Amendment

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Protocol Title: A PILOT STUDY OF TUMOR VACCINATION AND/OR RHIL-7 FOLLOWING STANDARD MULTIMODALITY THERAPY IN PATIENTS WITH HIGH RISK PEDIATRIC SOLID TUMORS

SIGNATURES

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* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: Expedited

DEC Clearance Date: 12/18/2015

Protocol Version Date: 12/09/2015

Date of IRB Submission
PRMC Protocol #: P6941
CC Protocol #: 07-C-0206 N
Revision Date: 12/9/2015

A PILOT STUDY OF TUMOR VACCINATION AND/OR R-HIL-7 FOLLOWING STANDARD MULTIMODALITY THERAPY IN PATIENTS WITH HIGH RISK PEDIATRIC SOLID TUMORS

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Non-NIH Associate Investigators: Crystal L. Mackall, M.D., Stanford Cancer Institute, Stanford, CA*

**no direct patient care responsibilities*

INVESTIGATIONAL AGENTS:

Drug Name:	Tumor Purged/CD25 Depleted Lymphocytes	Tumor lysate/KLH pulsed dendritic cell vaccine	IL-4	KLH	Miltenyi CliniMACS® System	Mab 8H9	Endotoxin
IND Number:	13366 Withdrawn	13366 Withdrawn	13366 Withdrawn	13366 Withdrawn	13366 Withdrawn	13366 Withdrawn	13366 Withdrawn
Sponsor:	Crystal L. Mackall, MD	Crystal L. Mackall, MD	Crystal L. Mackall, MD	Commercially available	Crystal L. Mackall, MD	Crystal L. Mackall, MD	Crystal L. Mackall, MD

Drug Name:	CYT107 (rhIL-7)
IND Number:	13366 Withdrawn
Sponsor:	Crystal L. Mackall, MD

Pharmaceutical Partner: Cytheris Inc., Rockville, MD, USA
Therese Croughs, MD, Chief Medical Officer

Non-NIH Associate Investigator Roles:
Crystal L. Mackall, M.D. (Stanford Cancer Institute, Stanford, CA) is a former NIH employee who was previously the PI of this study before leaving NIH. She remains on the study as an outside investigator to assist with data analysis.

PRECIS

Background:

- Patients with recurrent or metastatic pediatric solid tumors experience low survival rates, but using current standard therapies, many patients with these diseases are rendered into a state of minimal residual disease associated with lymphopenia.
- Lymphopenic hosts show augmented immune reactivity, which may be favorable for inducing antitumor immune responses.
- Cohort 1 completed treatment on this study and received a multicomponent regimen comprising immune reconstitution plus tumor vaccine. Patients with metastatic Ewing sarcoma treated with this regimen had favorable clinical outcomes compared to historical controls. RhIL7 administered upon completion of therapy may be capable of providing immune reconstitution without the complexity associated with the multicomponent regimen.

Objectives:

- Cohort 1: To determine whether ˜CD25 and 8H9 depleted autologous lymphocytes plus tumor lysate/KLH pulsed dendritic cell vaccines ± r-hIL7 (CYT107) can induce immune responses to tumor lysate in this patient population rendered lymphopenic by cytotoxic therapy. (Completed and no longer enrolling with Amendment L)
- Cohort 1: To assess the safety of administering lymphocytes depleted of CD4+CD25+ suppressor T cells ± r-hIL7 (CYT107) to lymphopenic hosts. (Completed and no longer enrolling with Amendment L)
- Cohort 2: To estimate and informally compare the overall survival between patients enrolled on this cohort and comparable patients with metastatic Ewing sarcoma from cohort 1, and secondarily to compare immune reconstitution parameters between this cohort and those with metastatic Ewing sarcoma treated on Cohort 1.

Eligibility:

- Cohort 1: Patients with metastatic or recurrent pediatric solid tumors of the following histologies: Ewing's sarcoma family of tumors, rhabdomyosarcoma, neuroblastoma, synovial cell sarcoma, desmoplastic small round cell tumor, undifferentiated sarcoma, embryonal sarcoma. (Completed and no longer enrolling with Amendment L)
- Cohort 1: Patients must have sufficient accessible tumor for biopsy to generate tumor lysate. (Completed and no longer enrolling with Amendment L)
- Cohort 1: Patients must meet eligibility criteria upon enrollment and upon completion of standard therapy prior to administration of immunotherapy as significant time will have elapsed between the time points. (Completed and no longer enrolling with Amendment L)
- Cohort 2: Patients with metastatic Ewing sarcoma who have achieved "no evidence of disease" from upfront therapy and are enrolled within 8 weeks of completion of upfront therapy.

Design:

- Cohort 1: Immunotherapy consists of one autologous lymphocyte infusion depleted of CD25+ suppressive T cells and depleted of contaminating tumor cells plus 6 sequential tumor lysate/KLH pulsed dendritic cell vaccines. No cytokine is administered on Arm A and r-hIL7 (CYT107) is administered on Arm B. (Completed and no longer enrolling with Amendment L)
- Cohort 1: Patients will be evaluated for immune responses to tumor lysates using ex vivo assays and DTH. (Completed and no longer enrolling with Amendment L)
- Cohort 1: The trial uses a one-stage design targeting a response rate of 50%. Up to 47 patients will be treated. (Completed and no longer enrolling with Amendment L)
- Cohort 1: Stopping rules will take effect if excessive toxicity is observed. (Completed and no longer enrolling with Amendment L)
- Cohort 2: Patients will receive rhIL7 20 mcg/kg/dose SQ every 2 weeks x 4 and be monitored for clinical and immunology endpoints. Up to 15 patients will be treated.

STUDY SCHEMA

COHORT 1 (Completed and no longer enrolling with Amendment L)**: CLINICAL PRESENTATION: NEWLY DIAGNOSED METASTATIC OR LATE RECURRENCE OF ELIGIBLE PEDIATRIC SOLID TUMORS**

- *Apheresis*
  - *PBMC elutriated into lymphocyte and monocyte fractions*
  - *Lymphocytes 8H9 and CD25 purged, then cryopreserved*
  - *Monocytes cryopreserved in 6 aliquots*
- *Tumor biopsy or surgery, then cryopreservation of tumor lysate aliquots*

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STANDARD THERAPY

- *"Best standard therapy" dictated and administered by patient's referring oncology team.*
- *Immunotherapy initiated upon hematopoietic recovery (ANC > 750, platelets > 50,000) from the last cycle of "standard therapy" when acute side effects of standard therapy have resolved and if adequate performance status.*

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IMMUNOTHERAPY

- *Baseline: apheresis/flow cytometry for immune endpoint baseline*
- *Day 0: CYT107 20 mcg/kg/dose SQ (approx 48h prior to vaccine #1), DTH responses for immune endpoint monitoring (baseline skin tests)*
- *Day 2: Tumor lysate/KLH pulsed dendritic cell vaccine #1*
- *Day 2: Infuse 8H9/CD25 depleted autologous lymphocyte infusion*
- *Day 14 (± 7 days): CYT107 20 mcg/kg/dose SQ (approx 48h prior to vaccine #2)*
- *Day 16 (± 7 days): Tumor lysate/KLH pulsed dendritic cell vaccine #2*
- *Day 28 (± 7 days)1: CYT107 20 mcg/kg/dose SQ (approx 48h prior to vaccine #3)*
- *Day 30 (± 7 days): Tumor lysate/KLH pulsed dendritic cell vaccine #3*
- *Day 42 (± 7 days): CYT107 20 mcg/kg/dose SQ (approx 48h prior to vaccine #4), apheresis/flow cytometry/ DTH responses for immune endpoint monitoring (skin tests); and radiographic studies for clinical restaging*
- *Day 44, 56, 70 (± 7 days)Tumor lysate/KLH pulsed dendritic cell vaccine #4, 5, 6*
- *Day 84, 126 (± 7 days): apheresis/flow cytometry/DTH responses for immune endpoint monitoring (skin tests)*
- *Day 126 (± 7 days): radiographic studies for clinical restaging*

COHORT 2:

ENROLLMENT/ELIGIBILITY

- *Metastatic Ewing sarcoma following standard frontline therapy dictated by referring institution.*
- *Patients who have received subsequent salvage regimens due to progressive, unresponsive or recurrent disease are **not** eligible.*
- *Patients must be in a state of "no evidence of disease" at the time of enrollment as determined using standard imaging and clinical studies.*
- *Patient must enroll within 8 week of completing standard frontline therapy.*
- *Patients must have recovered from acute toxicities of standard frontline therapy (ANC > 750, platelets > 50,000) and have adequate performance status.*

IMMUNOTHERAPY

- *Baseline: apheresis/flow cytometry*
- *Day 0 (within 7 days of enrollment): CYT107 #1, 20 mcg/kg/dose SQ*
- *Day 14 (± 7 days): CYT107 #2, 20 mcg/kg/dose SQ*
- *Day 28 (± 7 days): CYT107, #3, 20 mcg/kg/dose SQ*
- *Day 42 (± 7 days) apheresis, flow cytometry and radiographic studies for clinical restaging, then CYT107 #4, 20 mcg/kg/dose SQ*
- *Day 84(± 7 days): apheresis, flow cytometry*
- *Day 126 (± 14 days): apheresis/flow cytometry and radiographic studies for clinical restaging*

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary

• Cohort 1: To determine whether immune responses to tumor lysates can be induced in lymphopenic patients with metastatic or recurrent pediatric solid tumors by combining autologous lymphocyte infusions depleted of regulatory T cells (Tregs) with tumor lysate/keyhole limpet hemocyanin (lysate/KLH) pulsed dendritic cell (DC) vaccination ± r-hIL-7. (Completed and no longer enrolling with Amendment L)

• Cohort 1: To assess the feasibility of obtaining sufficient tumor samples for tumor lysate pulsed dendritic cell vaccination in patients with metastatic or recurrent ESFT, rhabdomyosarcoma and neuroblastoma. (Completed and no longer enrolling with Amendment L)

• Cohort 1: To assess the toxicity of combining 8H9/αCD25 purged autologous lymphocyte infusions (ALI) with lysate/KLH pulsed DC vaccination ± r-hIL-7 in lymphopenic hosts. (Completed and no longer enrolling with Amendment L)

• Cohort 2: To estimate and informally compare the overall survival between patients enrolled on this cohort and those with metastatic Ewing sarcoma treated on Cohort 1.

1.1.2 Secondary

• Cohort 1: To identify immunogenic tumor antigens in pediatric solid tumors by monitoring immune responses to candidate genes following tumor lysate pulsed dendritic cell vaccination Completed and no longer enrolling with Amendment L)

• Cohort 1: To evaluate immune responses to a dendritic cell lysate/KLH vaccine ± concomitant r-hIL-7 (CYT107) therapy (Completed and no longer enrolling with Amendment L)

• Cohort 1: To determine whether 8H9 based purging can render lymphocyte products free of contaminating tumor cells as measured by RT-PCR for patients with rhabdomyosarcoma and ESFT. (Completed and no longer enrolling with Amendment L)

• Cohort 1: To determine the event free and overall survival rate for patients with metastatic or recurrent pediatric sarcomas or neuroblastoma administered 8H9/αCD25 purged ALI with tumor lysate/KLH pulsed dendritic cell vaccination following standard multimodality therapy(Completed and no longer enrolling with Amendment L)

• Cohort 1: To determine whether lymphopenic patients who receive 8H9/αCD25+ depleted ALI demonstrate diminished reconstitution of CD4+CD25+ Treg cells compared to historical controls. (Completed and no longer enrolling with Amendment L)

• Cohort 1: To acquire paired tumor specimens and lymphocytes from patients with pediatric solid tumors for studies of tumor: host immunobiology. (Completed and no longer enrolling with Amendment L)

• Cohort 2: To compare immune reconstitution patterns in patients treated with a multicomponent regimen as per Cohort 1 and those treated with rhIL7 alone (Cohort 2).

1.2 BACKGROUND AND RATIONALE

1.2.1 Treatment and Prognosis of Metastatic Pediatric Sarcomas and Neuroblastoma

Multiagent chemotherapy has dramatically improved survival for ESFT and rhabdomyosarcoma, but outcome for patients with metastatic and recurrent disease remains poor. Current standard regimens cure 60-70% of patients who present without clinically apparent metastatic disease but <20% of those with clinically apparent metastatic disease at presentation are cured[1-3]. Despite poor overall survival, >90% of patients with localized or metastatic ESFT and rhabdomyosarcoma experience complete or very good partial responses to standard chemotherapy, therefore even patients with incurable metastatic disease are often rendered free of visible disease using standard multiagent chemotherapy and radiation therapy and/or surgery. For patients with recurrent disease, those with early recurrence (within 1 yr. following completion of chemotherapy) typically have chemoresistant disease, whereas those with recurrences > 1 year after completing standard chemotherapy often show responses to re-treatment with standard, frontline agents. Some second-line therapies have also shown activity for these diseases, but the "salvage" regimens developed thus far for ESFT and rhabdomyosarcoma have not resulted in substantial cure rates[4,5]. The clinical outcomes for patients with high risk or recurrent neuroblastoma in many ways parallel the results seen in patients with rhabdomyosarcoma and ESFT. Neuroblastoma is a chemosensitive tumor and most patients with high-risk disease can be rendered into a state of minimal tumor burden, however the overall survival rate for patients with Stage IV neuroblastoma (>1 yr. of age) is <40%. Similarly, patients who have recurrent disease following standard frontline therapy also have dismal outcomes[6,7]. Therefore, clinical outcomes for patients with metastatic or recurrent ESFT, rhabdomyosarcoma, and neuroblastoma remain unsatisfactory and new treatment strategies are needed. Similarly, while synovial cell sarcoma, undifferentiated or embryonal sarcoma, and desmoplastic small round cell tumor are considered among the group that are deemed "chemosensitive", outcomes are poor for patients with metastatic or recurrent disease[8-10]. Despite low overall survival rates, many patients sustain a very good partial or complete response to standard multimodality therapy comprising multiagent chemotherapy and radiation therapy and/or surgery. This protocol will seek to add immunotherapy to standard therapy following completion of frontline therapy for patients with primary metastatic disease and following best salvage therapy for patients with recurrent disease. Primary endpoints will be biologic as most patients will not have evaluable disease. This protocol will build upon the information learned in our previous trial wherein immunotherapy was administered following completion of intensive chemotherapy in patients with pediatric sarcomas.

1.2.2 Pediatric Sarcomas and Neuroblastoma as Candidate Diseases for Immunotherapy

A primary goal of the Immunology Section of the Pediatric Oncology Branch is to develop immunotherapy that can consolidate clinical remissions induced by standard therapy for pediatric cancer. This approach is promising for several reasons. First, resistance to one chemotherapy agent often confers resistance to multiple agents (e.g. multidrug resistance), but evidence thus far has suggested that resistance to chemotherapy does not confer resistance to immune effector mechanisms. This can be inferred from the fact that several malignancies that are highly sensitive to immune based therapies (e.g. chronic myelogenous leukemia, renal cell carcinoma and malignant melanoma) are chemoresistant.

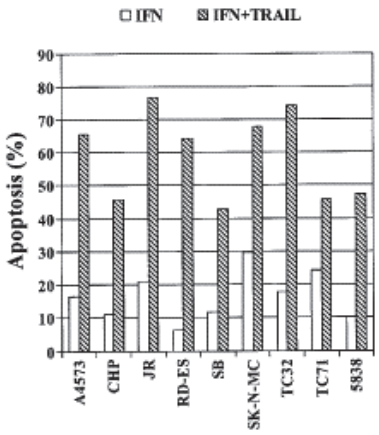


Figure 1. *Sensitivity of ESFT cell lines to apoptosis by TRAIL plus IFNgamma. Cells were preincubated with 2000 U/ml rIFNg for 60h, then treated with 100 ng/ml TRAIL for 24 hr. Equal killing is seen in chemoresistant lines (JR and SB) compared to the rest of the lines which are chemosensitive. (Adapted from Kontny et al., Cell Death and Differentiation, May 2001)*

Furthermore, we have demonstrated *in vitro* that chemoresistant ESFT cell lines are equally susceptible to lysis via the death receptor agonist TRAIL compared to chemosensitive ESFT cell lines (*figure 1*). TRAIL is one mechanism by which immune cells can kill tumors, thus demonstrating that resistance to chemotherapy induced cell death does not necessary imply resistance to immune mediated cell death. Thus, tumor cells that survive front line chemotherapy theoretically remain susceptible to immunologic based killing[11]. Second, immunotherapy has been well tolerated thus far, and side effects that are anticipated following immunotherapy are not likely to be overlapping with chronic or cumulative toxicity induced by cytotoxic chemotherapy[12-15]. Therefore, it is likely that immunotherapy can be administered to heavily pre-treated patients with pediatric sarcomas and neuroblastoma without undue toxicity. Third, one of the major side effects of the standard therapies administered for the diseases targeted in this study is profound and long lasting lymphopenia[16,17]. While at face value this may be expected to diminish the host's capacity to generate antitumor immune reactivity, emerging science has demonstrated that changes in immune physiology induced by lymphopenia can be exploited to amplify the effects immune based therapy[18] (discussed further below). This protocol will seek to exploit the physiologic response to lymphopenia as a means to amplify immune responses to tumor associated antigens.

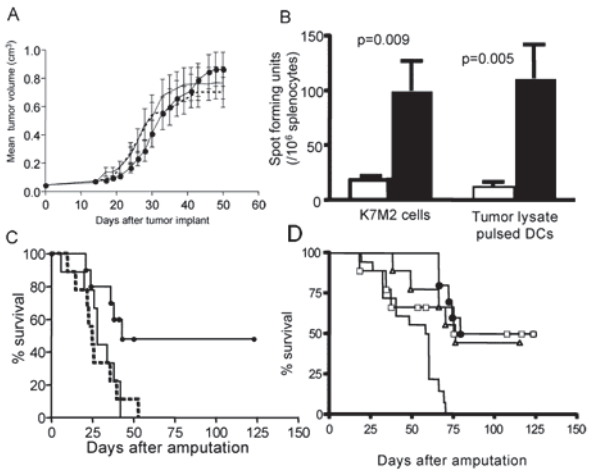
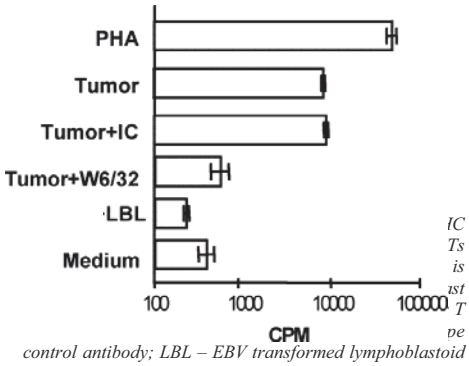


***Figure 2**. Panel A: Mean tumor volume of primary osteosarcomas developing following K7M2 fragment implantation in normal Balb/c mice (solid line with filled circles, n=10), SCID mice (plain line, n=10) and SCIDBg mice (dashed line, n=10). Panel B: Tumor bearing mice show immune responses to K7M2 tumor cells and tumor lysate pulsed DCs. Responders (filled bars) were splenocytes from n=5 Balb/c mice with bulky (>0.8-1.2 cm^3) primary tumors or from n=6 naïve Balb/c mice (open bars). Mean IFNγ spot forming units (SFUs) measured by Elispot in response to irradiated K7M2 cell (10 Gy) stimulators (R:S = 2.5:1) and syngeneic marrow derived dendritic cells incubated with tumor lysate (R:S = 10:1). Panel C: Immunodeficient mice have an increased metastatic death rate. Shown is survival of Balb/c mice, SCID mice and SCIDBg mice following amputation of implanted tumors. All non-surviving animals showed evidence for metastatic osteosarcoma at necropsy whereas surviving animals were euthanized at Day 125 and showed no evidence for metastatic disease. Significant differences exist between Balb/c vs. SCID (p=0.007) and Balb/c vs. SCIDBg (p=0.002). Panel D: Significant differences in survival are observed between control SCID mice vs. SCID receiving LN early (p=0.001), and between control SCID mice vs. those receiving 25x10^6 LN cells IV on the day following amputation (LN late, p=0.01).*

Fourth, data from a variety of studies have demonstrated that even though patients with cancer demonstrate a failure of endogenous immunity to control tumor growth, animals and patients with cancers of multiple different histologies show evidence for immune priming and immune responsiveness, which persists in the face of progressive tumor growth[19-23]. Thus, even when primary tumors evade immune responses, therapies that could augment low level residual immune reactivity directed toward tumor antigens may prove beneficial in the setting of minimal residual disease. This concept is illustrated by results obtained in our laboratory using a murine osteosarcoma model[24]. As show in *figure 2A*, primary tumor implants grow equally well in the presence or absence of an intact immune system, illustrating the capacity for primary tumors to evade an incipient immune response. Despite progressive tumor growth, animals show immune priming to the tumor (*figure 2B*). However, when evaluating animals rendered into a state of minimal residual disease via amputation, mice with normal immune systems show significantly improved outcomes compared to mice with lymphocyte depletion (*figure 2C)*. Further, when syngeneic T cells are administered to lymphopenic animals in the setting of minimal residual disease, recurrent metastatic disease can be prevented (*figure 2D*). Therefore, weak immune responses that are unable to control primary tumor growth can potentially control minimal residual and/or metastatic disease.



IC Ts is ist T pe control antibody; LBL – EBV transformed lymphoblastoid cell line. (Zhang, Cancer Biology and Therapy 2003)

In studies undertaken using lymphocytes collected from patients with progressive ESFTs, we demonstrated that circulating T cells recognize autologous tumor, undergo MHC restricted proliferation and mediate robust tumor lysis[25] (*figure 3*). Similarly, patients with neuroblastoma demonstrate evidence for neuroblastoma directed immune reactivity, which is largely directed toward the anti-apoptotic molecule survivin[26]. Therefore both patients with ESFT and neuroblastoma appear to have some level of immune responsiveness at the

time of clinical presentation, despite the presence of progressively growing tumor. Rhabdomyosarcoma, synovial sarcoma, desmoplastic small round cell tumor and embryonal sarcomas have not yet been studied for the ability to prime immune responses, however Geiger et al. reported a complete response in fibrosarcoma to dendritic cell based immune therapy[27] providing further evidence that the general principles identified in other tumor histologies can reasonably be hypothesized to pertain to other pediatric solid tumors as well. This trial will seek to harness and amplify immune reactivity, which is likely to be present at diagnosis, through the administration of autologous lymphocyte infusions in the setting of minimal residual disease. Further, by obtaining paired lymphocyte and tumor specimens as is a secondary objective for this trial, we will also attempt to extend studies investigating immune responses for patients with pediatric solid tumors.

1.2.3 Lymphopenia and Immunotherapy

Following lymphocyte depletion, T cells can be regenerated via one of two primary pathways: thymic-dependent regeneration and thymic-independent homeostatic peripheral expansion. The thymic-dependent pathway represents a recapitulation of ontogeny, resulting in the generation of naïve T cells with diverse T cell receptor specificities. Because of age-, therapy- and disease -associated changes in the thymus of patients with pediatric cancer, thymic-dependent pathways of T cell regeneration are typically delayed and incomplete [28]. We have demonstrated this directly for patients treated with cytoxan based regimens for ESFT and rhabdomyosarcoma[29]. Because therapies for neuroblastoma include high dose cytoxan and high dose alkylator administered as part of autologous transplantation, both of which induces thymic damage[30], even young neuroblastoma patients enrolled on this trial are predicted to demonstrate profound lymphopenia at the completion of therapy and sustain thymic insufficiency for at least 6 months following completion of primary therapy[31,32]. Therefore, thymic-independent T cell regeneration is likely to dominate T cell regeneration during the period when immunotherapy is administered in this trial. We hypothesize that the first several months post-chemotherapy represents a window of opportunity for the use of immunotherapy in these diseases, since the tumor burden is at its lowest point and the lymphopenia induced alterations in immune physiology gives rise to a process of T cell regeneration termed "homeostatic peripheral expansion" which can potentially be exploited in the context of immunotherapy.

