaneous self-administration. The last

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dose of IFN γ will be given 24-48 hours prior to surgery (absolutely not more than 72 hours).

10 patients will be treated on this regimen.

Paraffin embedded tissue will be tested for class I MHC, class II MHC and NY-ESO-1. Immune infiltrates in tumors will be studied using IHC staining for CD3, CD4, CD8, CD57, CD68 and FoxP3. Antibody responses will be examined by Sacha Gnajatic (Tisch Cancer Center). In patients with the HLA types A0201 and A2402 antigen specific T cell responses to NY-ESO-1 will be examined using MHC tetramers in the peripheral blood. In patients with the HLA type A0201, PRAME, MAGE family and CAMEL specific T cell responses will also be examined using MHC tetramers. In patients with large tumors that are resected, immune infiltrates will be examined directly by flow cytometry for markers of T cell memory (CD45RO, CD27, CD28, CCR7, CD62L, CD127) and when possible look for tumor infiltrating antigen specific T cells using MHC tetramers. When enough tumor is available RNA will also be extracted for possible future analysis.

Toxicity will be recorded and graded according to the NCI CTCAE v 4.0 at each visit.

7. MANAGEMENT OF TOXICITIES AND COMPLICATIONS

7.1 Criteria for Discontinuation of Therapy

Toxicity grading will be evaluated according to guidelines in NCI Common Toxicity Criteria version 4.0 [36]. Therapy will be discontinued in the following circumstances:

1. life threatening toxicity that is potentially a result of IFN γ
2. toxicity resulting in hospitalization
3. Grade 3 toxicity
4. Unexpected Grade 2 or greater toxicity
5. Expected Grade 2 toxicity lasting for over 48 hours at Grade 2 or requiring hospitalization with the exception of grade 2 cytopenias which may last >48 hours.

Expected Toxicities:

- i. Flu-like symptoms (headache, muscle ache, joint ache, chills)
- ii. Redness, pain and swelling at injection site
- iii. fatigue
- iv. rash
- v. nausea/emesis
- vi. abdominal pain
- vii. diarrhea
- viii. fever
- ix. cytopenia (anemia, lymphopenia, leukopenia may last >48 hours)

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If expected toxicities progress to grade 3 (including cytopenias), the patient will be discontinued from study.

Patients discontinued from the study will not be considered in any subsequent data analysis other than toxicity data.

7.2 Concomitant Therapy

All medical problems that should occur on the trial should be treated according to the standard of care. Patients are not allowed to have anti-cancer therapy (chemotherapy, radiation, biologics) while on study. If a patient developed any medical issues that require steroids, this will be reported in all analysis of data.

7.3 Premature Discontinuation

Subjects who do not complete the study medication will be considered to have prematurely discontinued the study. The reasons for premature discontinuation (for example, voluntary withdrawal, toxicity, death) must be reported on the case report form. A subject may re-enter the study after premature discontinuation only by approval of the Principal Investigator. If possible, final study evaluations should be completed at the time of discontinuation. Potential reasons for premature discontinuation include:

7.3.1 The development of a life-threatening infection.

7.3.2 Judgment of the principal investigator that the patient is too ill to continue.

7.3.3 Patient noncompliance with study therapy and/or clinic appointments.

7.3.4 Pregnancy.

7.3.5 Voluntary withdrawal; a patient may remove himself/herself from the study at any time without prejudice.

7.3.6 Significant and rapid progression of sarcoma requiring alternative medical or surgical intervention including, but not limited to, the development of CNS metastasis.

7.3.7 Toxicity as per section 7.1.

7.3.8 Termination of the study by the principal investigator, Institutional Review Office or the Food and Drug Administration.

8. SCHEDULE OF EVALUATIONS

8.1 General Toxicity Assessment

Patients will have vital signs taken, a physical exam, a comprehensive chemistry panel and a complete blood count and differential at each planned visit. Visits will occur prior to starting IFN γ , every other week while on IFN γ , and two weeks after surgery. All adverse events \geq grade 2 will be recorded and graded according to the NCI CTCAE v 4.0.

8.2 Assessment of Tumor

Paraffin embedded tumor will be tested for MHC and other studies using IHC (see Section 6 Investigational Plan). If sufficient tumor is available some will be taken for RNA and T cell infiltrates will be expanded from the tumor for analysis. If still more tumor is available tumor cell lines may be attempted to be established and infiltrates may be analyzed by flow cytometry directly at the direction of the PI.

8.3 Schedule of Evaluations

History and Physical Exam: Before starting IFN γ , 24-48 hours after first IFN γ dose, every 2 weeks while on IFN γ , within 48 hours prior to surgery (if not already part of the every other weekly evaluations), 2 weeks following surgery.

CBC with differential and platelet count, CMP, Hepatic panel, and research labs – With each history and physical exam

Tumor tissue – to be obtained at time of surgery or biopsy

Research labs will typically include 40mL in green top (heparinized) tubes and 10 mL in red top (non-heparinized) tubes and will typically be drawn only at each history and physical exam. However, up to 60 mL in green top tubes and up to 20 mL in red top tubes may be drawn as often as every week if there is a scientific rationale to sample more blood for an individual patient at the discretion of the PI.

9. REPORTING ADVERSE EVENTS

All unexpected and serious adverse events that may be due to study treatment or intervention must be reported to the FHCRC Institutional Review Office as soon as possible but within at least 10 calendar days of the investigator learning of the event.