Human and animal studies have demonstrated multiple qualitative changes in T cells in hosts who undergo immune reconstitution via homeostatic peripheral expansion. First, there is widespread lymphocyte activation, accompanied by increasing cycling of peripheral T cell populations. Murine models demonstrate that homeostatic peripheral expansion comprises cytokine driven expansion of memory populations and the combination of antigen and cytokine driven expansion of naive populations[33]. We have demonstrated that lymphopenia results in elevated levels of the homeostatic cytokine IL-7, which serves to induce peripheral T cell cycling and lowers the threshold for T cell receptor activation[34] (*figure 4*). There are also qualitative changes in the antigens

that induce immune responses during lymphopenia. Whereas self-antigens are typically ignored in normal healthy host as a result of self-tolerance, low avidity self-antigens become proliferative stimuli during lymphopenia[33]. Therefore, cancer patients rendered lymphopenic by cytotoxic chemotherapy show augmented peripheral T cell cycling that is driven by a combination of cytokine, cognate antigen and self antigen. This altered immune reactivity is predicted to result in augmented immune reactivity toward weak antigens, which antitumor immune responses represent. Consistent with this, Dummer et al. demonstrated that lymphopenic mice show improved responses to tumor antigens compared to T cell replete mice[35], Dudley et al. demonstrated that adoptive cell therapy for malignant melanoma is more effective in lymphopenic compared to T cell replete humans[15], and as discussed above (*figure 2d*), we have observed that murine sarcomas are prevented from metastasizing if T cells are supplied to lymphopenic hosts for homeostatic peripheral expansion.

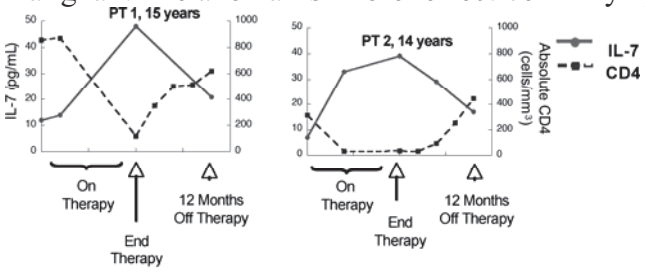


Figure 4:Inverse relationship between circulating IL-7 and CD4 counts following chemotherapy for childhood cancer. As CD4 counts recover following completion of therapy, IL-7 levels fall to baseline.

Using murine models, we have undertaken extensive studies to delineate the requirements for successful induction of immune responses during homeostatic peripheral expansion. Among critical factors is the accessibility of adequate lymphocyte inocula, which can serve as a nidus from which homeostatic expansion can proceed. In murine models, approximately 10% of the total T cell repertoire was sufficient to render hosts fully immunocompetent to respond to both CD4+ and CD8+ restricted antigens[36]. Our murine modeling has also demonstrated that optimal antigen presentation is critical for inducing immune responses during lymphopenia[37]. The strict APC requirements in lymphopenic hosts can be illustrated by that fact that B cells, which represent "semi-professional" APCS are adequate to prime responses to weak antigens in T cell replete mice, but induce tolerance when they present antigens to lymphopenic mice (*figure 5*). Similarly, bone marrow derived macrophages are immunostimulatory in normal mice but induce antigen specific tolerance in lymphopenic hosts. On the other hand, fully mature IL12 producing dendritic cells induce robust immune activation in lymphopenic mice. Therefore, while lymphopenic hosts demonstrate augmented immune reactivity, they have more stringent APC requirements than normal hosts, with a potential for augmented tolerance induction if suboptimal APCs are used (*Fry et al, manuscript in preparation*). Therefore, in this trial, we will attempt to combine a lymphocyte inocula which approaches 10% of the T cell repertoire with a tumor vaccine (described in more detail below) which utilizes activated

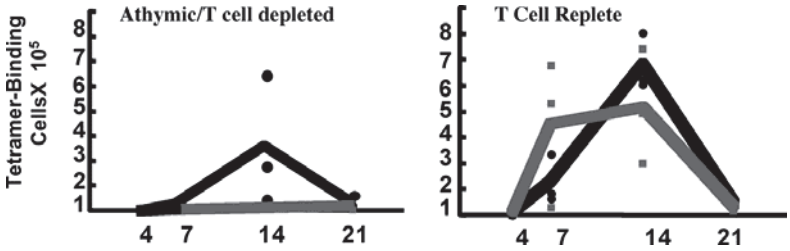


Figure 5: *HY Reactive T cells are clonally deleted following immunization with male spleen in athymic TCD mice but are expanded following immunization with male spleen in T cell replete mice. Grey data points from mice immunized with male spleen, black from mice immunized with male DCs.*

dendritic cells which produce IL-12, and are therefore predicted to be potent inducers of T cell immune responses.

In summary, patients treated with cytotoxic chemotherapy for ESFT, alveolar rhabdomyosarcoma and neuroblastoma universally sustain T cell depletion. While at face value this may limit overall immunocompetence, it can also provide a starting point for specific tumor directed immunotherapy which may be more effective when administered in this clinical setting. This protocol will seek to administer autologous lymphocyte infusions and tumor lysate based dendritic cell vaccines to patients rendered lymphopenic by chemotherapy for pediatric solid tumors. R-hIL-7 will be incorporated to enhance the rate of immune reconstitution in this populations and to increase the responsiveness to dendritic cell vaccinations.

1.2.4 Findings from 97-C-0052 which have Informed the Development of This Trial

97-C-0052 was a clinical trial of immunotherapy in patients with alveolar rhabdomyosarcoma and ESFT that was recently completed in the POB. This trial was similar to the study proposed here in that it involved the administration of autologous lymphocyte infusions and serial tumor vaccines to patients following completion of chemotherapy. The experience with this study provided the following pieces of information that have informed the development of this trial. First, 97-C-0052 confirmed that it is feasible to accrue patients to a trial following the initial diagnosis of metastatic disease or following a late recurrence to collect the cells necessary for use in the immunotherapy. This is critical since all of the diseases targeted in this trial are rare and therefore accrual is always a challenge. For potential immunotherapy on 97-C-0052, 60 patients with ESFT or alveolar rhabdomysarcoma came to the NIH Clinical Center and were apheresed over the course of 6 years, for an accrual rate of 10/year. Of these sixty, 29 (48%) received immunotherapy, 8 (13%) were subsequently deemed ineligible because they did not have the targeted translocation, 14 (23%) were not able to receive immunotherapy because of disease progression or death during primary therapy and 9 (15%) declined to return for immunotherapy. Because this trial will enroll based upon histology and will not require a specific chromosomal translocation for enrollment, is can be anticipated that approximately 60% of patients who undergo apheresis will ultimately receive immunotherapy. Furthermore, patients with neuroblastoma will also be eligible for this study and even though accrual for neuroblastoma may not be as brisk at that for ESFT and rhabdomyosarcoma due the absence of a referral base for this disease, we anticipate that it is likely that 3-4 patients/year with neuroblastoma will be referred for this trial. Therefore, we anticipate that we are likely to be able to enroll 13-14 patients/year on this trial with 60% of these ultimately receiving immunotherapy, which is approximately 8 patients/year.

Our experience with 97-C-0052 also demonstrated that patients could be effectively traveled to the NIH for apheresis prior to starting primary chemotherapy without undue delay. This is most relevant for newly diagnosed patients, and on 97-C-0052, of 34 newly diagnosed patients with metastatic ESFT or rhabdomyosarcoma who traveled to the NIH Clinical Center for apheresis and for whom data is available, the median time from diagnosis to initiation of chemotherapy was 10 days (range 1-24 days) and the median time from arrival at NIH to initiation of chemotherapy was 5 days (range 1-10 days). Because both the previous and the current proposed trial will only enroll patients with metastatic

disease who are clinically stable, such a delay is deemed acceptable considering there is no evidence that such a delay ultimately effects outcome and since patients are being enrolled and treated on this trial with the prospect of direct benefit by adding immune based therapy to their standard regimen.

The experience with 97-C-0052 also demonstrated expected T cell yields from apheresis. A 2 blood volume apheresis was used on that trial (e.g. 10L for 70kg patients), and we harvested a median of 7.2 x 10^9 lymphocytes, which calculated to a median of 1.2 x 10^8 lymphocytes/kg. Considering approximately 60% of peripheral blood lymphocytes are T cells, we estimate that we collected a median of 74 x 10^6 T cells/kg. Using the generally accepted estimate of 1 x 10^{11} T cells (approx 1.4 x 10^9/kg) in normal humans, we harvested approximately 5% of the total cell repertoire. Because our murine studies suggest that inocula of somewhere between 1-10% provides maximal immune reconstitution benefit, we will increase the amount of blood apheresed on this trial to 3-4 blood volumes to target a 10% T cell harvest.

On 97-C-0052, using elutriated monocytes from the initial apheresis, we were consistently able to generate sizable numbers of dendritic cells following GM-CSF and IL-4 culture with CD40 ligand maturation. Using CD40 ligand as the sole maturational agent a median of 84% (range 49%-100%) of the dendritic cells expressed CD83. However, because many HLA-A2+ patients did not demonstrate significant immune responses to the E7 peptide which is known to be an avid HLA-A2 binder and because we have recently identified CD83+ dendritic cells which are not immunostimulatory due to a lack of IL12 production in studies in our laboratory[38], we have undertaken preclinical studies aimed at enhancing the immunostimulatory properties of the dendritic cell populations with a focus on the capacity to produce IL-12. Preclinical studies performed in collaboration with the Department of Transfusion Medicine have demonstrated that dendritic cells matured with LPS plus IFNγ are the most optimal producers of IL12 and produce substantially more IL12 than CD40L, alone which was used in protocol 97-C-0052.

Table 1.	Cytokine secretion (pg/ml) Mean ± SEM	
Maturation Agent	IL-12	IL-10
None	10 ± 10	43 ± 12
CD40 Ligand	3 ± 2	94 ± 18
LPS 10 ng/ml/IFN 1000u/ml (n=6 expts.)	4200±2150	1100±270

Furthermore, they show enhanced motility suggesting that they may be better able to traffic to draining lymph nodes than CD40L matured DCs. Finally, LPS matured DCs have recently been administered intranodally in clinical trials. Preliminary evidence has demonstrated immune reactivity to Class I restricted epitopes in 80% and to Class II restricted epitopes in 90% of patients so treated without any evidence for toxicity[39]. Therefore, for this trial we plan to utilize LPS/IFNγ matured GM-CSF/IL-4 generated monocyte derived DCs.

With regard to immune reconstitution, 97-C-0052 confirmed that evidence for thymic-dependent reconstitution does not occur during the first 6 months in patients with ESFT and rhabdomyosarcoma. As noted above, because patients with neuroblastoma also receive dose intensive preparative regimens for autologous transplants, we believe a similar pattern in likely for neuroblastoma patients enrolled on this trial as well, despite their expected young age. 97-C-0052 also demonstrated that IL2 served to enhance CD4+ immune reconstitution but that this occurred through expansion of regulatory T cells[40]. Furthermore, lymphopenia induced homeostatic expansion itself augmented the cycling

and the frequency of these cells (described in detail below). Therefore, based upon the results from 97-C-0052 we propose to alter our DC maturation cocktail to augment IL-12 production by the dendritic cells administered and we will seek to deplete regulatory T cells prior to adoptive transfer of autologous lymphocytes.

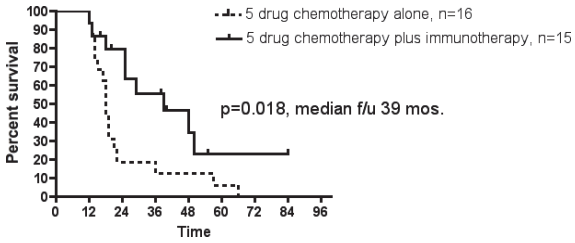


***Figure 6**. Overall survival of patients enrolled on 97-C-0052 with newly diagnosed metastatic ESFT and rhabdomyosarcoma compared to a group of historical controls treated in the POB with the same five drug regimen.*

97-C-0052 also confirmed previous work by ourselves and others that lymphocyte populations harvested from patients with metastatic disease are likely to contain contaminating tumor cells in a fraction of patients. Of the lymphocytes infused in 97-C-0052, 32% were found to be RT-PCR positive for tumor. These results were discussed with the patients prior to infusion and all agreed to receive the products. While it is not clear that such contamination is the primary factor determining relapse in patients treated in this manner, we would like to be able to offer cell populations that have been purged of contaminating tumor to the greatest extent possible. For this reason, this trial will incorporate a new moAb 8H9 based depletion as described below.

With regard to immune responses to the vaccines administered, a total of 29 patients were treated with immunotherapy, but only 25 are evaluable for immune responses to the vaccine since 4 patients had rapid progression following vaccine #1, which prevented return for sample acquisition following the vaccine. A total of five out of 25 patients tested demonstrated response to the fusion protein at presentation although these were generally weak and were seen in only one assay (e.g. cytokine production but not proliferation or cytolysis). Following immunization, immune responses, which met the criteria for positivity, were only seen in nine of 25 patients tested, and these were not robust (typically IFNγ production which was approximately two-fold above background), nor were they observed in more than one assay or sustained over time. Importantly however, all patients demonstrated robust flu responses indicating some level of overall immune competence. For this reason, this new trial will not use peptides to immunize nor will it seek to target the translocation region, which appears unlikely to be immunogenic enough to provide robust immune reactivity. Rather, we will use whole tumor lysate that we anticipate may be able to boost existent immune responses in these patients with pediatric sarcomas. Further, we will use KLH as an immune tracer to monitor responses to a non-tumor antigen and as well for it's immune boosting properties as a helper epitope[41,42]. Furthermore, as described below, by careful evaluation of immune responses in this population using candidate antigens, we hope that this trial will also serve to better define tumor antigens in these diseases.

With regard to clinical outcome, the five year survival of the 30 patients treated on 97-C-0052 is 43% with a median f/u of 7 years from the time of enrollment on the trial. This is favorable for a population of patients with metastatic and recurrent sarcomas. However it should be noted that some patients enrolled on this trial demonstrated an indolent clinical course as they had disease which had recurred > 1 yr. following completion of primary therapy. Therefore, it is possible that the eligibility criteria used could contribute to the favorable survival rate. To more accurately assess patient outcomes

compared to a similar control group, we have compared survival of newly diagnosed patients enrolled on this trial to a historical group treated with the same five-drug regimen. Using a non-intention to treat analysis (e.g. only patient which survived long enough to receive the immunotherapy are included), we observed 6/15 survivors with a median f/u of 39 months. This compares favorably with a historical control group of patients diagnosed with metastatic ESFT and rhabdomyosarcoma treated previously in the POB with the same five drug regimen which patients enrolled on this trial received (*figure 6*). Because immune responses to the peptide pulsed vaccine could not be consistently measured, our current hypothesis is that the favorable clinical outcome could relate to the adoptive T cell transfer which may allow restoration of some endogenous anti-tumor immunity during the early months following completion of chemotherapy. Although this hypothesis remains speculative and confirmation of benefit from ALI would require a randomized clinical trial, the favorable outcome for overall survival that we observed in 97-C-0052 demonstrates that patients treated with adoptive immunotherapy involving transfer of autologous lymphocytes collected prior to therapy are not likely to have inferior outcomes to patients receiving standard therapies for metastatic and recurrent sarcomas.

1.2.5 Expansion of Regulatory T Cells During Lymphopenia and Following IL-2 Therapy

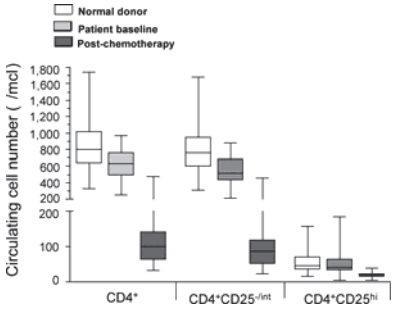


***Figure** 7. Cyclophosphamide induces profound depletion of both CD25-/int and CD25hi subsets with no selectively for CD25hidepletion.*

Cyclophosphamide is administered to essentially all patients with ESFT, high risk rhabdomyosarcoma, and neuroblastoma. This agent efficiently kills resting mature T cells and therefore is profoundly immunosuppressive. Several studies conducted by our group have demonstrated that lymphopenia is reproducibly induced in patients treated with multiagent cyclophosphamide-containing chemotherapy for pediatric sarcomas[16,29]. In the recently completed immunotherapy study (97-C-0052), patients enrolled with metastatic pediatric ESFT or alveolar RMS or with late recurrence of these diseases presented with mean presentation CD4+ counts of 616±44 cells/mcl but had mean CD4+ counts of 125±23 cells/mcl following completion of the "standard therapy" portion of the trial. CD8+ cells were less profoundly depleted. The trial enrolled patients from age 6-40 (median 18 yrs.) treated for pediatric sarcomas, and only the youngest patient enrolled in the trial showed evidence for thymic-dependent T cell regeneration during the period of follow-up and this did not occur until greater than six months following completion of chemotherapy[40].

CD4+ cells can be divided into a major population (approx. 95% of total CD4+ T cells) of non-regulatory cells, which are CD25- or CD25int and small numbers (approx. 5% of the total CD4+ pool in humans) of CD4+CD25hi cells. Within the CD4+CD25hi compartment, there are sizable numbers of regulatory (e.g. suppressor) cells that induce broad, antigen-non-specific immune suppression. Many murine models have demonstrated that CD4+CD25+ regulatory T cells can suppress antitumor immunity[43,44] and studies in human cancer patients have demonstrated that CD4+CD25+ regulatory cells infiltrate human tumors[45], have specificity for human tumor antigens[46] and can suppress antitumor immunity[47]. Interestingly, in growing tumor infiltrating lymphocytes from one

patient with ESFT in our laboratory, the CD4+ cell line generated expressed high levels of CD25 and mediated suppression (unpublished data). Therefore, emerging evidence suggests that CD4+ Tregs may be important inhibitors of effective antitumor immunity.

In patients treated on 97-C-0052, we carefully evaluated changes in CD4+CD25+ regulatory cells during lymphodepletion and immune reconstitution. These studies led to four major conclusions[40]. First, although cyclophosphamide based therapy has been historically described as being capable of selectively "depleting" suppressor T cells, we observed no selectivity in cyclophosphamide's effects on Treg depletion. Rather, patients receiving intensive cyclophosphamide therapy sustained profound depletion of both Treg and non-Treg CD4s (*figure 7*). Therefore, without some means to augment immune reconstitution of cells within the non-regulatory CD4+ repertoire, cyclophosphamide therapy alone simply induces pan-CD4 depletion which severely limits overall immune competence. The second observation was that the process of homeostatic peripheral expansion favors expansion of CD4+CD25+ regulatory T cells. We observed relative increases in the frequency of CD25hi vs. CD25-/int cells in lymphopenic patients compared to normal hosts (*figure 8*), due to augmented cycling of regulatory T cells during lymphopenia *in vivo* (*figure 9*). These results were consistent with previous studies demonstrating that CD4+CD25+ Treg cells show specificity for self-antigens and that self-antigens play a large role in driving homeostatic peripheral expansion. Third, the CD4+CD25+ Treg expansion observed occurred via homeostatic peripheral expansion, rather than via thymic-dependent pathways. Thus, it is logical to assume that high dose cyclophosphamide therapy (which depletes both CD4+ Tregs and non-Tregs) combined with the addback of lymphocytes, which have been depleted of CD4+CD25+ Tregs, will reduce the absolute number and percentage of Tregs generated during immune reconstitution. Fourth, the studies of CD4+ immune reconstitution from patients enrolled on 97-C-0052 demonstrated that IL2 potently expands regulatory T cells *in vivo*.

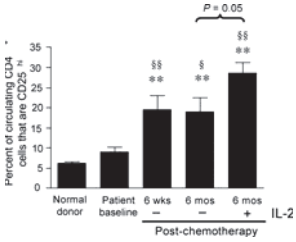


Figure 8. *Patients treated with chemotherapy show increased frequencies of CD25hi Treg cells compared to normal hosts or patients pre-therapy. IL2 further expands the CD4+CD25hi Treg subset.*

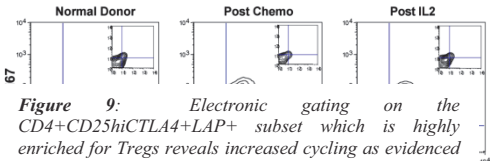


Figure 9*: Electronic gating on the CD4+CD25hiCTLA4+LAP+ subset which is highly enriched for Tregs reveals increased cycling as evidenced by Ki-67 expression post-chemotherapy and post-IL2 therapy consistent with homeostatic peripheral expansion of Tregs.*

Our working hypothesis therefore is that there are antagonistic forces at play during lymphopenia, which have evolved to maintain self-tolerance in this setting. Elevations in available IL7 serve to augment both strong and weak immune responses but there is also a selective survival/expansion of CD4+CD25hi cells, which likely limit autoimmunity in this setting. We postulate that combining lymphopenia-induced elevations in IL7 with an adoptive transfer of CD25 depleted T cells will result in robust expansion of tumor reactive T cell populations in lymphopenic hosts. Indeed, lymphopenic mice administered Treg depleted syngeneic lymphocytes frequently develop organ specific autoimmunity, and the goal of immunotherapy for cancer essentially represents the induction of organ-specific autoimmunity directed toward tumor tissues. Importantly, in murine models, organ specific autoimmunity in animals with lymphopenia who receive Treg depleted lymphocytes results in inflammatory colitis[48]. As yet, no colitis has been observed in studies of adoptive T cell transfer using CD25 depleted lymphocytes in humans[49] and

therefore the risk of humans remains unknown but it is presumed to be low. Patients will be monitored closely for clinical signs or symptoms of colitis, the study will be placed on hold and modifications and/or closure of the study will be discussed with the IRB and FDA.

In summary, in an attempt to augment immune reactivity in patients with high risk pediatric solid tumors, we will adoptively transfer CD25 depleted lymphocytes to lymphopenic patients with pediatric sarcomas and neuroblastoma. For patients who present with substantial lymphopenia (CD4+ cells < 200 cells/mcl) upon completion of standard therapy, added cytotoxic therapy will not be administered; however for patients who receive a regimen which does not result in CD4+ levels < 200 cells/mcl, additional chemotherapy will be administered in an attempt to render the patients lymphopenic prior to initiation of the immunotherapy phase of this trial. The immunotherapy on this trial will consist of both the adoptive transfer of autologous Treg depleted lymphocytes as well as serial tumor vaccines.

The cytotoxic chemotherapy that will be administered to patients who do not experience CD4+ lymphopenia below 200 cells/mcl upon completion of cytoreductive therapy will be comprised of cyclophosphamide 1800 mg/m2/d x2d and fludarabine 25 mg/m2/d x 3d. We have some experience with the combination of cyclophosphamide/fludarabine administered as part of the EPOCH regimen on POB NCI 02-C-0259 to a similar patient population of children and young adults who have completed dose intensive chemotherapy for pediatric sarcomas. The EPOCH regimen is comprised of Fludarabine 25 mg/m2 per day x3; Etoposide 50 mg/m2 per day x4d; Doxorubicin 10 mg/m2 per day x4d; Vincristine 0.4 mg/m2 per day x4d; Cyclophosphamide 750 mg/m2 x1d; Prednisone 60 mg/m2 per day in x5d. In a review of 51 consecutive cycles of fludarabine/cyclophosphamide chemotherapy as part of the EPOCH regimen administered to 24 patients with pediatric sarcomas enrolled on POB NCI 02-C-0259, we primarily observed myelosuppression without undue infectious or bleeding risk. Specifically, we observed 5 episodes of grade IV thrombocytopenia and 5 episodes of grade IV neutropenia and 1 episode each of grade IV: abdominal pain, hypocalcemia, diarrhea, low hemoglobin, myalgia and prolonged PTT. No grade IV infectious or bleeding events occurred. Grade 3 neutropenia was observed in 4 cycles, however only two cases of febrile neutropenia occurred and 2 cases of Grade III infection without neutropenia occurred. There was one episode of Grade 3 hemorrhage related to epistaxis. Therefore, while myelosuppression is expected with this regimen, the degree of myelosuppression observed in a similar patient population given a similar regimen gave rise to toxicities that are commonly seen with the cytotoxic therapy administered to this patient population.

In an attempt to avoid excessive toxicity from this regimen as part of this research study, a stopping rule is in place whereby if at any point during the course of the trial, 2 patients develop unacceptable Grade IV toxicity related to the cyclophosphamide/fludarabine regimen, no further patients will receive this regimen. However, because it is clear that this regimen will induce significant myelosuppression, and is not substantially different from the myelosuppression experienced by this patient population as a result of the intensive chemotherapy that comprises standard therapy for these diseases the following toxicities Grade IV expected toxicities are excluded from this early stopping rule: neutropenia, thrombocytopenia, lymphopenia, leukopenia, anemia and infection with or without neutropenia.

The approach used in this trial to deplete Tregs cells will be an immunomagnetic

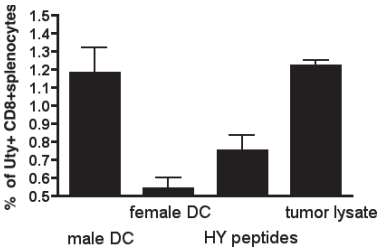


Figure 10. *Animals were immunized with dendritic cells from male mice, female mice unpulsed, female DC pulsed with a cocktail of HY specific peptides and female DC pulsed with tumor lysate from a tumor which expresses HY. Increased responses (cells binding the Uty tetrmaer) to the Uty immunodominant epitope are observed using lysate compared to peptide pulsing.*

bead based depletion procedure pioneered by Miltenyi Corp. to deplete CD25+ cells from autologous lymphocyte infusions. Using studies undertaken by Powell et al. this procedure results in >95% depletion of CD25+ cells and in substantial decreases in foxp3 expression within the CD4+ compartment[50]. While some activated CD4+ and CD8+ cells expressing CD25+ will also be depleted with this method, activated CD25+ cells represent a minority of the peripheral T cell pool and will presumably be replenished by homeostatic peripheral expansion of CD25- CD4+ and CD8+ cells. Therefore, depletion of the small numbers of activated CD25+ cells from the transferred inocula is not predicted to impact host immune competence. In contrast, if the peripheral progenitor pool for CD4+CD25hi cells is depleted from the inocula, which will undergo homeostatic peripheral expansion, we predict that patients will not generate substantial numbers of CD4+CD25+ Tregs. In an attempt to prevent colitis, which has developed in similarly treated animals, we will treat patients with oral metronidazole for 6 weeks starting one week prior to administration of Treg depleted lymphocyte infusion. The incidence of colitis or other autoimmune reactivity will be closely monitored as part of the toxicity endpoints for the trial, and if individual patients develop significant toxicity appropriate clinical intervention and if needed, immunosuppression will be initiated. If substantial numbers of patients enrolled on the trial develop colitis or undue toxicity, the trial will be modified as this will represent a stopping point as discussed in the statistical section.

1.2.6 Tumor Lysate Based Dendritic Cell Vaccines

Clinical studies in tumor immunotherapy continue to evolve rapidly by integrating insights from basic immunology, mouse cancer models and ongoing clinical trials. Many different approaches for delivering tumor vaccines are currently in clinical trials, with most demonstrating reproducible expansions of tumor reactive T cells *in vivo,* and no clearly superior method yet identified[51]. For pediatric solid tumors, one major challenge in developing an effective tumor vaccine is the limited information currently available regarding optimal tumor antigens for vaccine targeting[52]. Current paradigms hold that most tumors are likely to express multiple tumor antigens comprising a) developmental (oncofetal) antigens b) tumor-specific mutations c) universal antigens expressed in dividing tissues and/or d) cancer-testis antigens. For pediatric tumors targeted in this trial, a variety of candidate tumor antigens have been identified using microarrays or in some cases by identifying antitumor reactivity directed toward candidate antigens[53-55], but whether there are "single best targets" and which target antigens can induce responses which lead to tumor regression or prevent tumor recurrence remain unknown. Furthermore, even if a very strong antigen tumor regression antigen were identified, it is likely to dominate immune responses for only one HLA-allele and therefore is not likely to be an applicable target across individuals. Because pediatric tumors are rare diseases, we have reasoned that identification of immunodominant epitopes for individual histologies via a reductionist approach may not be as fruitful as immunizing with a broad array of antigens and allowing immunoreactivity to be naturally individualized, as will

occur if an array of potential antigens are provided via a whole cell vaccine.

Using dendritic cells as delivery vehicles with inherent adjuvant activity, it is feasible to administer tumor vaccines that expose the patient to a broad antigenic pool present in an autologous tumor. In order to determine whether immune responses to a known immunodominant epitope can be generated with equal potency via whole cell vaccination as compared to peptide based vaccination, we compared immune responses in a murine model using a cocktail of immunodominant peptides vs. responses induced to "whole cell" dendritic cells vaccines. Remarkably, using a mouse model of an HY expressing tumor and measuring responses to an immunodominant Class I restricted epitope which has been mapped for the HY antigen, we observed that immunization using any one of a variety of whole cell techniques was equal to, and in many cases superior to, immunizing with dendritic cells which have been pulsed with immunodominant peptides (*figure 10*). Thus, not only can the whole cell vaccination provide for sensitization of both CD4+ and CD8+ cells toward multiple antigens simultaneously in patients with diverse HLA types in tumors wherein immunodominant antigens have not been defined, comparison studies suggest that the magnitude of the immune responses are equal or superior to responses induced using dendritic cells pulsed with peptides (*Fry, manuscript submitted*). Thus, we believe that using a whole cell vaccine is a more promising approach to pursue at the current time for immunizing patients against pediatric solid tumors than immunizing for one specific antigen, which may or may not be immunodominant in a given individual. Importantly, the use of a whole cell approach will also allow us to "mine" for immunodominant antigens by measuring responses to candidate genes in the patient population immunized in this trial. Thus, an important secondary objective of this trial is to use immune responses from patients treated with a whole cell vaccine to provide the much needed information regarding tumor antigens for the tumors targeted in this trial.

Importantly, tumor lysate pulsed DCs have already been used by several investigators in the context of tumor immunotherapy and have been shown to be capable of inducing responses to tumor antigens[14,56,57], including patients with pediatric solid tumors[13]. For instance, using tumor lysate plus KLH pulsed dendritic cell vaccination in a trial for children with recurrent neuroblastoma, sarcoma and renal malignancies (one Wilm's, one renal cell carcinoma), Geiger et al. enrolled 10 patients who completed all three planned vaccines given 14 days apart (five more patients did not complete therapy). Of those who completed all three vaccine, immune responses (as evidenced by delayed type hypersensitivity and/or immune responses ex vivo) to KLH was observed in 7/10 patients and to autologous tumor lysate in 3/7 patients. A partial clinical response was seen in one patient with fibrosarcoma[27] and five patients showed stable disease[13]. There was no significant toxicity reported in this trial. This protocol will hopefully add to these results by using a more mature dendritic cell population (the dendritic cells used in the Geiger trial were GM-CSF plus IL-4 alone without further maturation) and will seek to exploit the altered immune physiology of lymphopenia with adoptive transfer as well.

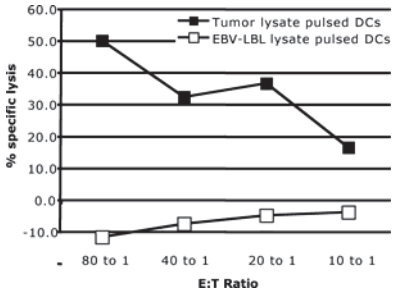


Figure 11. Tumor infiltrating lymphocytes obtained from a patient with ESFT were activated with artificial APCs bearing 4-1BBL and anti-CD3. Expanded cells were tested for lysis against autologous dendritic cells pulsed with autologous tumor lysate vs. lysate from an autologous EBV-LBL line. (Europium assay).

In order to determine whether tumor lysates contained relevant tumor antigens in

ESFT, which is a major focus on this trial, we used an approach developed in our laboratory to generate tumor specific immune effectors and tested whether tumor lysates provided appropriate targets for cell lysis. CD8+ T cells harvested from patients with Ewing's sarcoma, which are activated via αCD3 and 4-1BBL using artificial APCs, lyse autologous and allogeneic ESFT cell lines[25]. The mechanism responsible for this observation appears to be the capacity for the artificial APC to selectively expand memory T cells[58] thus expanding tumor reactive T cells that recognize as yet undefined tumor antigens. In order to test whether from ESFT tumor lysates contain immune recognizable tumor antigens, we tested whether autologous dendritic cells pulsed with ESFT tumor cell lysates could serve as targets for CD3/4-1BB expanded CD8+ T cells, and compared this to lysis observed when autologous dendritic cells were pulsed with lysates from an EBV immortalized B cell line. Note that both the tumor lines and the EBV lines were autologous to the effector T cells under study. As shown in *figure 11* using tumor infiltrating lymphocytes obtained from a patient with ESFT, we observed substantial killing when autologous dendritic cells were pulsed with autologous tumor lysate but not when dendritic cells were pulsed with lysates from EBV-LBL. We observed similar results with 3/3 patients from whom autologous peripheral blood T cells were tested for reactivity against autologous tumor lysate pulsed DCs. Therefore, although the specificity of the antigens, which are responsible for tumor lysate based lysis in ESFT are as yet unknown, this data demonstrates that tumor lysates do contain antigens, which are recognizable by T cells from patients with ESFT.

In summary, three lines of evidence suggest that tumor lysates represent a promising approach for dendritic cell based tumor vaccines in pediatric solid tumors. First, the acquisition of lysates in a standardized fashion is likely to be clinically feasible, as they have been used successfully in several previous clinical trials including a previous trial for patients with pediatric sarcomas wherein immune responses were observed following tumor lysate based dendritic cell vaccination. Second, using a sophisticated animal model of dendritic cell based vaccination where multiple tumor antigens could be tracked, we saw superior antitumor reactivity using tumor lysate compared to peptide-based vaccination. Third, we observe direct evidence that tumor lysates from ESFTs can serve as targets for lytic T cells, thus implying that tumor antigens are present in these products.

The methods used for producing tumor cell lysates will be based upon work done by Nestle et al. in the context of clinical trials[57]. In collaboration with associate investigators on this trial from the Department of Interventional Radiology, patients will undergo core biopsies of the primary or recurrent tumor. In the interventional radiology suite, the cytopathologist will provide an initial microscopic review of the tissue and confirm that tumor is present in what is likely to at least 70% of the specimen. Based upon this preliminary reading, using a standard operating procedure and good manufacturing practices developed by the Department of Transfusion Medicine, tumor tissue will be homogenenized, freeze-thawed 4 times, irradiated to 12000 cGy, centrifuged and the supernatant will be harvested as lysate. The lysate will be assessed for protein content and used at a final concentration of 50 mcg/ml for dendritic cell pulsing, for DTH analysis and for immune response analysis using *in vitro* assays.

One major issue to be addressed in this trial is the feasibility of generating adequate amounts of tumor lysate for vaccinations and if possible, for DTH and *in vitro* response monitoring. It is not known what the minimal dose of dendritic cells are that can reliably

induce vaccine responses however the work by Thurner et al.[59] demonstrated that subcutaneous and intradermal injection appeared to be superior to IV injection with 8/11 patients demonstrating CTL responses to the melanoma peptides used and 6/11 patients showing clinical activity. In that study, 1.5 x 10^6 DC were injected SQ at two sites (total 3 x 10^6/SQ vaccination) and 1 x 10^5 DCs were injected at 10 ID sites (total 1 x 10^6 cells injected ID/vaccination). We believe that the maximum number of injections our patients will tolerate is 6 injections/vaccine and therefore we have attempted to at least the same number of cells as reported by the Thurner study. Briefly, the minimum vaccine cell dose will be vaccines 1 x 10^6 cells/SQ injection x 3 and 1 x 10^5/ID injection x 3 as these doses are very close to that delivered in the Thurner report. The minimum requirement for the lysate to be deemed "adequate" is a sufficient amount for 3 complete immunizations (each immunization comprising 6 injections delivered at approximately 14d intervals). Assuming that 50 mcg/ml of lysate will be incubated in a minimum of 7.5 ml for dendritic cell loading, then a minimum tumor lysate is 350 mcg/vaccine or 1050 mcg for the generation of three vaccines. The target would be 2100 mcg for 6 vaccines, plus 300 mcg for DTH testing and approximately 700 mcg for in vitro studies for a total target of 3.1 mg of lysate, but a minimal acceptable level for feasibility will be 1050 mcg. If insufficient lysate is available for DTH responses, then KLH responses alone will be assessed for DTH, and for *in vitro* responses, KLH alone and allogeneic lysates (with appropriate controls, see Section 5.2.B) will be used. The methods for manufacturing the dendritic cell populations used in this trial have been derived based upon ongoing collaborations with the Department of Transfusion Medicine at the NIH Clinical Center which is focused upon generating efficient, standardized manufacturing processes for generating optimal monocyte derived dendritic cells (discussed above)[60].

In pilot studies done in collaboration with DTM to develop the standard operating procedure for creating lysates, Ewing's sarcoma xenografts, which had reached a size of 1.5 cm in immunodeficient mice, underwent core biopsy for acquisition of tumor lysates. A total of 10 tumors were biopsied with a mean of 5.8 mg of lysate acquired/tumor. Therefore it appears feasible to acquire the target tumor lysate using core biopsies of tumors as planned in this trial. With regard to patients with bone marrow replacement, we also performed a pilot study to assess the yield of lysate from a patient treated at the POB with replacement of marrow by neuroblastoma. Three cc of bone marrow yielded 60 x 10^6 cells which yielded 350 mcg of lysate. Therefore, we would anticipate that 100 cc of bone marrow aspirate would yield approximately 10.5 mg of tumor lysate which would be adequate for 6 vaccines and *in vitro* studies.

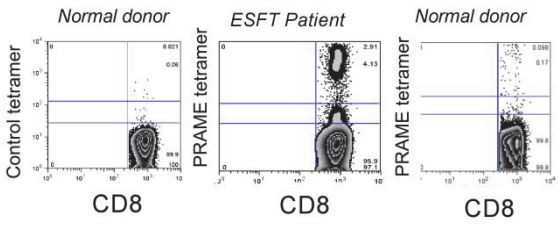


Figure 12. *PBL from an HLA-A2+ patient with ESFT (ESFT) or an HLA-A2+ normal donor were expanded using CD3/4-1BBL and assessed for binding to a tetramer bearing the 425-433 peptide from PRAME. The control tetramer was derived from TAX, and HTLV1 peptide.*

1.2.7 Identification of Tumor Antigens and Measurement of Antitumor Reactivity

A primary goal of this trial is to induce immune responses to tumor antigens present

in pediatric solid tumors. For the purpose of primary protocol endpoints, we will assess responses to KLH and to tumor lysate. Importantly however, this trial will provide an important opportunity to extend our current knowledge of clinically relevant tumor antigens in these diseases. Even if whole cell approaches become the optimal method for vaccination against pediatric solid tumors, molecular tumor antigen identification is important for monitoring immune responses and could eventually result in vaccines that could be produced and standardized across individuals. Using state-of-the-art approaches to monitor for responses to candidate tumor antigens in this trial, we hope to accomplish a secondary goal of "honing in" on antigens expressed by these tumors. The approach to identifying tumor antigens using lymphocytes harvested from patients treated on this trial will involve multiple steps, several of which have already begun in the Mackall laboratory. First, candidate tumor antigens have been identified for these diseases based upon published evidence for expression of tumor antigens found in other disease. For instance, survivin is expressed in neuroblastoma and ESFT, WT-1 is expressed in rhabdomyosarcoma and ESFT; PRAME is expressed in ESFT and neuroblastoma. Furthermore, by microarray analysis of ESFT and rhabdomyosarcoma, we have identified expression of survivin, WT-1, PRAME and hTERT in both of these tumors (Snyder, manuscript in preparation). Each of these genes has been identified as a tumor antigen in another disease and therefore is a candidate tumor antigen for the diseases under study in this trial. From the published list of existent tumor antigens, we have already produced HLA-A2 binding peptides from 6 antigens (NY-ESO-1, PRAME, WT-1, Survivin, hTERT and CYP1) and created tetramers to screen for immune reactivity in patients with ESFT at the time of clinical presentation, following chemotherapy and following immunization, using both non-expanded and anti-CD3/4-1BBL expanded PBL. Pilot studies have demonstrated that HLA-A2+ patients with ESFT show evidence for higher levels of expanded T cells to all six tumor antigens compared to healthy A2+ individuals. For instance, as shown in *figure 12*, anti-CD3/4-1BBL T cell expansion of PBL from a patient with ESFT shows significant expansions of cells that bind the PRAME-specific tetramer whereas minimal numbers are seen in the normal HLA-A2+ donor. Because ESFT patients have thus far demonstrated expanded PRAME reactive repertoires compared to normal donors, we believe the PRAME is likely to be a relevant tumor antigen in this disease. Ongoing studies are underway to determine whether PRAME reactive cells can recognize and lyse tumors and similar results have been seen with the other five tumor associated antigen tested. Using cells collected from patients enrolled on this trial, we will seek to extend these studies to evaluate the responses to candidate antigens following tumor lysate based vaccines in this trial in hopes of defining antigens which are immunodominant in ESFT. We will also seek to extend these studies to encompass epitopes for HLA-A1 and A3 from these same antigens, thus potentially encompassing the vast majority of individuals enrolled on this trial.

We are also in the process of identifying new candidate tumor antigens for these diseases based upon clues obtained from microarray data already available from several large databases generated from associate investigators on this trial. By comparing microarray data to that seen in normal tissues, we select for genes that are relatively overexpressed in one or more of the pediatric sarcomas. We then screen these genes for candidate peptides capable of binding to common HLA-A alleles (HLA-A2, HLA-A3, etc.) using computer algorithms, which are readily available (BIMAS, SINFIKL). Once

candidate peptides are generated, evidence for binding will be sought using a T2 assay, and peptides that bind to specific HLA alleles are then studied for immune reactivity using Elispot and tetramer analysis. Using this approach, we recently evaluated gene expression of 22,000 genes in ESFT, identified five genes of interest based upon a lack of expression in normal tissues, then identified approximately 80 peptides from these five genes which were predicted to bind to HLA-A2 with high affinity and finally identified 8 peptides which bound to T2 in a flow based assay. These peptides are currently being used to prepare tetramers and screening will then be performed in patients with ESFT. Using a similar approach for rhabdomyosarcoma and neuroblastomas as well as extending our analysis to HLA-A1 and A3, we hope to extend our knowledge of tumor antigen expression in pediatric sarcomas based upon PBL responses to tumor lysates vaccination used in this trial. Using a combination of techniques including tetramers, Elispot and intracellular cytokine production and cytolysis assays, this work will serve as an important basis from which we will seek to establish tumor antigens in these tumors so that future immunotherapy trials may use endpoints, which are more standardized and more readily available than measuring immune responses to "tumor lysate".

1.2.8 Risk of Tumor Cell Contamination of Cellular Products

The infusion of autologous lymphocytes harvested prior to or during treatment for cancer carries a theoretical risk for tumor cell contamination of the products. Contamination of bone marrow and peripheral blood populations with tumor cells of various histologies has been observed in a variety of trials, and genetic marking studies have shown that these cells can contribute to tumor relapse[61]. We and others have observed that approximately 30% of products harvested from patients who present with metastatic Ewing's sarcoma are contaminated with tumor as measured by RT-PCR for the tumor specific translocation[62,63]. As mentioned earlier, 32% of lymphocyte products that were infused on protocol 97-C-0052 showed evidence for nucleic acid derived from tumor cells as evidenced by polymerase chain reaction specific for the tumor specific translocation. No correlation could be observed in these small numbers of patients between outcomes and PCR positivity. For instance, of the patients who received products, which were PCR positive, one is a long term survivor without recurrence, one is a long term survivor but had recurrence four years after completing immunotherapy, one had a local recurrence approximately 6 months after completing immunotherapy. Furthermore, while we have tried to grow tumor cell lines from contaminated blood products using selection techniques to enrich for tumor, we have not been successful in growing tumors from the peripheral blood. Therefore, we have no clear evidence that clonogenic tumor is present in the peripheral blood of patients who demonstrate PCR positivity. Furthermore, purging of biologic products of contaminating tumor cells has not been demonstrated to improve survival in autologous BMT trials, most likely because the most common source for tumor recurrence is a reservoir, which remains within these high risk patients. Regardless of our inability to directly demonstrate risk due to tumor contamination of lymphocyte products, in an investigational trial such as this, we believe it is preferable to eradicate contaminating tumor cells to the greatest extent possible prior to infusion. To this end, we have developed a purging strategy for remove pediatric sarcoma cells from hematopoietic and lymphocyte populations using the moAb 8H9[63]. This moAb binds to surface gp96

which is expressed on the vast majority of sarcoma populations (essentially all neuroblastomas, ESFTs and rhabdomyosarcomas, undifferentiated sarcomas, desmoplastic small round cell tumors and 2 of 3 synovial tumor lines tested) and on many epithelial tumors as well[38]. Importantly, it does not bind to the surface of normal cells and is currently being used in an imaging trial at Memorial Sloan Kettering without any evidence for significant toxicity. We have developed a purging technique using 8H9 and using a single antibody in an immunomagnetic bead approach, we can accomplish 3-5 logs of tumor cell depletion[63]. No significant toxicity to normal lymphocytes or to stem cells was observed with this approach. Based upon these preclinical studies, we propose to use 8H9 in this trial to purge contaminating tumor cells from the lymphocyte products. This trial will be the first to use moAb 8H9 as an *ex vivo* purging agent.

Using qRT-PCR, we have developed assays in the laboratory for EWS-Fli1 translocations (type 1 and type 2), EWS-Erg and PAX3-FKHR, which allow detection of one cell in one million cells. In this trial, we will seek to determine whether purging using the Miltenyi based immunomagnetic selection approach, we can render all products from patients with ESFT or alveolar rhabdomyosarcoma negative for tumor contamination using qRT-PCR. Since most products are contaminated at levels that approximate 1:100,000 to 1:1,000,000 cells, and we are not likely to infuse more than 2 x 10^{10} cells on this trial, the maximal numbers of tumor cells which would be infused if 2 x 10^{10} cells RT-PCR- cells were given would be 200 cells. While we cannot prove that this is a safe dose, we do know that the clinical outcomes of patients treated on our previous trial where purging was not performed were favorable and that some patients who did receive contaminated products showed either long term disease free survival or local recurrence, neither of which imply high level contamination with clonogenic cells. Therefore while we will seek to improve the quality of our lymphocyte product by reducing tumor contamination below the limit of detection, we will not require PCR negativity for infusion and this will remain a secondary objective of this trial. The 8H9 depletion will occur in a single step simultaneous to the CD25 depletion. Our laboratory currently has a standardized assay for RT-PCR monitoring and serves as the reference laboratory for the Children's Oncology Group trial, which is investigating whether minimal residual disease correlates with prognosis. The monitoring is not CLIA certified and will not be used to guide clinical decisions. For patients with embryonal rhabdomyosarcoma and neuroblastoma, 8H9 purging will also be performed, but PCR will not be performed as a validated methodology to document purging is not available to our group.