9.1 Definition of an Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment.

1. Life-threatening Adverse Event

Any adverse event that places the patient, in view of the investigator, at immediate risk of death from the reaction.

2. Unexpected Adverse Event

An unexpected adverse event is an adverse event that is not described in the study protocol or informed consent.

3. Serious Adverse Event (SAE)

An SAE is any adverse event occurring that results in any of the following outcomes:

- death,
- a life-threatening adverse event (real risk of dying),
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity,
- a congenital anomaly,
- requires intervention to prevent permanent impairment of damage.

9.2 Attribution

- Related – includes adverse events that are definitely, probably, or possibly related to the medical treatment or procedure.
- Not Related – includes adverse events are doubtfully related or clearly not related to the medical treatment or procedure.

9.3 Procedure for Reporting Serious Adverse Events

The FHCRC Serious Adverse Event (SAE) Report Form will be completed for all serious adverse events (unexpected which may be related to study treatment) that meet the expedited reporting requirements. The SAE form will be faxed to the IRO at (206) 667-6831. All available information should be submitted but it is acceptable to fax an incomplete report form at the initial report. A completed report should be faxed as soon as possible but must be received within 10 calendar days.

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Unexpected serious adverse events that do not meet the requirement for expedited reporting must be reported to the IRB as part of the annual renewal of the protocol.

Serious Adverse Events will also be reported to Horizon Pharma, who will now be supplying Interferon Gamma for the study.

9.4 Evaluation and Reporting of Adverse Events:

Patients are monitored for the development of end organ damage by assessing adverse events with serum chemistries, liver function studies, complete blood counts, and physical exams performed accordingly as explained in **Section 8.3**.

We will report any adverse events related to IFN γ that are unexpected (see expected toxicities in 7.1), serious (grade 2 or higher) or result in the discontinuation of therapy. See **Section 7.1** regarding criterion for discontinuation of therapy. IFN γ is an approved drug that has been used in hundreds of cancer patients and has an established record of expected toxicities. All adverse events for all systems are graded on a scale of 1-5 and attribution is assigned, using the NCI Common Terminology Criteria for Adverse Events (version 4).

10. STATISTICAL CONSIDERATIONS

To date, 6 patients were treated with IFN γ at a dose of 100mcg/m², five of whom had adequate biopsy collection for MHC evaluation. All had a marked increase in MHC. However, we have also observed increased tumor infiltration with PD-L1+, CD11B+ cells. We are now increasing the planned enrollment of the study to 10 patients. For these additional four patients, it would be highly relevant to observe marked increase in these macrophages (effect size >2.5). Four patients gives us over 90% power to detect such a large increase with a two-tailed alpha of 0.05. These additional patients will also allow for more detailed visual illustration of CD3+ infiltration and MHC expression in anticipation of an up-coming follow-up study.

11. ADMINISTRATIVE CONSIDERATIONS

11.1 Institutional Review Board

In accordance with federal regulations (21 CFR 312.66), an Institutional Review Board (IRB) that complies with regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

11.2 Consent

The Principal Investigator or his associate must explain verbally and in writing the nature, duration, and purpose of the study and possible consequences of treatment. Patients must also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. In accordance with federal regulations (21

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CFR 312), all patients must sign the IRB-approved consent form in the presence of a witness.

11.3 Termination of Study

The Principal Investigators reserve the right to terminate this study at any time. The FDA may also terminate the study.

APPENDIX A

ECOG / Zubrod Performance Status

- | | |
|---|--|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours |
| 3 | Capable of only limited self-care, confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair |
| 5 | Dead |

Karnofsky Performance Status

- | | |
|-----|--|
| 100 | Normal no complaints; no evidence of disease. |
| 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| 80 | Normal activity with effort; some signs or symptoms of disease. |
| 70 | Cares for self; unable to carry on normal activity or to do active work. |
| 60 | Requires occasional assistance, but is able to care for most of his personal needs |
| 50 | Requires considerable assistance and frequent medical care. |
| 40 | Disabled; requires special care and assistance. |
| 30 | Severely disabled; hospital admission is indicated although death not imminent. |
| 20 | Very sick; hospital admission necessary; active supportive treatment necessary. |
| 10 | Moribund; fatal processes progressing rapidly. |
| 0 | Dead |

APPENDIX B

DATA AND SAFETY MONITORING PLAN

The study will comply with the requirements of the FHCRC Data and Safety Monitoring Plan (DSMP).

REPORTING ADVERSE EVENTS

Definitions:

Adverse Event: Any harm or untoward medical occurrence in a research participant administered a medical product, medical treatment or procedure even if it does not necessarily have a causal relationship with the product, treatment, or procedure. An adverse event can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medical product, medical treatment or procedure whether or not it is considered to be related.

- An adverse event is “unexpected” when its specificity and severity are not accurately reflected in the informed consent document.
- An adverse event is “related to the research procedures” if in the opinion of the principal investigator, it was more likely than not caused by the research procedures or if it is more likely that not that the event affects the rights and welfare of current participants).

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction.

Serious Adverse Event (SAE) – Any adverse event occurring that results in any of the following outcomes:

- death
- a life-threatening adverse event (real risk of dying)
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly
- requires intervention to prevent permanent impairment of damage

Expedited Reporting Requirements to the IRB

All unexpected *and* serious adverse events, which may be due to study treatment or intervention, *must* be reported to the FHCRC Institutional Review Office per their current reporting requirements.

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