1.2.9 Study Progress: Addition of r-hIL7 to Enhance the Effectiveness of Consolidative Immunotherapy

As of July 2009, a total of 14 patients were enrolled on study and 4 patients completed the immunotherapy portion of this trial, with a 5th patient completing immunotherapy using suboptimal tumor lysate on a single patient exemption. All therapy has been well tolerated with Grade I colitis observed in one patient that was transient and did not require intervention. Adequate tumor for lysate generation was obtained on 13/14 patients therefore the first objective of the trial has been met. With regard to the immune response endpoints, DTH skin testing has shown evidence for immune responses to KLH in 5/5 patients, the control neoantigen incorporated into this study. Biologic response

evaluation has been completed for 3 of 4 patients who have completed the immunotherapy. In all cases, vaccine induced robust KLH specific immune responses have been observed using Elispot analysis of interferon gamma production at weeks 8 and week 14, and in one patient this was sustained at week 20. In patient #1, a positive response to tumor lysate was observed although it was low in quantity and only marginally met the criteria for positivity. In the subsequent 2 patients, no evidence for response to tumor lysate has been observed by DTH or by Elispot. Thus both the DTH and the in vitro analysis of immune response suggest that the immunogenicity of the tumor lysate it less than we would hope.

One approach to enhance immune responses in lymphopenic hosts is pharmacologic administration of r-hIL-7. We have shown in murine models that lymphopenic mice receiving r-hIL-7 show enhanced immune responses leading to skin graft rejection[36]. Moreover, non-lymphopenic mice treated with r-hIL-7 and dendritic cell vaccines show increased responses to both CD4+ and CD8+ immunodominant and subdominant antigens[64]. R-hIL-7 therapy administered with vaccines resulted in enhanced long term immunity and resistance to tumor challenge as well, whereas similar effects were not seen following rhIL-2 or rhIL-15 therapy[64]. Furthermore, we demonstrated that r-hIL7 can diminish the relative frequency of regulatory T cells by preferential expansion of non-Tregs and that this enhances the effectiveness of adoptive immunotherapy in mouse models.

Studies in humans have confirmed that r-hIL-7 can be administered safely to both lymphoreplete and lymphopenic patients and substantially increases CD4+ and CD8+ T cell numbers, primarily through effects on homeostatic peripheral expansion[65,66,68]. This is accompanied by increases in the size of lymphoid tissues demonstrating that the changes observed in the peripheral blood reflect increase in total body lymphocyte mass. In two trials conducted in the NCI Center for Cancer Research, biologic activity of r-hIL7 (CYT 99 007) was seen at repeated doses of 10 mcg/kg/dose and above and repeated doses of 60 mcg/kg/dose were administered without DLT[66]. Grade I/II constitutional symptoms were commonly seen 6-8 hours following injection and Grade I local reactions with erythema, pruritis and induration at the injection sites were observed. Rapidly reversible grade 3 liver enzyme elevation was observed in one patient given 30 mcg/kg and grade 3 chest pain with troponin elevation was observed in one patient given 60 mcg/kg. Recent studies have also shown that r-hIL-7 substantially increases T cell numbers in lymphopenic hosts with HIV infection[67] and it was similarly well tolerated. In this series, biologic activity was seen at single doses of 3 mcg/kgdose and above and dose limiting toxicities of Grade 3 skin induration at the injection site and transient grade liver transaminitis were seen at single doses of 60 mcg/kg/dose. In this study, 30 mcg/kg was designated as the MTD. Interestingly, pharmacokinetic data has demonstrated that IL7 levels are increased inversely related to lymphocyte counts suggesting that lymphopenic patients may have a greater biologic exposure than lymphoreplete patients treated with rhIL7.

Currently, Cytheris is developing CYT107 (glycosylated r-hIL-7) mainly in two types of medical settings:

- In lymphopenic immune-compromised patients, whatever the origin of the lymphopenia, CYT107 is expected to improve and speed up immune reconstitution limiting the risk of infection and in oncology, the risk of tumor relapse.
- In diseases associated with insufficient cellular immune responses to clear the

antigen, such as cancer and chronic viral infections, CYT107 is expected to improve the number and quality of T cells available for the response and partly reverse the tolerance to these antigens.

As of today, six clinical phase I/IIa studies have been initiated with CYT107 and more than 50 patients have been treated with CYT107

All these studies have a phase I or I/IIa dose-escalation design. Their primary endpoint is an evaluation of the safety of biological active doses of CYT107. Dose Limiting Toxicity (DLT) and the Maximal Tolerated Dose (MTD) will be identified during this safety assessment. However, the MTD will not be sought systematically by continuing dose escalation above a dose, which appears to be safe and fully active in pre-clinical studies.

Secondary objectives are to characterize the pharmacokinetics and pharmacodynamics and compare CYT107 effects with equivalent weekly doses of CYT 99 007 by comparing the increase in CD3, CD4, CD8 T cells induced by CYT107 in this trial and CYT 99 007 in previous phase I trials. These studies are also designed to evaluate the impact of IL-7 on the immune system, notably the immune specific response.

The early data gathered in a variety of patients treated with CYT107 confirm the safety and activity results of studies and experience performed with the previous generation of recombinant IL-7, CYT 99 007. The safety profile and preliminary activity measured in the ongoing clinical studies are satisfactory and consistent through all the studies. For further details, see Investigators Brochure.

Based upon preclinical data demonstrating that r-hIL7 can enhance vaccine responses and diminish Treg frequencies and clinical data demonstrating safety and biologic activity of r-hIL7 therapy, we will incorporate r-hIL7 (CYT107) therapy into a second arm for Cohort 1, which will be designated Arm B. Patients on Arm B will receive treatment as per the schema shown on page 3. Because rhIL7 can enhance T cell expansion even in hosts that are not lymphopenic, hosts treated on Arm B will not be required to receive lymphodepleting chemotherapy if their CD4 count is >200 cells/mcl upon completion of standard therapy. R-hIL7 (CYT107) will be administered subcutaneously at a dose of 20 mcg/kg approximately 48 prior to each of the first 4 dendritic cell vaccines. This dose was chosen because it has been shown to be safe in three clinical trials and to have biologic activity. In addition, because we anticipate that most patients treated with r-hIL7 on this study will be lymphopenic at the time of administration, this relatively low dose is predicted to provide reasonable systemic exposure. Biologic endpoint studies will remain the same although patients treated on Cohort 1, Arm A vs. Arm B will be evaluated separately for biologic and clinical outcomes. In addition, pharmacokinetic analyses for CYT107 will be performed during the first dose and following the first dose (prior to, 2, 4, 6, 24 and 48 hours post) and then prior to the second, the third and the fourth dose. Immunogenicity analyses will be performed prior to the first dose, at D35 ± 7 days and D49± 7 days, 7 days after the last dose of the agent. Samples will also be drawn at Day 84 and Day 126 after 3 of 7 patients were positive for neutralizing antibodies (7 of 14 patients were positive for binding antibodies). If the Day 49 sample is positive for neutralizing antibodies, the cryopreserved samples will be analyzed and samples will be drawn at each follow up time point until the results are negative.

1.2.10 Findings from Cohort 1 which have Led to the Addition of Cohort 2

A total of 45 patients were enrolled on Cohort 1 of which 30 patients initiated immunotherapy. The other 15 were unable to receive immunotherapy due to insufficient collections (n=4), disease progression/death (n=7), patient choice (n=3) or toxicity of primary therapy (n=1). Of the 30 patients who received immunotherapy, 5 were treated on Cohort 1, Arm A and received autologous lymphocyte infusions, plus dendritic cell vaccination and 25 patients on Arm B, and received autologous lymphocyte infusions, dendritic cell vaccination plus rhIL7.

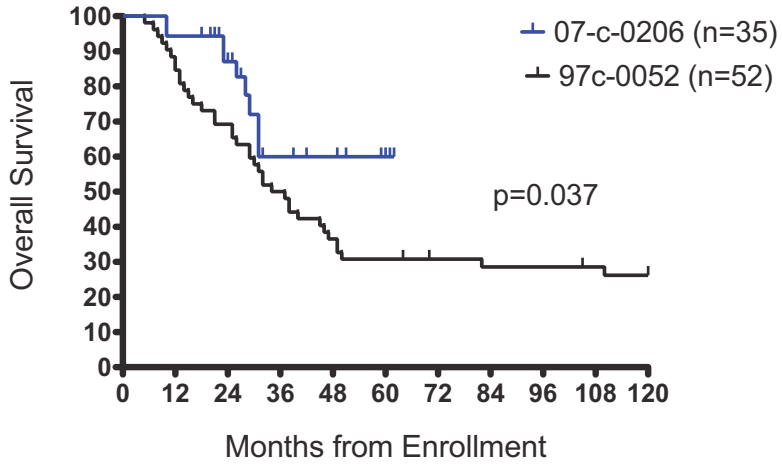
Results for Primary Objectives for Cohort 1 are summarized below:
Objective 1: Immune responses to tumor lysates were observed in 65% of patients overall. In 35% of patients, the response could be attributed to the vaccine since it was not preexisting prior to vaccination. There was a suggestion that the absolute value of vaccine responding cells is increased by rhIL7, and an increase in the frequency of responding patients with rhIL7 (20% response rate in Cohort 1, Arm A and 38% response rate in Cohort 1, Arm B) but this was not statistically significant. Given that there were only 5 patients in the non-IL7 group, we believe the study was underpowered to answer this endpoint.

Objective 2: Sufficient tumor was harvested to generate lysate in 41 of 44 patients, therefore it was judged to be feasible.

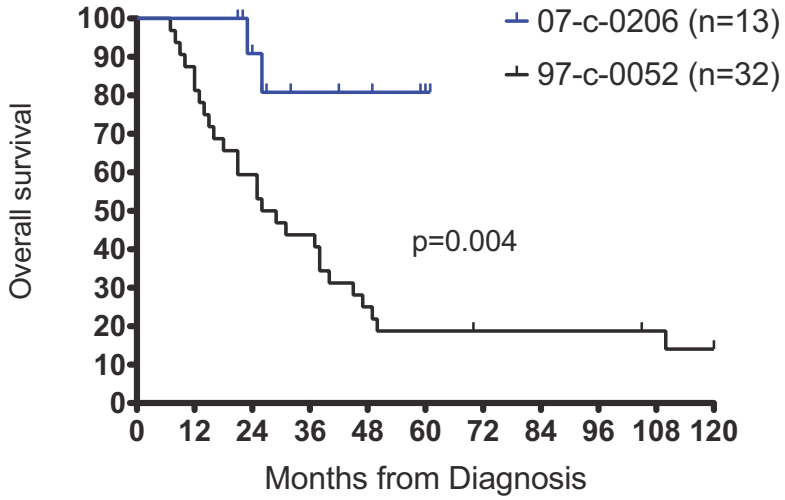
Objective 3: The therapy was very well tolerated, with Grade 3 or 4 toxicities limited to: 1 grade 3 allergic reaction definitely related to rhIL7, three grade 3 transaminitis likely related to rhIL7, 1 grade 4 fever likely related to the immunotherapy regimen. We also observed transient lymphopenia grade 3 or 4 in 10 patients, although this is judged to be a biomarker of rhIL7 activity due to lymphocyte trafficking effects and not a toxicity per se.

Among the secondary objectives, two remarkable observations were made:

We observed very favorable overall survival in patients with newly diagnosed or recurrent Ewing sarcoma and rhabdomyosarcoma compared to our previous study (97-C-0052) and compared to historical controls. Shown below is an intent-to-treat Kaplan Meier survival graph showing outcomes for all patients with Ewing sarcoma and rhabdomyosarcoma enrolled on the two consecutive POB immunotherapy trials. Note that eligibility criteria were essentially identical.



The results were even more impressive when we restricted our analysis to patients with newly diagnosed metastatic Ewing sarcoma and rhabdomyosarcoma, a group with uniformly dismal survival in historical trials. Again this is an intent-to-treat analysis. It is important to note only 1 patient treated with newly diagnosed metastatic disease had rhabdomyosarcoma, thus Ewing sarcoma made up the vast majority of patients with this favorable outcome on 07-C-0206.



Given these favorable clinical results, we seek to gain further experience with consolidation regimens designed to induce immune reconstitution ± tumor vaccine. At the same time, the regimen as currently configured is too complex for export, which would limit availability to a wider swath of patients and would preclude a definitive randomized trial. Thus, through this amendment, we seek to determine whether a simpler regimen, which involves rhIL7 alone administered after completion of up front therapy may be sufficient to induce similarly favorable survival results.

We will also intensively study patients enrollment on Cohort 2 to determine whether

lymphopenic patients who receive rhIL7 can undergo significant immune reconstitution and to determine whether regulatory T cell regeneration may be inhibited. This interest is fueled by the observation of significantly reduced regulatory T cell populations in patients treated on 07-C-0206 Cohort 1, Arm B compared to patients treated without rhIL7 (97-C-0052 and 07-C-0206 Cohort 1, Arm A). This appears to be driven in large part by rhIL7's capacity to preferentially expand non-Treg populations. We also observed that patients with very high levels of Tregs early after completion of standard frontline chemotherapy were at very high risk of relapse. Thus, it remains possible that agents such as rhIL7, which can diminish Treg regeneration in the early phases of immune reconstitution, could prevent tumor recurrence. Patients treated on Cohort 2 therefore will be followed closely for Treg regeneration and further analysis of a potential relationship between Tregs and clinical outcome will be undertaken.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Cohort 1: Inclusion Criteria for Apheresis/Tumor biopsy portion of the trial (Completed and no longer enrolling with Amendment L)

2.1.1.A Diagnosis

- rhabdomyosarcoma: embryonal or alveolar
- Ewing's sarcoma family of tumors (ESFT), which include: classical, atypical, and extraosseous ESFT, peripheral primitive neuroectodermal tumors, peripheral neuroepithelioma, primitive sarcoma of bone, and ectomesenchymoma.
- neuroblastoma: may be diagnosed via histology or the standard clinical evidence for increased catecholamines in the urine plus tumor cells in the bone marrow
- undifferentiated or embryonal sarcoma
- desmoplastic small round cell tumor
- synovial cell sarcoma

2.1.1.B Extent of Disease/Previous Therapy

- Initial presentation: Stage IV or metastatic disease, enrolled prior to any cytoreductive therapy.
- Recurrent Disease:
    - Patient >5yo must have recovered CD4 count to >350 cells/mm3 OR have disease free interval > one year from completion of cytotoxic therapy
    - Patients <5yo must have recovered CD4 count to >350 cells/mm3 OR have disease free interval > six months from completion of cytotoxic therapy
- Multiple recurrences are allowable as long as CD4 count or disease-free intervals have been met.

2.1.1.C Age/Weight

- >18 mos. and ≤ 35 years at the time of initial diagnosis
- > 10 kg at the time of apheresis. Patients between 10-15 kg. must be approved by the apheresis unit prior to enrollment on protocol.

2.1.1.D Informed Consent

All patients or their legal guardians (if the patient is <18 years old) must sign a document of informed consent (screening protocol) prior to performing studies to determine patient eligibility. After confirmation of patient eligibility all patients or their legal guardians must sign the protocol specific informed consent to document their understanding of the investigational nature and the risks of this study before any protocol related studies are performed (other than the studies which were performed to determine patient eligibility).

2.1.1.E Laboratory Parameters

- renal function: creatinine clearance > 60 mL/min/1.73m^2 or normal age adjusted serum creatinine (≤ 5 yrs. ≤ 0.8 mg/ml; 5-10 yrs. ≤1.0 mg/ml; 10-15 yrs. ≤ 1.2 mg/ml; >15 yrs. ≤ 1.5 mg/ml)
- liver function: AST and ALT < 2.5x ULN, bilirubin < 5 ULN
- hematologic function: platelets > 50,000 cells/mcl, Hgb > 9.0 gms/dl, PT < 1.5 ULN. Patients may receive transfusion if necessary to reach the pre-apheresis hematology parameters. If elevated PT is concluded to be due to a circulating anticoagulant then patient may enroll despite the abnormal laboratory finding.

2.1.1.F Accessibility of Tissue to Generate Tumor Lysates

Patients must have adequate tumor bulk accessible to biopsy in order to generate the tumor lysate (at least 2 cm diameter). Procedures employed to acquire biopsies for tumor lysates will be limited to percutaneous biopsies or open biopsies of readily accessible lesions. Patients should not undergo biopsies, which will later compromise the ability to render function preserving local therapy (e.g. limb salvage therapy). To prevent this, all bone biopsies should be performed in consultation with the orthopedic consultant on the case. For patients with bone marrow involvement, bone marrow aspirates may be used as a source of tumor for tumor lysates. Patients are not eligible if, in the opinion of the principal and associate investigators, tumor biopsy would entail extensive surgery such as thoracotomy or laparotomy, or if the tumor site places the patient a substantial risk from the biopsy procedure. NCI Laboratory of Pathology will review all tumor specimens for diagnosis.

2.1.2 Cohort 1: Exclusion Criteria for Apheresis/tumor biopsy portion of the trial (Completed and no longer enrolling with Amendment L)

2.1.2.A Other conditions

- Clinically significant unrelated systemic illness, such as serious infections, autoimmunity or organ dysfunction, which in the judgment of the Principal or Associate Investigators would compromise the patient's ability to tolerate the investigational agents or are likely to interfere with the study procedures or results.
- Previous allogeneic stem cell or allogeneic bone marrow transplantation.
- Conditions related to tumor, which require emergency treatment (airway compression, spinal cord compression) since enrollment would delay initiation of such therapy.
- Women who are pregnant or lactating.
- Corticosteroids initiated at the time of tumor diagnosis or recurrence for treatment

of nerve compression or other symptoms *is permitted* during this phase of the trial, but will not be permitted during the immunotherapy phase, with the exception of a self limited course of steroids as described in Section 2.1.4.A.

- Patients with a history of CNS metastases from cancer are not excluded provided that the metastatic CNS disease has been effectively treated and there is no evidence of active CNS disease as evidenced by stable clinical findings and stable radiographic findings for a period of 6 weeks.
- Patients with human immunodeficiency virus infection, hepatitis B, or hepatitis C due to confounding effects on immune system.

2.1.3 Cohort 1: Inclusion Criteria for Immunotherapy portion of the trial (Completed and no longer enrolling with Amendment L)

2.1.3.A Informed Consent

Because significant time will have elapsed between apheresis/tumor biopsy and the initiation of immunotherapy, all patients or their legal guardians (if the patient is <18 years old) must sign a second informed consent to document their understanding of the investigational nature and the risks of this study before any protocol related studies are performed (other than the studies which were performed to determine patient eligibility).

2.1.3.B Time and Recovery from Cytotoxic Therapy

At least 3 weeks should have elapsed since the last cycle of cytotoxic therapy or since the last dose of radiation therapy, at least 4 weeks must have elapsed since the patient has received any investigational therapy or antibodies, and at least 7 days since the last dose of biologics (i.e. rapamycin or sorafinib), and patients should have recovered from toxic side effects of previous therapy to a grade 1 or less, with the exception of the following:

- Hematological toxicity: recovery to levels required in Section 2.1.1.E.
- Low electrolyte levels (Such individuals should receive appropriate supplementation)
- For patients on anticoagulant therapy or with pre-existing coagulation abnormalities, PT, PTT must return to baseline.
- Liver function tests must resolve to values required in Section 2.1.1.E.
- Grade 3 hypoalbuminemia
- Alopecia
- Sterility

2.1.3.C Laboratory Parameters

• renal function: creatinine clearance > 60 mL/min/1.73m^2 or normal age adjusted serum creatinine (≤ 5 yrs. ≤ 0.8 mg/ml; 5-10 yrs. ≤1.0 mg/ml; 10-15 yrs. ≤ 1.2 mg/ml; >15 yrs. ≤ 1.5 mg/ml)
• liver function: AST and ALT < 2.5x ULN, bilirubin < 1.5 ULN
• hematologic function: ANC > 750 cells/mcl, platelets > 50,000 cells/mcl

2.1.3.D Birth Control

Subjects of childbearing or child-fathering potential must be willing to use a medically acceptable form of birth control, which includes abstinence, while they are being treated

on this study.

2.1.4 Cohort 1: Exclusion Criteria for Immunotherapy Portion of the Trial (Completed and no longer enrolling with Amendment L)

2.1.4.A Other conditions

• Clinically significant unrelated systemic illness, such as serious infections or organ dysfunction, which in the judgment of the Principal or Associate Investigators would compromise the patient's ability to tolerate the investigational agents or are likely to interfere with the study procedures or results.

• Persistent or progressive cancer following the completion of the standard therapy phase of the trial will not, in and of itself, preclude receipt of immunotherapy. However, patients will not receive immunotherapy if they have an ECOG performance status performance status of 3 or 4 or, for children ≤10 years of age, Lansky ≤50 (Appendix III). Furthermore, patients will be removed from the trial if they develop requirements for anti-neoplastic therapy (e.g. radiation therapy) for progressive disease during the trial as discussed in Section 3.8.

•Women who are pregnant or lactating.

•Patients with human immunodeficiency virus infection, hepatitis B, or hepatitis C infection due to confounding effects on immune function.

•Patients who require chronic daily oral corticosteroid or other immunosuppressive therapy. Topical or inhaled corticosteroids are permitted. Also, a time limited course of steroids does for an unrelated medical condition (e.g. allergic reaction, poison ivy) will not preclude receipt of immunotherapy provided that two weeks elapse between the last dose of systemic corticosteroids and initiation of immunotherapy.

•Patients who are receiving other biologic therapies including cytokines or growth factors not specified by the protocol. Herbal supplements will not result in exclusion but should be noted and reviewed with the PI.

•Patients with a history of CNS metastases from cancer are not excluded provided that the metastatic CNS disease has been effectively treated and there is no evidence of active CNS disease as evidenced by stable clinical findings and stable radiographic findings for a period of 6 weeks.

• Excluded from Arm B:

Patients with history of autoimmune disease (excluding thyroiditis on chronic thyroid replacement therapy) or active auto-immune disease, due to a risk of exacerbation of autoimmunity with r-hIL7. Patients with a history of B cell malignancy due to a risk for growth with rhIL7 therapy.

QTc prolongation defined as a QTc greater than or equal to 470 ms or a prior history of cardiovascular disease, arrhythmias, or significant ECG abnormalities.

2.1.5 Cohort 2: Inclusion Criteria

2.1.5.A Diagnosis:

• Ewing sarcoma (ES), which include: classical, atypical, and extraosseous ES, peripheral

primitive neuroectodermal tumors, peripheral neuroepithelioma

2.1.5.B Extent of Disease/Previous Therapy

• Patients must have completed standard frontline therapy for newly diagnosed metastatic disease. Lung, bone, bone marrow or other metastases are sufficient to qualify as metastatic disease. Standard frontline therapy is comprised of a regimen that includes (but is not limited to) multiple cycles of vincristine, adriamycin, ifosfamide and etoposide. Local therapy as dictated by the treating institutions. Patients may have received autologous stem cell transplantation or other investigational agents as part of their primary therapy.

• Patients must be judged as being in a state of "no evidence of disease" at the time of enrollment. We realize that there is sometimes difficulty in definitively determining whether previously irradiated or surgerized sites are truly sterile, but the judgment should be based on standard imaging using the best judgment of the referring physician and Principal Investigator or her designee.

2.1.5.C Age

• Patients must have been ≤ 35 years at the time of initial diagnosis.

2.1.5.D Informed Consent

• All patients or their legal guardians (if the patient is <18 years old) must sign a document of informed consent (screening protocol) prior to performing studies to determine patient eligibility. After confirmation of patient eligibility all patients or their legal guardians must sign the protocol specific informed consent to document their understanding of the investigational nature and the risks of this study before any protocol related studies are performed (other than the studies which were performed to determine patient eligibility).

2.1.5.E Time and Recovery from Cytotoxic Therapy

At the time of enrollment, at least 3 weeks and no more than 8 weeks should have elapsed since the last cycle of cytotoxic therapy or since the last dose of radiation therapy, and patients should have recovered from toxic side effects of previous therapy to a grade 1 or less, with the exception of the following:

- Hematological toxicity: recovery to levels required in Section 2.1.5.G.
- Low electrolyte levels (Such individuals should receive appropriate supplementation)
- For patients on anticoagulant therapy or with pre-existing coagulation abnormalities, PT, PTT must return to baseline.
- Liver function tests must resolve to values required in Section 2.1.5.G.
- Grade 3 hypoalbuminemia is permitted
- Alopecia is permitted
- Sterility is permitted

2.1.5.F Laboratory Parameters

• Renal function: creatinine clearance > 60 mL/min/1.73m^2 or normal age adjusted serum creatinine (≤ 5 yrs. ≤ 0.8 mg/ml; 5-10 yrs. ≤1.0 mg/ml; 10-15 yrs. ≤ 1.2 mg/ml; >15 yrs. ≤

1.5 mg/ml)
• Liver function: AST and ALT < 2.5x ULN, bilirubin < 1.5 ULN
• Hematologic function: ANC > 750 cells/mcl, platelets > 50,000 cells/mcl

2.1.5.G Birth Control

Subjects of childbearing or child-fathering potential must be willing to use a medically acceptable form of birth control, which includes abstinence, while they are being treated on this study.

2.1.5.H Performance Status

ECOG performance status of 0, 1, or 2 or for children < 10 years of age, Lansky of > 50 is required.

2.1.6 Cohort 2: Exclusion Criteria

2.1.6.A Disease status

Patients must have had a significant response to standard frontline therapy judged as a complete response or a very good partial response (especially for irradiated sites where CR often impossible to ascertain). Clear evidence for persistent or progressive cancer or the administration of a salvage regimen for persistent or progressive disease precludes enrollment on Cohort 2. Administration of consolidation such as autologous stem cell transplant without evidence for persistent or progressive disease does not preclude enrollment.

2.1.6.B Other conditions

• Clinically significant unrelated systemic illness, such as serious infections or organ dysfunction, which in the judgment of the Principal or Associate Investigators would compromise the patient's ability to tolerate the investigational agents or are likely to interfere with the study procedures or results.

• Women who are pregnant or lactating.

• Patients with human immunodeficiency virus infection, hepatitis B, or hepatitis C infection due to confounding effects on immune function.

• Patients who require chronic daily oral corticosteroid or other immunosuppressive therapy. Topical or inhaled corticosteroids are permitted. Also, a time limited course of steroids doses for an unrelated medical condition (e.g. allergic reaction, poison ivy) will not preclude receipt of immunotherapy provided that two weeks elapse between the last dose of systemic corticosteroids and initiation of immunotherapy.

• Patients who are receiving other biologic therapies including cytokines or growth factors not specified by the protocol. Herbal supplements will not result in exclusion but should be noted and reviewed with the PI.

• Patients with a history of CNS metastases from cancer are not excluded provided that the metastatic CNS disease has been effectively treated and there is no evidence of active CNS disease as evidenced by stable clinical findings and stable radiographic findings for a period of 6 weeks.

• Patients with history of autoimmune disease (excluding thyroiditis on chronic thyroid replacement therapy) or active auto-immune disease, due to a risk of exacerbation of autoimmunity with r-hIL7. Patients with a history of B cell malignancy due to a risk for growth with rhIL7 therapy.

• QTc prolongation defined as a QTc greater than or equal to 470 ms or a prior history of cardiovascular disease, arrhythmias, or significant ECG abnormalities.

2.2 Research Eligibility Evaluation

Pre-treatment evaluation should be performed prior to initial enrollment on the trial and then repeated prior to initiation of immunotherapy (except where indicated). The pretreatment and pre-immunotherapy evaluations are outlined in Appendix 1.

2.2.1 History and Physical Examination

Cohort 1 (Completed and no longer enrolling with Amendment L): To be performed within 1 week of initial enrollment and within 1 week of initiation of immunotherapy. Cohort 2: performed within 1 week of enrollment.

- Document signs and symptoms.
- Record height, weight and body surface area using the standard formula: $BSA=Weight\ (kg)^{0.425}$ x $Height\ (cm)^{0.725}/139.315$.
- ECG evaluation to assess QTc interval

2.2.2 Laboratory Evaluation

Cohort 1 (Completed and no longer enrolling with Amendment L): Within 1 week of initial enrollment and within 1 week of initiation of immunotherapy. Cohort 2: Within 1 week of enrollment:

- CBC with differential
- Electrolytes (Na, K, Cl, CO_2, Mg, Ca, phosphorus, uric acid, BUN, Cr)
- liver panel (bilirubin (including conjugated bilirubin), alkaline phosphates, AST/ALT)
- PT/PTT
- urinalysis
- thyroid function tests, including anti-thyroid antibodies
- 10 ml serum storage for cryopreservation
- Urine pregnancy test for females of childbearing potential
- All patients enrolled: Flow cytometry

Cohort 1 (Completed and no longer enrolling with Amendment L): Prior to apheresis, all patients will be tested for blood type, antibody screen, HIV, hepatitis B, and hepatitis C but results are not necessary for eligibility to the Apheresis/tumor biopsy portion of the trial. Results are required prior to enrollment on the Immunotherapy portion of the trial. In addition an RPR will be obtained for screening purposes.

Cohort 2: Prior to apheresis, all patients will be tested for blood type, antibody screen, HIV, hepatitis B, and hepatitis C and RPR prior to enrollment.

2.2.3 Tumor Staging Studies

2.2.3.A Radiographic studies

Cohort 1: (Completed and no longer enrolling with Amendment L)

- Radiographic studies of all sites of known disease including but not limited to CT scans or MRI of sites of known disease, CT scan of the chest, FDG PET scan should be performed within 3 weeks of initial enrollment. If PET scan is unavailable or would result in undue time delay in initiating therapy, radionuclide bone scan may be substituted. Radiographic studies prior to initial enrollment may be performed at the patient's referring hospital with electronic or hard copies of the radiographic studies sent to the Clinical Center.
- No protocol dictated studies are required prior to Step 1 immunotherapy, but should be obtained if clinically indicated.
- Full restaging to include imaging of sites of previous disease as well as CT scans of the chest and PET scans should be performed at baseline prior to beginning immunotherapy on this study. These studies should be performed at the NIH. Radionuclide bone scan is not required.

Cohort 2:

- Within 3 weeks prior to enrollment or within one week after enrollment, full staging to include imaging of sites of previous disease, as well as CT scans of the chest and PET scans. These studies may be performed at the home institution. Radionuclide bone scan is not required.

2.2.3.B Bone Marrow Analyses

Cohort 1: (Completed and no longer enrolling with Amendment L)

- For patients with newly diagnosed neuroblastoma, rhabdomyosarcoma or ESFT, a bone marrow aspiration/biopsy should be performed within 4 weeks of protocol enrollment. If the marrow is negative at the time of diagnosis, no further bone marrow studies are required prior to immunotherapy, unless clinically indicated. If the marrow is positive at the time of diagnosis, bone marrow aspiration and biopsy is recommended after cytotoxic treatment, and prior to initiation of immunotherapy, until at least one negative bone marrow study is documented.
- For patients with newly diagnosed synovial, desmoplastic or undifferentiated sarcoma, no bone marrow study is necessary due to the low rate of marrow spread in these diseases.
- For patients enrolled on trial following a disease recurrence, bone marrow analysis is not required if there are no signs or symptoms of bone marrow involvement and the patient has no history of bone marrow involvement. Such patients do not require bone marrow analyses prior to initiation of immunotherapy unless clinically indicated.
- Bone marrow aspirations and biopsies may be performed at the patient's referring hospital with copies of the bone marrow report sent to the Clinical Center for inclusion in the patient’s chart.

Cohort 2:

- A bone marrow aspiration/biopsy should have been performed at diagnosis. If the marrow was negative at the time of diagnosis, no further bone marrow studies are required prior to enrollment, unless clinically indicated. If the marrow is positive at the time of diagnosis, at least one negative bone marrow study must be documented prior to enrollment. If not marrow aspiration was performed at diagnosis and there is no clinical reason to suspect marrow involvement, then bone marrow aspirate/biopsy will not be required.

2.3 PATIENT REGISTRATION

For Cohort 1(Completed and no longer enrolling with Amendment L), patients will be registered with Central Registration prior to apheresis or tumor biopsy (301-402-1732). For Cohort 2, patients will be registered with Central Registration prior to enrollment (301-402-1732). Prior to entry on study, an M.D. investigator for this protocol who is in the Pediatric Oncology Branch must be notified; office telephone: 301-496-4256, or via page operator: 301-496-1211. A completed eligibility checklist and a signed consent form must be on file prior to entry on study.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Overall Trial Design

3.1.1.A Cohort 1 (Completed and no longer enrolling with Amendment L):

This is a pilot study of immunotherapy for high risk patients with pediatric solid tumors of the following histologies: Ewing's sarcoma family of tumors, rhabdomyosarcoma, neuroblastoma, synovial cell sarcoma, desmoplastic small round cell tumor, undifferentiated sarcoma, embryonal sarcoma. Patients are enrolled either at the time of initial diagnosis with metastatic disease or at the time of tumor recurrence following a treatment free interval. Upon enrollment, patients will undergo 1) a tumor biopsy for generation of tumor lysate 2) apheresis for autologous lymphocytes and monocytes which will be 8H9/αCD25 depleted, then cryopreserved for subsequent reinfusion. Endpoints are feasibility, biologic responses to the vaccine with or without r-IL-7 and toxicity. Patients then undergo "standard therapy" with a goal of rendering them into a state of no evident disease, (which is anticipated in the majority of patients). The standard therapy will be determined by the primary oncology team caring for the patient and is not protocol directed. Immunotherapy begins upon recovery from the last cycle of standard therapy when CD4+ counts are measured. Patients in Arm A with a CD4+ count < 200 cells/mcl will proceed directly to step 2. Patients in Arm A with a CD4+ count > 200 cells/mcl will receive cyclophosphamide/fludarabine to induce a CD4+ count < 200 cells/mcl. Patients in Arm B only will not receive cyclophosphamide/fludarabine regardless of CD4 count.

Patients then undergo pre-immunotherapy radiographic and immune studies, then receive 8H9/αCD25 depleted autologous lymphocyte infusion and a total of 6 tumor lysate/KLH pulsed dendritic cell vaccines ± r-hIL-7 (each vaccine comprises 6 injections) every 2 weeks. Protocol enrollment is based upon a Phase II statistical design powered to detect whether 50% of patients demonstrate a response to the vaccine, with an early stopping rule if we do not obtain adequate samples to prepare lysates in the majority of patients or

if excessive toxicity is observed. The primary endpoints are biologic as most patients will not have clinical evidence of disease at the time immunotherapy is administered. After 6 patients have been treated on Arm A, all subsequent patients will be treated on Arm B (vaccine + CYT107), and will be evaluated separately for biologic and clinical outcomes compared to patients enrolled on Arm A (vaccine only).

3.1.1.B Cohort 2:

Patients with metastatic Ewing sarcoma who received standard frontline therapy for their disease at their local institution may be enrolled upon completion of therapy to receive rhIL7 as a single maneuver to induce immune reconstitution. Patients must be judged to be in a state of "no evidence of disease" at the time of enrollment and cannot have been previously treated for progressive or recurrent disease. They must enroll within 8 weeks of completing standard frontline therapy for their disease. They will be followed for clinical endpoints and immune reconstitution endpoints.

3.2 Cohort 1 Treatment Administration (Completed and no longer enrolling with Amendment L)

3.2.1 Pre-Standard Therapy

Enrollment on this protocol requires that the patient come to the NIH Clinical Center for tumor biopsy and apheresis. For newly diagnosed patients, this will occur prior to initiation of standard frontline therapy and for patients with a late recurrence of disease, this will occur prior to the initiation of cytotoxic chemotherapy or radiation therapy for recurrent disease. If surgery is performed prior to referral, patients remain eligible if sufficient tumor is available to obtain biopsies for tumor lysate generation. Every attempt will be made to limit the delay in therapy involved in this phase, especially for newly diagnosed patients. The estimated delay in initiating therapy is anticipated to be under two weeks for most patients allowing for travel time and time to undergo the biopsy and apheresis.

3.2.1.A Tissue Acquisition to Generate Tumor Lysate

Patients must undergo biopsy or excision to acquire tissue for the generation of the tumor lysate. Except for patients in whom the tumor source is bone marrow, this will be done in collaboration with the primary physician or surgeon. For patients who have unresectable disease or disease for which outcome is not improved by resection, in which case the sole purpose of tissue acquisition is for enrollment on this investigational trial, or for which benefit to individual patients is not known, risks associated with acquisition of tumor tissue will be minimized. In this case, procedures should be limited to percutaneous biopsy or open biopsy of readily accessible lesions and patients should not be subjected to extensive surgeries such as thoracotomy or laparotomy. However, for patients with disease status or histologies (e.g. synovial sarcoma) where excision of primary or metastatic sites is deemed to have potential therapeutic benefit, then tumor lysate may be obtained from tissue obtained at the time of excision. For these patients, thoracotomy or open procedures are acceptable if the patient's treating team recommends the procedure for therapeutic intent. Importantly, for patients with bone tumors, the biopsy should be performed in consultation with the orthopedic oncology involved with the case to minimize the chance that the biopsy procedure will adversely affect future attempts at limb salvage.

It is expected that patients will routinely receive sedation and/or anesthesia for percutaneous procedures and standard anesthesia for open procedures. Standard techniques will be used for percutaneous biopsies and may include CT and / or ultrasound guidance with a goal of acquiring 5 mg of tumor tissue for use in generating the tumor lysate, which will serve as the immunogen on this trial. In some cases, biopsies may be expedited and facilitated with the use of navigation tools such as an automated laser angle selector connected to CT scan, or a standard needle guide connected to a protractor to determine which exact angle the biopsy needle will be inserted. These guiding techniques may occur as maneuvers to facilitate the biopsy, which will take place in the usual conventional fashion, with standard, disposable, conventional spring-loaded biopsy equipment. Such guiding techniques may add to the reliability of tissue acquisition from specific spatial coordinates of a tumor target.

Tumor tissue will be reviewed by a cytopathologist or cytopathology technician in attendance to confirm that an adequate sample of tumor is present in the tissue biopsy. This will be performed in real time using "diff quick" staining of a smeared slide in the biopsy suite. If designated as an adequate sample by the cytopathologist/cytopathology technician in attendance (estimated to be <50% normal tissue), the biopsies will immediately be placed into sterile PBS, and tumor cells will be dispersed with a 19-gauge needle to create a single cell suspension. A sample of dispersed pooled biopsy specimen will be sent to cytopathology for formal quantitation of the percent contaminating normal cells using a cytospin prep, but this will not be available in real time. Cells will be lysed by 5 freeze thaw cycles (freeze on liquid nitrogen, thaw at room temperature), and then centrifuged (10 min. at 600 rpm) to remove particulate matter. Supernatants are collected, filtered through a 0.2 micron filter and protein content determined using a Bio-Rad assay. Aliquots of 0.5 mg/vial will be frozen at -80ºC until used as described in 3.2.4.E. Prior to release of the lysate for use as an immunogen, a report from cytopathology documenting <50% normal tissue must be documented. If the tissue is >50% normal tissue, then the tumor lysate will not be used as a vaccine and either the biopsy will be repeated or the patient will be removed from study.

For patients with overt bone marrow involvement (at least 50% replaced with tumor), tissue acquisition will consist of bone marrow aspiration with the goal of acquiring 100 cc of bone marrow aspirate. This will be done with appropriate anesthetic support using the standard operating procedure, which is currently in place for a marrow harvest. Following ficoll-hypaque centrifugation, a sample will be transferred to cytology for enumeration of tumor content as part of the lysate release criteria (see Section 3.2.4.E) and the rest of the aspirate will be processed via freeze/thaw, centrifugation, protein enumerations and cryopreservation as described above.

3.2.1.B Initial Apheresis for Collection of Autologous Lymphocytes for Infusion

Large volume leukapheresis will be performed in the NIH DTM. For patients who are > 50 kg, 15 to 25 liters will be processed per procedure; in patients ≤ 50 kg, 6-8 blood-volumes will be processed per procedure with a goal of the procedure being collection of 3.0×10^8 nucleated cells/kg. Citrate anticoagulant will be used in subjects greater than 18 kg. For subjects less than 18 kg, low-dose heparin may be added. Prophylactic intravenous $CaCl_2$ and $MgSO_4$ infusions will be administered at the discretion of the DTM physician. Bilateral peripheral venous access will be used whenever possible. Alternatively, a

temporary femoral central venous catheter (CVC) will be placed for collection. If this is necessary, the patient will be admitted to the Clinical Center for CVC placement prior to starting apheresis. The CVC will be inserted by Critical Care or Interventional Radiology staff with the assistance of anesthesiology as clinically indicated. The patient will remain hospitalized until the apheresis is complete and the CVC is removed. Studies to rule out blood borne infection (HIV, anti-HCV, anti-HBS, HbsAg) will be performed upon initiation of apheresis.

Lymphocytes will be separated from monocytes via countercurrent centrifugal elutriation. The lymphocyte fraction will then be depleted of CD25 cells using the Miltenyi CliniMACS® System and depletion of cells based upon 8H9 binding of cells will be performed using the Elutra Cell Separation System. Pre- (5%) and post-selection (5%) aliquots will be sent to FCRDC for cryopreservation in the Frederick repository. These cells will be used for RT-PCR to monitor for tumor contamination, for baseline immune endpoint monitoring and for research studies aimed at characterizing the immunologic reactivity of patients during lymphopenia. Post-selection but prior to cryopreservation, apheresis fractions will be monitored for sterility, endotoxin and mycoplasma as detailed in the DTM standard operating procedures. Post-selection FACS analyses for determination of T cell and B cell content, and CD4+CD25+ cell content will be performed. The product to be infused will be cryopreserved using DTM standard operating procedures.

The monocyte rich fraction will be collected as the antigen presenting cell product. Aliquots of antigen presenting cells not to exceed 10% of the total APC product will be sent to the Frederick repository to be used in baseline immune endpoint monitoring and for research studies. The remaining monocytes will be divided into at least 6 aliquots of 1 x 10^5/kg- 1 x 10^8/kg and cryopreserved using DTM standard operating procedure.

3.2.2 Cohort 1: Standard Therapy

Patients will undergo standard therapy for treatment of their primary or recurrent malignancy as directed by their primary oncology team. In some cases, this will occur at the Pediatric Oncology Branch, but patients may also receive treatment at referring institutions. Standard therapy is not dictated by this protocol per se, however for newly diagnosed patients, primary therapy must comprise a regimen, which is considered to be standard front line therapy for their disease. Patients may receive investigational agents during the standard therapy phase of their treatment, but research protocols for which long term survival outcomes are primary endpoints would not be appropriate since the immunotherapy may impact long term outcome. For patients with recurrent disease, the standard therapies administered will vary depending upon individual patient circumstances and may involve salvage chemotherapy regimens, retreatment with drugs, which the patient has previously received, investigational drugs, radiation therapy and/or surgery.

3.2.3 *Cohort 1: Immunotherapy (Lymphodepletion) in Arm A ONLY*

Based upon our clinical experience and the published experience with the diseases targeted in this trial, it is anticipated that most patients will demonstrate profound lymphopenia upon completion of standard therapy. However, in exceptional circumstances, such as when there may be delays following completion of standard therapy before initiation of immunotherapy or when standard therapy induced no (e.g. surgery alone) or minimal (e.g.

limited radiation or non-lympholytic chemotherapy) lymphocyte depletion, patients may not be lymphopenic. The goal for patients treated on Arm A of this trial is to initiate immunotherapy in lymphopenic hosts, therefore we will plan to administer cytotoxic/lympholytic therapy in patients who present following completion of standard therapy with a CD4 count > 200 cells/mcl in an attempt to render them lymphopenic to this level. Upon recovery from all toxic side effects of standard therapy, Step 1 is initiated by measurement of a peripheral blood CD4+ count. Patients will be treated according to the following schema:

If CD4+ cells are < 200 cells/mcl, patients will proceed directly to immunotherapy.
If CD4+ cells are ≥ 200 cells/mcl, patients undergo lymphodepletion:

- Patients should be hydrated at a rate of 125 ml/m^2/hr beginning at least one hour prior to and during cyclophosphamide administration. Prior to initiation of chemotherapy, patients must demonstrate a urine output of 2 ml/kg/hr for at least one hour or one void.
- Hour 0: fludarabine 25 mg/m^2/dose in 100 ml D_5W over 30 minutes on Days 1, 2 and 3.
- Hour 1: cyclophosphamide 1800 mg/m^2/dose and mesna 360 mg/m^2/dose in 100 ml D5W IV over 1 hour on days 1 and 2
- Hours 2-20: mesna 360 mg/m^2/dose IV over 15 min. immediately after the cyclophosphamide infusion, then every 3 hrs. for an additional 6 doses PO or IV over 15 minutes on days 1 and 2 (total 14 doses). Oral mesna is supplied as scored 400 mg tablets. Adequate oral hydration (minimum 500 mL PO q 6 hr must be maintained).
- Routine antiemetics should be administered. Corticosteroids may be used as antiemetics if clinically indicated.
- Pegfilgrastim 100 mcg/kg (max 6 mg) x 1 dose should be administered 24-36 hours after completion of fludarabine. For patients < 55 kg, daily filgrastim at a dose of 5 mcg/kg should be administered until the ANC is > 1000 cells/mcl.

Upon hematopoietic recovery (ANC ≥ 1000 cells/mcl, platelets ≥ 50,000 cells/mcl), the CD4+ count will be measured and patients will proceed to immunotherapy regardless of their CD4+ count.

For patients treated on Cohort 1, Arm B, where CYT107 will be coadministered and wherein lymphopenia is not essential to amplify immune responses, chemotherapy will not be administered prior to initiation of immunotherapy regardless of the CD4+ count.

3.2.4 Immunotherapy

3.2.4.A Baseline Small Volume Apheresis to Obtain Cells for Immune Endpoint Monitoring

- Patients will undergo a small volume apheresis (approx. one to two blood volumes) to obtain peripheral blood lymphocytes that will serve as the post-standard therapy, pre-vaccination sample for immune endpoint monitoring. This may occur anytime

within one week prior to the lymphocyte infusion.

- Every attempt will be made to use an indwelling catheter or peripheral lines for the small volume aphereses. If this is not feasible and a central line is required, then this may be placed to acquire cells prior to immunotherapy and at the evaluation for immunotherapy timepoints (approximately 8 weeks after the last vaccine), but not at the intervening time points. Because these two time points are critical time points for response criteria in this trial, these will be determined as the minimally acceptable data necessary in order to determine whether an immune response to the vaccine was induced.

3.2.4.B Pre-immunotherapy radiographic imaging (within 3 weeks of starting immunotherapy)

- Radiographic studies of all sites of previous disease including but not limited to CT scans or MRI, CT scan of the chest (including simultaneous evaluation of lymph nodes and thymus in patients in Arm B) and FDG PET scan. These studies should be performed at the NIH Clinical Center.

3.2.4.C Baseline DTH Testing (prior to starting dendritic cell vaccine)

- 50 mcg of KLH in 0.1 ml and 50 mcg of tumor lysate in 0.1 ml will be provided in separate prefilled tuberculin syringes to the outpatient clinic.
- Patients will receive intradermal injections of each on the volar aspect of the forearm.
- Approximately 48 hours later, the site will be inspected for induration and the result recorded in the patient's medical record.
- A positive response is induration exceeding 5 mm.

3.2.4.D Immunotherapy Dose #1

This vaccine should be administered on the same day as the ALI, at least one hour prior to administration of ALI.

- *Production of Dendritic Cells (detailed manufacturing procedures contained in DTM Standard Operating Procedures)*:
  - Prior to the scheduled administration of the tumor lysate pulsed dendritic cell vaccine, one cryopreserved monocyte enriched apheresis fraction will be thawed, washed and evaluated for cell count and viability.
  - Thawed monocytes will be placed into culture at 37°C and cultured with rhIL-4 and rhGM-CSF.
  - After 96 hours in culture, DCs will be concentrated and thawed tumor lysate (prepared as described in Section 3.2.1.a) will be added to the immature dendritic cells at a concentration of 50 mcg/ml[57]. At the same time, KLH will be added at a concentration of 50 mcg/ml. This will be incubated for two hours at 37°C.
  - Additional media will then be added to bring DCs to an appropriate culture concentration (approx. 1.5 x 10^6 cells/ml) and clinical grade LPS (20 ng/ml) and IFNγ (1000 u/ml) will be added for the final 24-48 hours of culture.

- *Release Criteria & Quality control*: Focus will be upon general product

characterization and ensuring patient safety in this Phase I/II trial. Specific requirements will be included in the IND submitted to the FDA and the standard operating procedures in the Department of Transfusion Medicine.

- The following information will be contained on the certificate of analysis:
  - Total cell number as determined using an automated counter.
  - Viability using Trypan blue dye exclusion with a minimum viability of 70% as a release criteria.
  - Negative gram stain must be documented
  - Endotoxin level must be <5 EU/ml (this will be achievable as the LPS will be washed out prior to release testing)
  - Sterility check will be placed into culture 48 hours prior to infusion and must show no growth.
- A sterility check will also taken from the final harvested culture (aliquots inoculated into 50 ml of broth medium containing Soybean-Casein Digest) but final results will not be available as a release criterion.
- Mycoplasma culture will also be sent from the final culture but results will not be available for use as a release criterion.
- Other assays will be carried out to further characterize the product so that relationships between product characteristics and patient outcomes can be evaluated in a retrospective manner. These will include phenotypic evaluation by flow cytometry (MHC Class I, MHC Class II, CD80, CD83, CD86).
- Tumor lysate should have been generated from a sample which was <50% comprised of normal tissue and >50% comprised of tumor which will be documented on a final report from cytopathology.

- *CYT107 (r-hIL7):*
  - CYT107 will be administered subcutaneously at a dose of 20 mcg/kg in the arm or the leg or abdomen. This dose is expected to be safe and is well below the 80% Phase II dose used in adults. It should be administered in an extremity that will not be used for vaccine administration to avoid confounding evaluation of local reactions. CYT107 may be administered as an outpatient but patients must be observed in the clinic for 6 hours. At which time blood (10 mL, red top tube) will be drawn (6 hours after CYT107 administration) for serum storage. After treatment of a patient under the age of 12, enrollment of subsequent patients under 12 years of age will be staggered by 4 weeks to allow for toxicity assessment two weeks after the 2nd dose of CYT107, as toxicity data is limited in this age group. Enrollment will continue in the absence of Grade 3 or greater toxicity (excluding hematologic toxicity) or grade 2 autoimmune toxicity (excluding rash, fever or injection site reactions). Toxicity data in Arm B will be analyzed for two strata, participants under 12 years of age, and participants 12 years of age and older.

    Dose for CYT107 is calculated based on patient weight.

    CYT107 Doses will be modified in obese patients (with BMI ≥ 35) as follows:

– In obese patients, a corrected weight will be used to calculate the final dose the patient will receive. This corrected dose will be calculated and used if the patient's actual weight gives a Body Mass Index (BMI) at the upper limit of normal (i.e. BMI ≥ 35).
– BMI = weight (kg) / height (m2)
– Corrected weight (kg) = 35 x height (m2)

A maximum of 0.45 mL can be collected from one vial. If 2 vials are needed (or any volume over 0.45 mL), divide the volume into two equal injections.

- ECG must be done pre and 3 hours post CYT107 administration, to ascertain for transient changes in the QTc interval that may be potentially related to study drug. Liver panel tests will be evaluated every 48-72 hours post administration for 1 week, and repeated daily for any elevated results, until levels return to grade I or baseline.
- No premedication is required. However, if recurring reactions such as fever, skin rash, or nausea/vomiting appear following dosing, as observed with some other cytokines, patients may be pre-medicated according to the standard guidelines established to administer these products:
    - -administration of acetaminophen and consumption of alcohol is absolutely prohibited within the 48 hours preceding the dose, and within the 48 hours following the dose
    - Omeprazole for gastro-duodenal ulcer prevention
    - Antihistamine (anti H-1)
    - If needed, anti-emetics and anti-diarrheals can also be administered.
- If there is evidence of autoimmune reaction of Grade 2 or greater, CYT107 will be stopped, excluding Grade 2 fever, vitiligo, skin rash, injection site reaction, or localized urticaria.
- Vital signs including temperature should be taken at 1, 3, and 6 hours after administration or more frequently if clinically indicated.

- *Vaccine Administration*
    - The vaccination will be prepared by placing the dendritic cells, which have been pulsed with lysate/KLH and matured, into syringes appropriate for subcutanous and intradermal injections.
    - Each vaccination consists of three subcutaneous injections (1-5 x 10^7 cells total/site) and three intradermal injections (1-5 x 10^6 cells/site) using the ventromedial portion of the upper arm or anterior thigh. Subsequent vaccines will be administered approximately every 14 days. Vaccinations should occur approximately 48 hours (40-50 hour range) after the CYT107 injection.
    - Sites may be prepared with anesthetic cream prior to injection to minimize discomfort.
    - The patient will be monitored for at least one hour with vital signs taken every 15 minutes to detect any drop in blood pressure or other acute toxicity

following vaccination.

- If there is evidence of an allergic reaction, appropriate supportive medical care should be initiated. If the reaction is Grade 2 or less, premedication with diphenhydramine (but not corticosteroids) may be used with subsequent vaccines. If the allergic reaction is Grade 3 (or grade 2 non-responsive despite premedication), no subsequent vaccinations will be administered. Patients may remain on study for follow-up.

3.2.4.E Lymphocyte infusion

- Pre-medicate with 0.5-1.0 mg/kg (max 50 mg) diphenhydramine IV and 10mg/kg (max 1000 mg) acetaminophen po 30-60 min. prior to the infusion.
- Lymphocytes collected by apheresis and processed as described in Section 3.2.1.a. will be thawed rapidly in a 37℃ water bath and infused over 15-30 minutes. 90% of the product collected will be administered (10% for research) with a minimum infusion dose of 1 x 10^6 cells/kg and no maximal dose.
- Potential adverse reactions include fever, allergic reactions, transient dyspnea, nausea or vomiting. Supplemental oxygen should be available at the bedside.
- Patients should be observed for a period of one hour with vital signs recorded every 15 minutes after completion of the infusion.
- If an allergic or other acute reaction occurs, studies appropriate for investigation of a transfusion reaction will be performed (urinalysis, CBC, Coomb's test).
- Any adverse reactions should prompt cessation of the infusion and should be reported to the principal investigator.

3.2.5 Subsequent Immunotherapy Doses

3.2.5.A Dose #2: Day 14 (± 7 days)

- CYT107 dose #2 should be administered as described in 3.2.4.d. Patients must be monitored in the clinic for 6 hours after administration with vital signs performed at 1, 3, and 6 hours post r-hIL-7 administration. Blood (10 mL, red top tube) will be drawn 6 hours after CYT107 administration for serum storage. ECG must be done pre and 3 hours post rhIL-7 administration. LFTs must be repeated every 48-72 hours for 1 week post rhIL-7 administration, and repeated daily for any elevations until resolved to grade 1 or baseline.
- Dendritic cell vaccine #2 as described in Section 3.2.4.d. This should occur approximately 14 days following vaccine #1.

3.2.5.B Dose #3: Day 28 (± 7 days)

- CYT107 dose #3 should be administered as described in 3.2.4.d. Patients must be monitored in the clinic for 6 hours after administration with vital signs performed at 1, 3, and 6 hours post r-hIL-7 administration. Blood (10 mL, red top tube) will be drawn 6 hours after CYT107 administration for serum storage. ECG must be done pre and 3 hours post rhIL-7 administration. LFTs must be repeated every 48-72 hours for 1 week post rhIL-7 administration, and repeated daily for any elevations until resolved to grade 1 or baseline.
- Dendritic cell vaccine #3 as described in Section 3.2.4.d. This should occur

approximately 14 days following vaccine #2.

3.2.5.C Dose #4: Day 42 (± 7 days)

- CYT107 dose #4 should be administered as described in 3.2.4.d. Patients must be monitored in the clinic for 6 hours after administration with vital signs performed at 1, 3, and 6 hours post r-hIL-7 administration. Blood (10 mL, red top tube) will be drawn 6 hours after CYT107 administration for serum storage. ECG must be done pre and 3 hours post rhIL-7 administration. LFTs must be repeated every 48-72 hours for 1 week post rhIL-7 administration, and repeated daily for any elevations until resolved to grade 1 or baseline.
- small volume apheresis for immune endpoint monitoring as described in Section 3.2.4.a.
- lymphocyte immunophenotyping
- radiographic imaging as described in 3.2.4.b.
- DTH testing as described in 3.2.4.c.
- Dendritic cell vaccine #4 as described in Section 3.2.4.d. This should occur approximately 14 days following vaccine #3.

3.2.5.D Dose #5: Day 56 (± 7 days)

- Dendritic cell vaccine #5 as described in Section 3.2.4.d. This should occur approximately 14 days following vaccine #4.
- ECG to assess QTc interval

3.2.5.E Dose #6: Day 70 (± 7 days)

- Dendritic cell vaccine #6 as described in 3.2.4.d. This should occur approximately 14 days following vaccine #5.

3.2.5.F Day 84 (± 7 days)

- small volume apheresis for immune endpoint monitoring as described in 3.2.4.a.
- lymphocyte immunophenotyping
- DTH testing as described in 3.2.4.c.

3.2.5.G Day 126 (± 7 days)

- small volume apheresis for immune endpoint monitoring as described in 3.2.4.a.
- lymphocyte immunophenotyping
- radiographic imaging as described in 3.2.4.b.
- DTH testing as described in 3.2.4.c.
- ECG to assess QTc interval

3.3 Cohort 2 Treatment Administration and Evaluations (See Appendix II)

3.3.1.A Small Volume Apheresis to Obtain Cells for Immune Endpoint Monitoring
Timepoints: Baseline, Day 42± 7 days, Day 84 ± 7 days, Day 126 ± 7 days:

- Patients will undergo a small volume apheresis (approx. one to two blood volumes) to obtain peripheral blood lymphocytes that will serve as the pre-rhIL7 sample for immune endpoint monitoring. This may occur anytime within one week prior to

rhIL7 dosing at baseline and Day 42.

- Every attempt will be made to use an indwelling catheter or peripheral lines for the small volume apheresis. If this is not feasible peripheral blood (1cc/kg) will be used in lieu of the apheresis sample for biologic endpoint monitoring.

3.3.1.B Radiographic imaging (within 3 weeks of enrollment)

- Radiographic studies of all sites of previous disease including but not limited to CT scans or MRI, CT scan of the chest (including simultaneous evaluation of lymph nodes and thymus) and FDG PET scan. These studies may be performed at the home institution.

3.3.1.C CYT107 (r-hIL7): Day 0, 14±7d, 28±7d, 42±7d

- CYT107 will be administered subcutaneously at a dose of 20 mcg/kg in the arm or the leg or abdomen. CYT107 may be administered as an outpatient but patients must be observed in the clinic for 6 hours.
- RESEARCH SAMPLE: Six hours (± 30 minutes) following CYT107 administration, 10mL (red top tube) will be drawn for serum storage.

CYT107 Administration Guidelines

- In obese patients, a corrected weight will be used to calculate the final dose of CYT107 the patient will receive. This corrected dose will be calculated and used if the patient's actual weight gives a Body Mass Index (BMI) at the upper limit of normal (i.e. BMI ≥ 35).– BMI = weight (kg) / height (m2)– Corrected weight (kg) = 35 x height (m2)
- A maximum of 0.45 mL can be collected from one vial. If 2 vials are needed (or any volume over 0.45 mL), divide the volume into two equal injections.
- ECG must be done pre and 3 hours post CYT107 administration, to ascertain for transient changes in the QTc interval that may be potentially related to study drug. Liver panel tests will be evaluated every 48-72 hours post administration for 1 week, and repeated daily for any elevated results, until levels return to grade I or baseline.

- No premedication is required. However, if recurring reactions such as fever, skin rash, or nausea/vomiting appear following dosing, as observed with some other

cytokines, patients may be pre-medicated according to the standard guidelines established to administer these products:

- Administration of acetaminophen and consumption of alcohol is absolutely prohibited within the 48 hours preceding the dose, and within the 48 hours following the dose
- Omeprazole for gastro-duodenal ulcer prevention
- Antihistamine (anti H-1)
- If needed, anti-emetics can also be administered.

- If there is evidence of autoimmune reaction of Grade 2 or greater, CYT107 will be stopped, excluding Grade 2 fever, vitiligo, skin rash, injection site reaction, or localized urticaria.
- Vital signs including temperature should be taken at 1, 3, and 6 hours (± 15 minutes) after administration or more frequently if clinically indicated.

3.4 TREATMENT MODIFICATIONS

Toxicity associated with the tumor vaccines is expected to be mild and limited to local induration and/or erythema. Patients may use non-steroidal anti-inflammatory agents for severe symptoms, but it is not expected that vaccine doses will need to be modified or eliminated due to local reactions. If severe reactions occur which in the judgment of the PI and the treating physicians require cessation of vaccine therapy (see section, 3.2.4.e., vaccine administration) patient will remain on protocol and be monitored for follow-up but subsequent vaccines will not be administered.

Any patient who develops Grade III or IV toxicity (excluding Grade III hematologic toxicity) possibly, probably or likely attributed to CYT107, will not receive further doses of CYT107 but will continue on study. CYT107 will be stopped for any Grade 2 or greater autoimmune toxicity, excluding those that do not affect vital organ functioning such as fever, vitiligo, skin rash, injection site reaction or localized urticaria. Because transient reversible liver enzyme elevations have been observed with r-hIL7, patients must avoid all exposure to alcohol and acetaminophen for 48 hours prior to CYT107 and post CYT107 administration. Grade III liver enzyme elevations 5-10x ULN occurring in patients in Arm B will not require discontinuation of CYT107 if it resolves within 72 hours to Grade 2 and to baseline or ≤ Grade 1 within three weeks. Subsequent doses of CYT107 and vaccines may be delayed by 7 days to allow for toxicity resolution. Grade III liver enzyme elevations >10x ULN regardless of duration will result in discontinuation of CYT107.

For patients in whom CYT107 is discontinued, subsequent vaccines may be administered if toxicity resolves to baseline or ≤ Grade 1 within three weeks. Subsequent vaccine administration may be delayed by 7 days to allow for toxicity resolution. If toxicity does not resolve to baseline or ≤ Grade 1 within three weeks, immunotherapy will be discontinued, and every attempt will be made to continue to monitor for toxicity resolution.

3.5 PHARMACOKINETIC/IMMUNOGENICITY STUDIES

No pharmacokinetic studies will be performed for patients treated on Cohort 1, Arm A. For patients treated on Cohort 1, Arm B, or Cohort 2, r-hIL7 pharmacokinetics and immunogenicity studies will be performed. See Appendix IV for sample collection, processing, and storage for these studies.

3.5.1 Pharmacokinetic Studies

Blood samples (1 mL, in EDTA (2 or 3 mL) tubes) will be drawn at the following time points:

- Baseline (prior to 1st dose of CYT107 administration)
- 2 hours
- 4 hours
- 6 hours
- Approximately 24 hours
- Approximately 48 hours, after 1st dose;
- Day 14 (± 7 days) (prior to the 2nd dose of CYT107)
- Day 28 (± 7 days) (prior to the 3nd dose of CYT107)
- Day 42 (± 7 days) (prior to the 4nd dose of CYT107)

3.5.2 Anti-IL7 antibody tests

Blood samples (1 mL, in Li Heparinate (2-3 mL pediatric) tubes) will be drawn to evaluate specific IL-7 binding via ELISA (screening for antibodies present) and if need be, neutralizing antibody studies, at the following time points:

- Baseline (prior to 1st dose of CYT107 administration)
- Day 35(± 7 days)
- Day 49 (± 7 days) (1 week after last dose of CYT107 administration)
- Day 84 and Day 126 (± 7 days): These samples will be drawn on all patients and cryopreserved, awaiting results of Day 49 testing.
- If Day 49 tests are positive, samples from Day 84 and Day 126 will be shipped to Cytheris for analysis and patients with positive results will continue to have samples drawn at follow up time points until results return negative.

Plasma from Baseline, Day 35 and Day 49 will be analyzed together using an ELISA assay to detect IL-7 binding antibodies. Results of the ELISA will be considered positive if the antibody titer is higher than 1/100.

The ELISA positive samples will be retested in a bio-assay designed to detect IL-7 neutralizing antibodies. Results will be regarded as positive if the antibody titer is higher than 1/400 in this assay. Detection of neutralizing antibodies with a titer > 1/200 and ≤ 1/400 will require the collection of an additional plasma sample around W 13, followed by immediate testing in the bioassay. Results of the neutralization assay will be considered positive if the antibody titer becomes higher than 1/400.

3.5.3 Limits on Blood Volumes for Research Purposes

The amount of blood drawn from adult patient subjects (those 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, in an 8 week period.

The amount of blood drawn for research purposes from pediatric patient subjects (those under 18 years of age) will not exceed 5 mL/kg in a single day, and no more than 9.5 mL/kg over an 8 week period.

3.6 On Treatment Evaluation

3.6.1 Routine Monitoring during Immunotherapy

3.6.1.A History and Physical:

- Cohort 1(Completed and no longer enrolling with Amendment L): Patients will have a history and physical examination, including lymph node and spleen, performed prior to each vaccine during immunotherapy and at each visit, with notation of all sites of known disease and any symptomatic complaints. Patients do not need to be seen by a physician in the weeks between vaccines unless clinically indicated.
- Cohort 2: Patients will have a history and physical examination, including lymph node and spleen, performed prior to each dose of CYT107 and at each visit, with notation of all sites of known disease and any symptomatic complaints. Patients do not need to be seen by a physician in the weeks between CYT107 injections unless clinically indicated.

3.6.1.B Routine Laboratory Studies: Immunotherapy

Cohort 1 (Completed and no longer enrolling with Amendment L):

- Patients should have the following routine labs prior to each vaccine (Day 0, 14, 28, 42, 56, 70) and at the Day 84 and 126 visits (±7 as per Section 3.2.5):
  - CBC with differential
  - Electrolytes (Na, K, Cl, CO_2, Mg, Ca, phosphorus, uric acid, BUN, Cr)
  - liver panel (bilirubin (including conjugated bilirubin), alkaline phosphatase, AST/ALT)
  - urinalysis
  - 10 ml serum storage (red top tube) for cryopreservation at each timepoint. When sampling occurs at designated treatment timepoints, samples should be drawn PRIOR to treatment administration. In addition, a sample will be collected 6 hours after each CYT107 administration. Samples will be sent to the NCI Frederick Clinical Support Laboratory.
  - Plasma Storage for PK and anti-IL-7 Ab (see Section 3.4)
  - Urine pregnancy test for females of childbearing potential
- Post CYT107 administration, patients will have liver panel (bilirubin (including conjugated bilirubin), alkaline phosphatase, AST/ALT) performed every 48-72 hours for 1 week. If elevated, liver panel will be repeated daily until levels return to grade 1 or baseline.
- Prior to vaccine administration on Day 42, Day 84 and Day 126 patients should also have:
  - thyroid function tests, including anti-thyroid antibodies

Cohort 2 (See Appendix II):

- Patients should have the following routine labs prior to each dose of CYT107 (Day 0, 14, 28, 42) and at the Day 84 and 126 visits ±7:
  - CBC with differential
  - Electrolytes (Na, K, Cl, CO_2, Mg, Ca, phosphorus, uric acid, BUN, Cr)
  - liver panel (bilirubin (including conjugated bilirubin), alkaline phosphatase, AST/ALT)
  - urinalysis
  - thyroid function tests including anti-thyroid antibodies
  - 10 ml serum storage (red top tube) for cryopreservation at each timepoint. When sampling occurs at designated treatment timepoints, samples should be drawn PRIOR to treatment administration. In addition, a sample will be collected 6 hours after each CYT107 administration. Samples will be sent to the NCI Frederick Clinical Support Laboratory.
  - Plasma Storage for PK and anti-IL-7 Ab (see Section 3.4)
  - Urine pregnancy test for females of childbearing potential
- Post CYT107 administration, patients will have liver panel (bilirubin (including conjugated bilirubin), alkaline phosphatase, AST/ALT) performed every 48-72 hours for 1 week. If elevated, liver panel will be repeated daily until levels return to grade 1 or baseline.

3.6.1.C Radiographic Imaging

- Spleen ultrasound will be performed before the first administration of CYT107, and within 2 weeks after the last administration of CYT107 to measure the spleen with accuracy
- Patients should have radiographic imaging of all sites of known disease (Including simultaneous assessment of lymph nodes and thymus) and an FDG PET scan during prior to Day 0, Day 42, and Day 126.

3.6.2 Immunologic Monitoring

3.6.2.A Immunophenotyping – Cohort 1 and Cohort 2

- Flow cytometry to monitor lymphocyte subsets will be performed prior at Immunotherapy Baseline, Day 42 (± 7 days), Day 84 (± 7 days) and Day 126 (± 7 days).
- 3 ml of blood should be collected into an EDTA tube and sent to the Clinical Pathology Flow Cytometry Lab, Bldg. 10, Rm 2C410, tel: 301-496-4879. A CBC must be performed on the same day that this specimen is drawn.

3.6.2.B Immune Response monitoring – Cohort 1 only (Completed and no longer enrolling with Amendment L)

- Monitoring for responses to tumor lysate and KLH will be performed by the Laboratory of Cell Mediated Immunity at FCRDC under the direction of Dr. Anatoli Malyguine as described in Section 5.2.
- The responses for baseline pre-therapy, Baseline and Day 126 should be obtained from the apheresis specimen. If possible, the responses for Day 42 (± 7 days) and Day 84 (± 7 days) should also be obtained from a small volume apheresis as

described in Section 3.2.4.b. Specimens collected via apheresis will be sent to the Frederick Clinical Support Laboratory. If an apheresis specimen cannot be collected, peripheral blood should be collected into green top heparinized tubes (1 cc/kg, max 50 cc) and sent to the Frederick laboratory. Attn: Anatoli Maguine, 301-846-5893 via scheduled courier.

- DTH monitoring will be performed as described in Section 3.2.4.c. at Baseline and Days 42, 84 and 126 (±7 as per Section 3.2.5).

3.7 Concurrent Therapies

- Patients should not receive concurrent cytoreductive (radiation therapy or chemotherapy), corticosteroid or other immunosuppressive therapy during the immunotherapy portion of the trial. A time limited course of corticosteroids for a medical condition unrelated to the cancer (e.g. allergic reaction, asthma, poison ivy) is permitted however at least two weeks should elapse between the period of the last dose of corticosteroids and the next vaccine administration. Non-steroidal antiinflammatory agents may be used if clinically indicated.
- Appropriate supportive care to include transfusions, antibiotics and other supportive care may be administered as clinically indicated.

3.8 Surgical & Radiation Therapy Guidelines

Patients will not receive concurrent surgical or radiation therapy for cancer during immunotherapy. If such anti-neoplastic therapy is required during this time, it will be administered according to standard treatment guidelines and the patient will be removed from study. Any surgery or radiation therapy for the primary tumor will be dictated by the patient's primary oncology team and will not be considered protocol directed therapy.

3.9 Off Treatment and Off Study Criteria

3.9.1 Off-Treatment Criteria

- Progressive disease during immunotherapy will not, in and of itself, require removal from study. However, if progressive neoplastic disease requires intervention (e.g. radiation therapy, surgery or chemotherapy) during immunotherapy, patients will be removed from study. Furthermore, if the patient develops a diminished performance status (e.g. ECOG 3 or 4 or Lansky ≤50) due to progressive disease, he/she will be removed from study.
- Patients may voluntarily withdraw from the treatment at any time.
- If toxicity possibly, probably or likely attributed to the investigational agent(s) (excluding Grade III hematologic toxicity) does not resolve to baseline or ≤ Grade 1 within three weeks, immunotherapy will be discontinued, however every attempt will be made to continue to monitor such individuals for toxicity and resolution of toxicity over time.
- Non-compliance or an inability to comply with the protocol requirements may render the patient unable to continue enrollment. This decision will be made by the principal investigator.
- For patients removed from treatment, every attempt will be made to complete and report

their observations as thoroughly as possible up to the date of removals. We will also attempt to continue to monitor such individuals for any toxicity that may occur related to the immunotherapy.

3.9.2 Off Study Criteria

Every attempt will be made to annually contact patients removed from this study to obtain survival data. Subjects will be removed from study for:

- Death
- Patients may voluntarily withdraw from the study at any time.
- Lost to follow up

3.10 POST-TREATMENT EVALUATION:

At the conclusion of treatment requirements (Day 126 [See Section 3.2.5.6]) patients may return to the care of their referring physician. The primary care giver will be requested to supply the study team with documentation of H &P and scan results, and send research blood samples according to the following table:

F/u timepoint (post Day 0) (± 30 days)	H&PE	Blood & Urine*	Chest CT	CT/MRI of primary	PET scan	Immune monitoring §	Flow£
WK 24 (6 mo)	X	X				X	
WK 30 (7.5 mo)	X	X	X	X	X	X	
WK 36 (9 mo)	X	X				X	
WK 42 (10.5 mo)	X	X	X	X	X	X	X
WK 54 (13.5 mo)	X	X	X	X	X	X	X
WK 70 (17.5 mo)	X	X	X	X	X	X	X
WK 90 (22.5 mo)	X	X	X	X	X	X	X
3 year	X	X	X	X	X	X	X
4 year	X	X	X	X	X	X	X
5 year	X	X	X	X	X	X	X

* CBC, differential, Na, K, Cl, CO_2, BUN, creatinine, albumin, Ca, Mg, Phos, SGOT, SGPT, bilirubin, alkaline phosphatase, uric acid, urinalysis, thyroid function tests and anti-thyroid antibodies.

§ 1 cc/kg (max 50 cc) collected in a green top tube and sent to to NIH.
Immune monitoring labs to be sent to arrive Tu-Th (NON gov't holidays). Please notify prior to sending.
Send to: Donna Bernstein
NIH/NCI/POB
9000 Rockville Pike
Building 10/Room 1W-3750
Bethesda, MD 20892
Fax: 301-451-5746

Phone: 301-435-7804
Email: bernsted@mail.nih.gov

£ Flowcytometry should be drawn in EDTA tube(s): 8 mL are required. CBC/Diff must be drawn the same day and results faxed to Donna Bernstein at 301-451-5746. THIS TEST MUST BE SCHEDULED IN ADVANCE WITH THE LAB AND **CANNOT** BE DONE ON A FRIDAY, so please notify the lab when planning to send the sample. Sample should arrive Tue-Thurs (NON gov't holidays).

4 SUPPORTIVE CARE

- PCP prophylaxis should be administered until 9 months following completion of cyclophosphamide/fludarabine, 6 month following completion of multiagent chemotherapy or 9 months following receipt of autologous stem cell transplantation.
  - The first choice is trimethoprim/sulfamethoxazole DS tablet (TMP160/SMX800), one tablet orally three times weekly on consecutive days. For children who cannot swallow the tablets, a suspension of trimethoprim 40 mg/sulfamethoxazole 200 mg/5 ml should be administered at a dose of 75 mg/m^2 BID three times weekly at a dose not to exceed the adult dose.
  - For patients who cannot tolerate TMP/SMX prophylaxis, alternative should be administered according to the "Guidelines for Infection Management in Allogeneic Transplant Recipients" which is available on the Pediatric Oncology Branch server.
  - Blood products may be administered as necessary for supportive care, but must be irradiated as per standard procedure for immunocompromised patients.
- Patients will be followed clinically for the development of autoimmune disease. Autoimmune colitis is expected to be the most likely site based upon the murine studies of Treg depletion during lymphopenia. If patients develop diarrhea which is <1000 cc/d (<15 cc/kg), then routine supportive care including intravenous fluids with monitoring for C. difficile colitis should be performed. If the diarrhea is >1000 cc/d (>15 cc/kg/d) or is accompanied by fever, severe abdominal pain or ileus, then evaluation for evidence of inflammatory autoimmune colitis (e.g. stool for leukocytes, radiographic imaging and consultation with a gastroenterologist) should be obtained. If autoimmune colitis occurs, every attempt to confirm this via biopsy should be made and treatment should be initiated with a corticosteroid based regimen in collaboration with gastrointestinal consultation.
- Other areas of the body could also incur autoimmune reactivity including (but not limited to) skin, liver, lungs, eyes, brain, etc. Patients will be monitored clinically with history and physical examination for the development of autoimmune reactions. If evidence for autoimmune inflammatory disease occurs, appropriate subspecialty consultation will be obtained and patients will be treated with immunosuppressive drugs to attempt to stop the reaction. First line will be corticosteroids either topically or systemically with weaning based upon clinical response. In other cases where autoimmunity has developed following immunotherapy for cancer, the autoimmune reactions have been controlled using supportive care and corticosteroids[69].

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

The NCI, POB research team will be responsible for collection and maintenance of the study data. All data from source documents will be recorded by the designated research nurse, or data manager onto individual case report forms. Toxicity will be entered into the electronic C3D database at least every two weeks. Response data will not be available until the patient has completed therapy at which time the assays will be run in a batch for each patient. Results will be recorded within 2 weeks of completion of immune response assays. Source documents will be defined as original documents, data and records which can include hospital records, clinical and office charts, lab data and information from patient diaries or evaluation checklists, pharmacy records, data from automated instruments, photographs or digital media and x-rays.

5.2 RESPONSE CRITERIA

5.2.1 Timing of Response Assessment

For Cohort 1(Completed and no longer enrolling with Amendment L), immune response to the tumor vaccine will compare responses from Baseline to those at the completion of immunotherapy (Day 126 (± 7 days)). Immune responses will also be monitored at the time of clinical presentation (baseline evaluation prior to initiation of standard therapy) and at Day 42 and Day 84 (± 7 days) time points, but the primary objective will be based upon changes in immune responses between baseline and Day 126 (± 7 days). For Cohort 2, immune response assessment will be limited to parameters of immune reconstitution, including but not limited to lymphocyte subsets and lymphocyte repertoire diversity. They will be monitored in an exploratory fashion with endpoints compared between patients treated on Cohort 1 and Cohort 2 based upon analysis upon initiation of immunotherapy, Day 42, Day 84 and Day 126.

5.2.2 Response Definition – Cohort 1 only (Completed and no longer enrolling with Amendment L). Patients on Cohort 2 will not be evaluated for vaccine responses.

A positive response to the tumor vaccine requires a positive reaction in at least one of the two assays described below:

1) The presence of a positive DTH reaction to the tumor lysate in a patient who did not show a positive DTH reaction prior to immunotherapy. A positive reaction is induration of at least 0.5 cm. Patients will also be analyzed for DTH reactions to KLH but a positive response to KLH will not meet the criteria for a positive immune response on this trial.
2) The presence of a net T cell response post-immunotherapy via Elispot that is at least twice that observed pre-immunotherapy and which represents an increase of at least 10 spots counted.
    - Elispot will be measured for IFNγ production and for granzyme B. Either IFNγ or granzyme B production that meets the criteria listed above will

constitute an immune response.

- Where possible, an immune response will be measured to autologous tumor lysate, which is pulsed onto autologous dendritic cells and/or autologous PBMC. Net response will be that which remains following subtraction of the response obtained using lysate from autologous EBV-LBL pulsed onto DCs or PBMC.
- If sufficient tumor lysate is not available to perform these studies, allogeneic tumor lysate may be utilized and an allogeneic lysate from the same individual's EBV-LBL will serve as the background for allogeneic reactivity. In this case, the reactivity will be measured to autologous DC or PBMC pulsed with allogeneic lysate minus the reactivity to autologous DC or PBMC pulsed with allogeneic EBV-LBL and the net reactivity (the reactivity to lysate minus the reactivity to EBV-LBL) must be two-fold and 10 spots greater post-immunotherapy than prior to immunotherapy.
- The procedure used for the Elispot analyses will follow Standard Operating Procedures developed and validated in the Laboratory of Cell Mediated Immunity at FCRDC under the direction of Dr. Anatoli Malyguine. This validation of inter- and intra-assay variability has been performed with peer review and meets acceptable criteria for responses in an early phase clinical trial such as this.

5.2.3 Clinical Response Assessment

Where present, measurable or evaluable disease will be followed during immunotherapy and will be reported descriptively with regard to clinical responses using the standard RECIST criteria. Because measurable or evaluable disease is not expected to be present in most patients, this will a descriptive rather than statistically based endpoint.

5.3 TOXICITY CRITERIA

This study will utilize the CTCAE version 3.0 for toxicity and adverse event reporting. A copy of the CTCAE version 3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). All appropriate treatment areas should have access to a copy of the CTCAE version 3.0.

5.4 STATISTICAL SECTION

5.4.1 Protocol Objectives and Design

5.4.1.A <u>Cohort 1 (Completed and no longer enrolling with Amendment L):</u>

1) To determine the feasibility of obtaining sufficient tumor samples for tumor lysate-pulsed dendritic cell vaccination in patients with metastatic or recurrent chemosensitive solid tumors. As of July 15, 2009, 13/14 patients enrolled have had sufficient lysate obtained to vaccinate on this trial, therefore this feasibility has been demonstrated.

2) To determine if patients who have sufficient tumor samples for tumor lysate pulsed dendritic cell vaccination will develop immune responses toward tumor and KLH ± r-hIL7

following immunization. As of May 2013, of 5 evaluable patients in Cohort 1, Arm A, one showed evidence for an immune response (20%). Of 21 patients in Cohort 1, Arm B, 8 showed evidence for an immune response (38%). The differences between Arm A and 2 were not significant by chi-square.

3) To determine if the treatment being administered in this protocol is safe and to report the toxicity experienced. As of May 2013, we had no persistent Grade 2, no Grade 3 or 4 toxicities in Cohort 1, Arm A. In Cohort 1, Arm B, there were no persistent Grade 2 toxicities. There was one grade 4 fever, likely caused by the immunotherapy regimen but this was transient. There was one grade 3 allergic reaction, definitely due to IL-7. There were also three grade 3 transaminitis observed, likely due to IL-7, all self-limited. Thus, we judged the regimen to be safe.

For Cohort 1, the study will evaluated the immune responses in patients with adequate tumor samples for 3 vaccines. Immune response will be determined according to the specifications detailed in section 5.2.

For patients treated on Cohort 1, Arm A, a one-stage design as utilized to evaluate immune response, because a two stage design would be impractical after taking into consideration the long time period, which elapses between enrollment and response. A two stage design could result in sizable numbers of patients being enrolled but being ineligible to receive therapy if the trial were to close due to limited responses in an initial cohort. A single stage design based on an exact binomial test for a single proportion, as determined using nQuery advisor version 5, will be used. It will be considered desirable if 50% of evaluable patients experience an immune response to the vaccine (p1=0.50) and an unacceptably low proportion if only 25% do so (p0=0.25). Using a one–sided alpha=0.10 level test with 90% power, a total of 28 patients may be accrued in order to evaluate immune response. Based upon our previous trial, it can be expected that approximately 25% of patients who enroll for apheresis and tumor lysate harvest will not return for immunotherapy due to death, disease progression or refusal. Although such patients will not be evaluate for the primary endpoints, the acquisition of the tumor biopsy and the apheresis will allow us to explore the secondary endpoint which involves identifying tumor antigens in these diseases, therefore we believe this level of anticipating rate of inevaluable patients is acceptable and unavoidable in these dire diseases. Therefore a total of 40 patients will be enrolled in order to have a reasonable chance (82% probability or greater, with 75% probability of returning) of treating 28 patients who will be evaluable for response to the immunotherapy. With 28 evaluable patients, if 0-10 of 28 have an immune response, this will be considered inadequate, while if 11 or more have an immune response, this will demonstrate an adequate immune response rate for further consideration, since the lower bound of a one-sided 90% confidence interval about 11/28 is 26.7% while that about 10/28 is 23.5%. As of July 15, 2009, a total of 4 patients had completed immunotherapy. Thus far, no evidence for DTH responses to tumor lysate have been observed although we have seen impressive DTH responses to KLH in all patients thus far. Similarly, of three patients in whom biologic responses have been completed, all patients have had impressive responses to KLH whereas only 1 has met the criteria for a positive response to tumor lysate. In that patient, the net increase in number of spots/million peripheral blood mononuclear cells

post-immunotherapy was 10 spots at week 8 and 13 spots at week 14, with no positivity seen at week 20. While this can be characterized as positive, it is a weak response. In contrast, KLH responses have been substantial averaging over 200 spots/million PBMC. Thus, we believe that these early results suggest that the potency of the tumor lysate response is not sufficient to warrant further development using the same regimen.

After a total of 5 evaluable patients were treated on Cohort 1, Arm A, and a total of 25 patients were treated on Cohort 1, Arm B, which was exactly the same regimen as Arm A except for the addition of CYT107 (r-hIL7) 20 mcg/kg administered before the first 4 vaccines. Patients treated on Arm B were evaluted separately from those on Arm A using the same basic statistical schema. Toxicity data in Arm B will be analyzed for two strata, participants under 12 years of age, and participants 12 years of age and older. Specifically, for patients treated on Arm B, a one-stage design was utilized for the trial to evaluate immune response. A single stage design based on an exact binomial test for a single proportion, as determined using nQuery advisor version 5, will be used. It will be considered desirable if 50% of evaluable patients experience an immune response to the vaccine (p1=0.50) and an unacceptably low proportion if only 25% do so (p0=0.25). Using a one–sided alpha=0.10 level test with 90% power, a total of 28 patients may be accrued in order to evaluate immune response. Based upon our previous trial, it can be expected that approximately 25% of patients who enroll for apheresis and tumor lysate harvest will not return for immunotherapy due to death, disease progression or refusal. Therefore a total of 47 patients will be enrolled in order to have a reasonable chance (82% probability or greater, with 75% probability of returning) of treating 28 patients who will be evaluable for response to the immunotherapy. With 28 evaluable patients, if 0-10 of 28 have an immune response, this will be considered inadequate, while if 11 or more have an immune response, this will demonstrate an adequate immune response rate for further consideration, since the lower bound of a one-sided 90% confidence interval about 11/28 is 26.7% while that about 10/28 is 23.5%.

As of May 2013, a total of 8/21 patients treated on Cohort 1, Arm B had evidence for an immune response to the vaccine, which is 38% and deemed interested for further study. Future plans are therefore to create an off-the-shelf vaccine derived from a Ewing tumor cell line that could be used to administer a tumor vaccine following completion of frontline therapy and avoid the labor, discomfort and expense associated with tumor lysate acquisition. The off-the-shelf vaccine is not yet available and therefore we will enroll subsequent cohorts on this trial sequentially, to maximally capture patients with metastatic Ewing sarcoma which is a rare disease and avoid long periods of non-enrollment on this regimen.

5.4.1.B Cohort 2

The objective of amendment L is to enroll a small number of patients with metastatic Ewing sarcoma following standard frontline therapy, to determine if the use of rhIL7 alone as a sole immunorestorative will be associated with a meaningfully high probability of survival.

This will be conducted as a small pilot arm within the trial, with an intent to enroll at least 11 evaluable patients based upon the following statistical justification. While more than

90% of patients with metastatic Ewing sarcoma are rendered into a state of no evidence of disease following standard frontline therapy, it is generally accepted that they have an approximately 20% 3 year survival from diagnosis due to relapse following completion of therapy. Thus, an improvement to an estimated 50% 3-year survival would be interesting for further study. Using the method of Brookmeyer and Crowley (ref: Brookmeyer R and Crowley, JJ. A confidence interval for the median survival time. *Biometrics,* 38, 29-41, 1982), with 11 patients enrolled in a single arm study, there would be 80% power to test whether the OS for the patients on Cohort 2 have a 50% 3 year survival from diagnosis rather than a 20% 3 year survival, using a one-tailed 0.10 alpha level significance test, assuming 12 months of accrual and an additional 36 or more months of follow-up. In practice, the Kaplan-Meier curve for OS will be constructed and presented, along with 80% and 95% confidence intervals at selected time points, particularly at approximately 36 months. Given the limited number of patients to be studied, these will be interpreted as pilot results and will be used to guide further development of the treatments in this population. In order to allow for a small number of inevaluable patients and to gain experience with this approach in this disease, up to 15 patients may be enrolled on Cohort 2 under this amendment.

5.4.2 Early Stopping Rules

Cohort 1 (Completed and no longer enrolling with Amendment L): An early stopping rule for feasibility based upon the ability to harvest adequate tumor for lysate generation will also be incorporated into the trial design. This will be done at an early enough point to ensure that no more than 14, about a third of the overall ceiling of 40 patients, are explored to ensure feasibility before proceeding with full efforts to accrue more patients onto the trial. It would be desirable and worthwhile undertaking the procedure outlined in this protocol if 85% of patients could produce sufficient tumor for evaluation. On the other hand, if only 50% are able to produce sufficient tumor for evaluation, this would be inadequate. If there is adequate material for 3 vaccinations for a given patient, this will be considered minimally adequate and feasible for the patient, while the goal is to produce enough for 6. Based on binomial probabilities, if 14 patients are enrolled and evaluated for tumor specimen adequacy, the probability of having 10 or more with adequate specimens will be 95.3% if the true probability of providing an adequate specimen is 85%; on the other hand, the probability of having 10 or more with adequate specimens will be 9.0% if the true probability of providing an adequate specimen is 50%. Thus, if 14 patients have been enrolled and fewer than 10 provide specimens that are adequate, then the trial will cease enrollment due to the limited feasibility of obtaining sufficient tumor samples for tumor lysate-pulsed dendritic cell vaccination. If this initial feasibility evaluation and stopping rule has not resulted in accrual termination, then all patients enrolled on the study will be used to determine an overall fraction of patients who are feasibly able to produce the required tumor specimens. A 95% confidence interval will be formed about this overall fraction of all enrolled patients who have adequate tumors for evaluation. As of July 16, 2009, 13/14 patients enrolled have had sufficient lysate obtained to vaccinate on this trial, therefore feasibility has been demonstrated and this stopping rule is now moot.

While the goal of this protocol is to administer immunotherapy to patients in Cohort 1, Arm A during lymphopenia as defined by a CD4+ count of < 200 cells/mcl, we will attempt

to minimize risk of the cytotoxic chemotherapy administered to this heavily pretreated population. Therefore, if at any point during the course of the trial, 2 patients develop unacceptable Grade IV toxicity related to the cyclophosphamide/fludarabine regimen, no further patients will receive this regimen. Grade IV expected toxicities that will be excluded from this early stopping rule include neutropenia, thrombocytopenia, lymphopenia, leukopenia, anemia and infection with or without neutropenia. If this occurs, patients with a CD4+ count > 200 cells/mcl will receive immunotherapy, but will be evaluated separately for the primary endpoints.

A potential toxicity anticipated from the immunotherapy used in this trial is autoimmunity and specifically, autoimmune colitis. Since this is a potentially serious condition, the study will continue to enroll patients according to the plan above provided that no more than one patient develops grade 2 autoimmunity not reversible within 72 hours, requiring immunosuppressive therapy, excluding those that do not affect vital organ functioning such as fever, vitiligo, skin rash, injection site reaction or localized urticaria. If a second patient develops this degree of toxicity, then the trial will require amendment before allowing any additional patients to be enrolled. Any toxicities of grade 2 or higher, which are at least possibly attributable to the treatment being evaluated in this protocol, will be reported by grade and type of toxicity.

5.4.3 Secondary Objectives

Event free and overall survival will be evaluated in patients who are able to receive at least 3 vaccinations. Since this pilot study is not designed to evaluate this as a specific endpoint, the evaluation will be reported as being exploratory but will include both survival in patients that received immunotherapy and survival of the entire cohort enrolled (e.g. intention-to-treat analysis). All other evaluations performed on these patients will be done with exploratory intent and any statistical tests performed to evaluate secondary objectives will be done without formal adjustment for multiple comparisons. The context in which the tests are being performed will be carefully reported.

5.4.4 Accrual Projection

Cohort 1 Completed and no longer enrolling with Amendment L): Based upon accrual rates seen in 97-C-0052, it is expected that 1 patient per month could enroll onto this trial; thus an accrual period of 3 – 4 years is anticipated.

Cohort 2: There are currently no competing trials currently open for metastatic Ewing sarcoma within the Children's Oncology Group and substantial interest in this study, therefore we expect to enroll 15 patients within one year.

5.5 DATA SAFETY MONITORING PLAN

The Principal Investigator will review all adverse events. Adverse events will be reported to and tracked by the NCI IRB. Continuing review reports will be submitted to the NCI IRB and the FDA every year. If trends are noted and/or risks warrant it, accrual will be interrupted and/or the protocol and/or consent document will be amended accordingly.

5.6 HANDLING OF TISSUE SPECIMENS FOR RESEARCH PURPOSES

Blood and tissue collected during the course of this study will follow the handling procedures established by the Central Repository for NCI/Frederick. Blood and tissue processed by the Department of Transfusion Medicine (DTM), Clinical Center will comply with the Standard Operating Procedures of DTM. All samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators either in the Central Repository for NCI/Frederick or in the investigator's laboratory (CRC, 1 NW) at specified temperatures with alarm systems in place. All samples (blood or tissue) are documented in a secure central computer database with identification and storage location, with computer backup according to established standards for the Central Repository for NCI/Frederick. Samples without patient identifiers (using only coded identification) will be shipped according to specifications provided by Cytheris Inc. for PK and Immunogenicity tests. Upon completion of these tests as specified in Section 3.4, excess samples will be disposed of as outlined below.

At the termination of this protocol, if additional studies are to be performed on any samples retaining patient identifiers, obtained during the conduct of this trial, a Request to Conduct Research for Stored Human Samples Specimens, or Data Collected in a Terminated NCI-IRB Protocol will be submitted. Otherwise, specimens will be disposed of in accordance with the environmental protection laws, regulations and guidelines of the Federal Government and the State of Maryland.

Any loss or unintentional destruction of the samples will be reported to the IRB. During the conduct of this study any new use of research samples must be reviewed and approved by the NCI IRB prior to implementation.
5.6.1.A.1

6 HUMAN SUBJECTS PROTECTIONS

6.1 RATIONALE FOR SUBJECT SELECTION

The rationale for limiting enrollment to Ewing sarcoma for Cohort 2 is based upon the fact that the driving force for this cohort is the favorable overall survival seen in patients with newly diagnosed metastatic small round blue cell tumors enrolled on Cohort 1 when compared to historical controls and when compared to the same populations enrolled on 97-C-0052. However, of the 12 patients with newly diagnosed metastatic disease who received immunotherapy on Cohort 1, 11 had metastatic Ewing sarcoma and only one had rhabdomyosarcoma. Therefore the vast majority of patients referred for this study have Ewing sarcoma. Furthermore, treatment and outcomes for metastatic Ewing sarcoma are more standard than for metastatic rhabdomyosarcoma, where differences in age and histologic subtype are more profound and make interpretations of results from small series more difficult. Thus, we have chosen to focus future development of this immune reconstitution based platform on patients with newly diagnosed metastatic Ewing sarcoma, as 1) outcomes are judged to be predictable across series and upfront regimens are highly standardized, 2) limited other options are available for this population thus diminishing problems of competing trials and 3) patients with this histology have accrued briskly to our trials and thus we have established a robust referral base for this disease, increasingly the feasibility of completing these studies efficiently.Patients from all racial and ethnic groups

are eligible for this trial if they meet the eligibility criteria. Efforts will be made to extend accrual to a representative population, but in a small pilot trial, it may be difficult if not impossible to achieve complete balance in this regard. The trial will be listed on the NCI and NIH websites and clinical trials search sites. Individuals with HIV disease are not eligible for this protocol, due to confounding effects of the immunodeficiency on vaccine responses. Individuals who are pregnant or lactating will not be candidates for this protocol, due to risk to the fetus or newborn.

6.2 PARTICIPATION OF CHILDREN

The age range of patients eligible for this trial is 18 months to 35 years at the time of diagnosis. Patients will be cared for by the physicians, nurses, and multidisciplinary support teams of the POB and CC. The POB has expertise in providing care for children and young adults with complex oncologic disorders and complications of therapy. Full pediatric support and subspecialty services are available at the NIH CC. The risks to the patients are related to the potential induction of autoimmunity as a result of tumor directed immunotherapy. The risk of autoimmunity due to dendritic cell vaccination alone appears to be low on the basis of several clinical reports, but this could be enhanced when administered to lymphopenic hosts with CD25 depleted autologous lymphocytes. If autoimmunity is induced, it is likely that it could be readily reversed using immunosuppression although the possibility of irreversible organ damage cannot be entirely excluded. The therapy also involves the risks associated with the cytotoxic agents cyclophosphamide and fludarabine which include infection, second malignancies, bone marrow damage and sterility. Although the overall approach is investigational, a central goal of this pilot trial is to determine the feasibility and efficacy of this approach in generating tumor specific immunity in pediatric sarcomas and neuroblastoma. Hence, patients will be enrolled and treated with therapeutic intent and the response to therapy will be monitored closely. Therefore, this protocol involves greater than minimal risk to children, but presents the prospect of direct benefit to individual subjects

6.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Although some patients will receive cytotoxic therapy as part of this trial, they will receive similar therapy as part of their primary treatment regimen therefore it is unlikely that the cytotoxic therapy administered here will significantly add to their risk of acute or late effects from these drugs. The primary risks to patients participating in this research study therefore are related to the potential induction of autoimmunity as a result of tumor directed immunotherapy. The risk of autoimmunity following tumor lysate pulsed dendritic cell vaccination alone appears to be negligible on the basis of several clinical reports, but this could be higher when DC vaccines are administered to lymphopenic hosts which have been given CD25 depleted autologous lymphocytes. In an attempt to mitigate the risk patients will be watched closely for the development of autoimmunity during the course of the trial. If autoimmunity is induced, appropriate subspecialists for the organs involved will be consulted and patients will be treated with immunosuppression. Based upon the responsiveness of organs to acute immune reactions in the context of GVHD and anti-CTLA4 therapy, we believe it is likely that autoimmunity that may be induced will be

reversible with corticosteroid therapy. However the possibility of irreversible organ damage cannot be entirely excluded.

Another potential risk to patients enrolled on this trial is the possibility that tumor cells contained in the lymphocyte products may be infused as part of the autologous lymphocyte infusion. Previous studies have demonstrated that approximately 30% of patients who have apheresis at the time of diagnosis will show levels of tumor contamination ranging from 1 cell in 1-10 million cells. This trial will use 8H9 in an attempt to purge contaminating tumor cells, an approach which we have demonstrated to result in approximately 4 log reduction in tumor contamination. Nonetheless, it remains possible that some contaminating cells will evade purging and be infused with the cellular product.

Another potential risk to patients enrolled on this trial (Cohort 1, Arm B) is the possibility that exogenous r-hIL-7 (CYT107) treatment will enhance the risk of autoimmunity following vaccines. The potential risks to patients enrolled in Cohort 2 are more limited and reflect those related to exogenous r-hIL-7 (CYT107) treatment without the vaccines. Previous and ongoing studies with CYT107 have shown a good safety profile. The most frequent adverse reactions are local injection site reactions, mainly grade 1 or 2. In rare cases, increased lymphadenopathy and/or hepatosplenomegaly, with or without pain, discomfort, or anatomic compromise of vital structures such as blood vessels or nerves have been reported. Increased liver function tests have also been reported at higher doses of IL-7 than those that will be used in this study. These risks will be assessed throughout the study period by history and physical examination, laboratory testing, and diagnostic studies.

Potential benefits to patients enrolled on this trial in Cohort 1 include the possibility that the cellular infusion will help to more rapidly restore natural immunity toward their tumors and the possibility that the vaccines may induce tumor directed immunity. Survival of patients treated on our previous trial using autologous lymphocyte infusions revealed favorable overall survivals compared to historical controls suggesting that immunotherapy involving autologous lymphocyte infusions is not harmful and may be beneficial. Thus patients treated on this trial will sustain a small risk of autoimmunity and tumor cell reinfusion but will also potentially benefit by experiencing an augmentation of their immune responses to their tumors.

The favorable overall survival seen in patients with newly diagnosed metastatic small round blue cell tumors enrolled on Cohort 1 when compared to historical controls and when compared to the same populations enrolled on 97-C-0052, have given us some indication of benefit. Cohort 2 will determine in a pilot fashion if the use of rhIL7 alone as a sole immunorestorative will be associated with a meaningfully high probability of survival as shown in Cohort 1.

6.4 DISCUSS WHY THE RISKS TO SUBJECTS ARE REASONABLE IN RELATION TO THE ANTICIPATED BENEFITS IN RELATION TO THE IMPORTANCE OF THE KNOWLEDGE THAT MAY REASONABLY BE EXPECTED TO RESULTS

Survival for the populations of patients eligible for enrollment on this trial ranges from <20-40%. Therefore, the population targeted has a very high risk of dying from their disease. Although the overall approach is investigational, a central goal of this pilot trial is to determine the feasibility and efficacy of this approach in generating tumor specific

immunity in pediatric sarcomas and neuroblastoma. Preclinical data and data from other studies suggest that immune responses to such therapies are likely to be induced and that such immune responses may be beneficial in preventing or delaying disease recurrence. Therefore, although the therapy is investigational, patients will be treated with therapeutic intent and the response to therapy will be monitored closely. This protocol involves greater than minimal risk to children, but presents the prospect of direct benefit to individual subjects.

6.5 CONSENT AND ASSENT PROCESSES AND DOCUMENTS

The investigational nature and research objectives of this trial, the procedures and treatment involved and their attendant risks and discomforts and potential benefits, and alternative therapies will be carefully explained to the patient and/or the patient's parents or guardian if he/she is a child. A signed informed consent document will be obtained prior to entry onto the study. The investigators are requesting a waiver from the IRB to allow only one parent to sign the informed consent to enter a child on the protocol. Because many patients must travel to the NIH from long distances at substantial expense, requiring both parents to be present for the consent process could be a financial hardship for many families. The PI or an associate investigator on the trial will obtain consent. Where deemed appropriate by the clinician and the child's parents or guardian, the child will be included in all discussions about the trial and verbal assent will be obtained. The parent or guardian will sign the designated line on the informed consent attesting to the fact that the child has given assent. The attached informed consent documents contain all elements required for consent. In addition, the principal investigator, associate investigator, or their designee will be available to answer all questions from patients, and their parents or guardians. Because substantial time is likely to have elapsed between the initial consent and protocol enrollment and the initiation of immunotherapy, the entire consent/assent process will be repeated and a new consent form signed prior to initiation of immunotherapy.

Telephone consent may be employed in order to obtain tissue from prospective subjects for the tumor lysate manufacture in Cohort 1. Patients who meet all other criteria for enrollment to the apheresis/biopsy portion of this study and who are unable to travel to the NIH for biopsy or surgery may be consented via telephone according to CC MAS 77-2(rev). In such cases, a protocol investigator will review the Consent form by telephone. The consent/assent signatures will be witnessed and a copy will be faxed to the investigator and the original sent by mail to the PI. Prospective subjects who consent to send such samples for outside testing will be reconsented at the NIH prior to evaluation for participation in the immunotherapy portion of the study. Subjects and their referring medical team will be notified of the results and records will be maintained with the protocol research files.

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY

MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Events

An Adverse Event (AE) is defined as any untoward or unfavorable medical occurrence in a human subject (physical or psychological), including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is also considered an AE.

AEs should be reported up to 30 days following the last dose of study drug. AEs that are considered treatment related, expected, continuing, but not resolvable by 30 days after treatment completion (e.g., alopecia) will not be followed after the 30-day period.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience

- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

A protocol deviation is any change, divergence, or departure from the IRB approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 ABNORMAL LABORATORY TEST RESULTS AND REPORTING OF ABNORMAL POST-RELEASE STUDIES ON CLINICAL PRODUCTS

All clinically important abnormal laboratory tests occurring during the study will be

repeated at appropriate intervals until they return either to baseline or to a level deemed acceptable by the investigator, or until a diagnosis that explains them is made. For abnormal results on cellular products released and administered to patients that are found following administration, the patient and the patient's caregiver will be notified of the finding and appropriate surveillance as medically indicated for infection will be performed. In addition, these findings will be included in the continuing review reports on this trial.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

7.3 NCI-IRB REPORTING

7.3.1 NCI-IRB Expedited Reporting of Unanticipated Problems, and Deaths

The Protocol PI will report to the NCI-IRB:

- All deaths, except deaths due to progressive disease
- All Protocol Violations or Deviations
- All Unanticipated Problems
- All serious non-compliance (non-compliance that increases risks or causes harm to participants, decreases potential benefits to participants, compromises the integrity of the NIH HRPP, or invalidates study data)

Reports must be received by the NCI-IRB within 7 working days of PI awareness via iRIS.

7.3.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
    - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
    - All Grade 5 events regardless of attribution;

- All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.3.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3.4 Serious Adverse Event Reporting on Cell Therapy Products to the NCI IRB and FDA (for Cohort 1):

a. If a cell product deviation (or any component thereof) occurs during or after its manufacture, a report of the event, and information relevant to the event, associated with the manufacturing will be submitted to the NCI IRB and the FDA by the PI, in collaboration with CPS/DTM, to include testing, processing, packing, labeling, or storage, or holding or distribution of the product, if the event meets the following criteria:
   (i) Represents a deviation from current good manufacturing practice, applicable regulations, applicable standards, or established specifications that may affect the safety, purity, or potency of that product; or
   (ii) Represents an unexpected or unforeseeable event that may affect the safety, purity, or potency of that product;
b. Cell product deviations will be reported as soon as possible but at a date not to exceed 45-calendar days from the date the PI/research team or CPS, DTM acquired information reasonably suggesting that a reportable event has occurred.
c. FDA reporting: Completed Form FDA-3486, Biological Product Deviation Report on cell product deviations will be sent the Director, Office of Compliance and Biologics Quality, Center for Biologics Evaluation and Research (HFM-600), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448.
d. NCI IRB reporting: Information may be sent via the NCI iRIS Application: **https://iris.nci.nih.gov/iMedris/**

7.4 IND Sponsor Reporting Criteria

An investigator must immediately report to the sponsor any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

7.4.1.A.1 Serious adverse events reporting to r-hIL-7 pharmaceutical company (Cytheris)

The Sponsor will provide the Safety Contact for Cytheris Pharmaceutical Group as specified in the Clinical Trial Agreement (CTA), via FAX, e-mail, or international courier, when copies of SAE reports are sent to the FDA. All SAE reports sent to Cytheris will include the investigator's assessment of causality. Reports will be sent only to the designated Safety Contact per the CTA.

7.4.1.A.2 Critical Events Notification for r-hIL-7 pharmaceutical company (Cytheris)

The sponsor of r-hIL-7 pharmaceutical company (Cytheris Inc) will be notified of any of the following critical events that could lead to discontinue r-hIL-7 treatment, if deemed appropriate:

- Any grade 3 or 4 AE or laboratory abnormality considered by the PI to be probably or definitely related to r-hIL-7/CYT107. The following exceptions are permitted:
  - A Grade 3 LFT increase which satisfies the following 3 conditions:
    1) The duration of these changes must remain limited. LFTs must have decreased:
       - to a maximum of 5 X ULN within the 7 days following the diagnosis of the grade 3 or
       - to a maximum of 2.5 X baseline level (without exceeding 5 X ULN) within the 14 days following the diagnosis of this grade 3
    2) Conjugated bilirubin and alkaline phosphatase should remain < 1.5 X ULN
    3) PT remains > 70%
  - Any grade ≥ 3 increase of LFTs should be monitored at least 2 times per week until LFTs decrease to grade 2 or lower.
- Any diagnosis of lymphoma confirmed by a pathologist
- Any adenopathy compromising or threatening organ function (e.g. mediastinal adenopathy inducing a respiratory distress; inguinal adenopathy inducing an edema of the lower limb; or any adenopathy threatening skin breakdown).

7.5 FDA Reporting Criteria

7.5.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

7.5.1.A Expedited reporting to the FDA

The Sponsor will notify FDA via phone, fax, or email of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information. This will be followed with a written report within 15 days using the MedWatch Form 3500a.

The study Sponsor will notify FDA in writing of any suspected adverse reaction that is both serious and unexpected as soon as possible but no later than 15 calendar days after

initial receipt of the information using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.
The study Sponsor will also report expeditiously as above:

- any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug
- clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.

7.5.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The study Sponsor will submit a brief report annually of the progress of the trial within 60 days of the anniversary date that the IND went into effect as indicated in 21CFR 312.33, and any associated FDA correspondences regarding the IND annual report.

The annual report to the FDA will include as a minimum a summary of the following adverse events:

a. Grade 2-5 adverse events temporally associated with each administration of the vaccine or DLI;
b. all biological product deviations;
c. all Grade 4 hematologic toxicities through 30 days after the last dose of drug;
d. all Grade 2-5 allergic adverse events through 30 days after the last dose of drug;
e. all other Grade 3-5 adverse events 30 days after the last dose of drug;
f. all SAEs, acute GVHD and chronic GVHD through off-study or end-of-study, whichever is earlier.

7.6 Withdrawal From Study

The reason for a patient withdrawing from the study will be recorded. A withdrawal occurs when an enrolled patient ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The investigator will record the reason for study withdrawal provide or arrange for appropriate follow-up (if required) for such patients, and document the course of the patient's condition.

7.7 Record Keeping

All patients must have signed an Informed Consent and an on-study confirmation of eligibility form filled out before entering on the study. An NCI electronic database will be used to report data. Complete records will be maintained on each patient; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports, or physician’s records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on a computer database from which formal analyses are done. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; flowsheets, specialty forms for pathology, radiation, or surgery; and off-study summary sheet, including a final assessment by the treating physician.

7.8 DATA AND SAFETY MONITORING PLAN

7.8.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis, (2-4 times per month, on a weekly basis) when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations and violations will be immediately reported to the IRB using iRIS and if applicable to the Sponsor.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.8.2 Sponsor Monitoring Plan

This trial will be monitored by personnel employed by Harris Technical Services on contract to the NCI, NIH. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

At least 25% of enrolled patients' will be randomly selected and monitored at least quarterly, based on accrual rate. The patients selected will have 100% source document verification done. Additional monitoring activities will include: adherence to protocol specified study eligibility, treatment plans, data collection for safety and efficacy, reporting and time frames of adverse events to the NCI IRB and FDA, and informed consent requirements. Written reports will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

8.1 SARGRAMOSTIM (COMMERCIAL; LEUKINE, GM-CSF™)

Formulation and Preparation: For this protocol, the lyophilized version of Leukine will be used. Each vial contains 250 mcg of sargramostim, 40-mg mannitol, 10-mg sucrose, NF and 1.2 mg tromethamine. Aseptically reconstitute a 250 mcg vial with 1.0mL Bacteriostatic Water for Injection, USP. During reconstitution, the diluent should be directed at the side of the vial and the contents gently swirled to avoid foaming. Do not agitate or shake. The reconstituted sargramostim solution is clear, colorless, isotonic with a pH of 7.4+/-0.3, and contains 250 mcg/ml of sargramostim.

Stability and storage: When reconstituted in this manner, the solution is stable for up to 20 days when stored at 4-8°C. Do not freeze. Unreconstituted vials should be stored as per

the manufacturer's direction.
Drug procurement: Sargramostim will be purchased for this study by the Clinical Center Pharmacy.
Administration: To be used in dendritic cell culture, not administered directly to patients. Cells will be extensively washed before administration.

8.2 Recombinant Human Interleukin-4 (investigational; cross file Cellgenix IND)

Background: Interleukin-4 (IL-4) exerts important effects on B cells, T cells, macrophages, eosinophils, hematopoietic progenitor cells, endothelial cells and promotes the maturation of dendritic cells. Recombinant human interleukin-4 (rhuIL-4) is produced by a strain of Escherichia coli bearing a genetically engineered plasmid that contains the rhuIL-4 gene. The complimentary DNA clone (cDNA), when expressed in E. coli yields a 130 amino acid protein with a molecular weight of 14kD. IL-4 is a highly purified (>97% chromatographically pure), sterile, water-soluble protein.
Formulation and Preparation: RhIL-4 is supplied in 50 mcg vials as a sterile lyophilized powder formulated without carrier proteins. Unreconstituted IL-4 should be kept at -20°C to -80°C. 1.2 mL of Sterile Water for Injection USP should be added to each vial of rhuIL-4 Sterile Powder for Injection. The vial should be gently agitated to completely dissolve the powder and should be inspected visually for discoloration and particulates prior to use.
Stability and Storage: The reconstituted product should be refrigerated at 2-8°C and should used within 24 hours.
Drug Procurement: This investigational agent will be purchased from Cellgenix and the Master File for this agent will be cross referenced.
Administration: To be used in dendritic cell culture, not administered directly to patients. Cells will be extensively washed before administration.
Incompatibility: None known in culture.

8.3 Clinical Center reference endotoxin [CCRE] (RE#2 Endotoxin, U.S. Standard Endotoxin)

Background: Reference Endotoxin is a purified lipopolysaccharide prepared from Escherichia coli O:113 (U.S. Standard Reference Endotoxin) and vialed under good manufacturing practice guidelines. The reference endotoxin was processed by the Clinical Center/NIH using the original bulk material extracted from E. coli O:113. The latter material has served as a national reference standard in assays of pyrogenicity using the Limulus amebocyte lysate.
Formulation and Preparation: Supplied as a clear, glass, 5 mL vial containing a sterile, white, lyophilized powder. Each vial contains 10,000 endotoxin units (EU) (approximately 1 mcg) of Reference Endotoxin, 10 mg of lactose, and 1 mg of polyethylene glycol 6000. A vehicle has also been prepared labeled as RE #2 Endotoxin Vehicle. It is supplied as a vial containing 11 mL of a sterile 0.9% sodium chloride solution. Each mL contains 400 mg lactose USP and 40 mcg polyethylene glycol 6000. 4.1 after reconstitution with SWI
Stability and Storage: The contents of the vial are under a vacuum. Vials should be reconstituted with 5 mL of Sterile Water for Injection, USP (SWI). Since the endotoxin does not go into solution readily, though it appears to be dissolved, it is necessary to

intermittently shake the vial for 30 minutes on a vortex shaker or a Metamix 100 (or equivalent). When reconstituted as recommended, each mL contains 200 nanograms of Reference Endotoxin, 2 mg of lactose, and 200 mcg of polyethylene glycol 6000.
Intact vials and reconstituted drug should be stored in the refrigerator (2℃ - 8℃). Stability studies with this preparation are in process. Other reference endotoxin preparations have had an expiration date 5 years from the date of manufacture. Reconstituted vials were stable for six months when stored in the freezer (10° to -20℃) and for two weeks when refrigerated (2℃ - 8℃). Since the vials do not contain a preservative, the reconstituted solution should be used within 24 hours. The reconstituted solution, once drawn up in a syringe for administration, should be stored in the refrigerator (2℃ - 8℃) for up to 24 hours if not administered immediately.
Procurement: The finished injectable dosage forms are manufactured by the Bureau of Biologics. Vials labeled US Standard Reference Endotoxin are vialed by MARP/NCI-FCRDC, Frederick, MD. This investigational agent will be supplied by the NCI repository.
Administration: A final concentration of 20 ng/ml will be used for dendritic cell maturation. To be used in dendritic cell culture, not administered directly to patients. Cells will be extensively washed before administration and release criteria of < 5 EU/ml (<0.5 ng/ml) will be in place to avoid administration of a toxic dose.
Incompatibility: None known in culture.

8.4 8H9 (INVESTIGATIONAL, CROSS-FILE INVESTIGATOR HELD IND DR. NAI-KONG CHEUNG, MSKCC)

Formulation and Preparation: 8H9 will be supplied as a vialed produce containing: 8H9 Mouse Monoclonal Antibody 2.0 mg Sodium Phosphate Dibasic, 7 H_20 11.05 mg, Citric acid anhydrous 5.65 mg, Sodium hydroxide*, Water for Injection, USP Q.S. to 1 mL. Each vial will contain 300 microliters of 8H9 at a concentration of 2 mg/ml.
Storage and Stability: 8H9 will be stored at -60 to -80°C in single dose vials (670 mcg/vial) which will be ordered by the DTM and used in their entirety during the purging procedure. Any unused portion will be discarded or kept for research. Stability studies thus far confirm stability for 12 months at -60 to -80°C.
Procurement: Was manufactured under GMP conditions by Goodwin Biotechnology, Inc. (Plantation, Florida) and currently vialed and stored in the Clinical Center Pharmacy.
Administration: Added directly to the Miltenyi selection column and cells will be washed before infusion. 8H9 will not be administered directly to patients in this trial.
Incompatibility: None known

8.5 ANTI-CD25 (INVESTIGATIONAL, CROSS-FILE MILTENYI BIOTECH IND)

Background: CD25 is expressed at high levels on a subpopulation of CD4+ cells which exert suppressive activity. In animal models, depletion of CD25+ cells enhances immune responsiveness, especially toward self antigens and tumor antigens. The anti-CD25 developed by Miltenyi was conjugated with paramagnetic particles in order to be used in the context of their immunomagnetic bead selection system. Evaluation in preclinical and clinical studies have demonstrated efficient depletion of regulatory or suppressor CD4+ T cells using this antibody in the Miltenyi selection system.
Formulation and Preparation: Anti-CD25 is conjugated to paramagnetic particles which

are used to deplete CD25+ cells using the CliniMACS system.
Storage and Stability: Lyophilized antibody can be stored at -60 to -80°C. Reconstituted antibody should be stored at 4°C.
Procurement: Anti-CD25 will be purchased from Miltenyi corp. who currently holds an IND for use of this moAb in the context of immunomagnetic particle based depletion. It will be purchased by the Cell Processing Section of the Department of Transfusion Medicine.
Administration: Added directly to the Miltenyi selection column. Cells will be washed before infusion. Anti-CD25 will not be administered directly to patients in this trial.
Incompatibility: None known.

8.6 IFN-GAMMA1B (COMMERCIAL, ACTIMMUNE)

Background: Interferon gamma is produced in E. Coli using recombinant DNA technology. It induces IL-12 production from matured dendritic cells. It is a highly purified sterile solution consisting of non-covalent dimmers of two identical 16,465 dalton monomers; with a specific activity of 20 million International Units (IU)/mg (2.10^6 IU per 0.5 mL) which is equivalent to 30 million units/mg.
Formulation and Preparation: Commercially available interferon gamma will be used. It is supplied as a 100 mcg/0.5ml sterile, clear, colorless solution filled in a single-dose vial for subcutaneous injection. Each 0.5 mL of *ACTIMMUNE* contains: 100 mcg (2 million IU) of interferon gamma-1b formulated in 20 mg mannitol, 0.36 mg sodium succinate, 0.05 mg polysorbate 20 and Sterile Water for Injections. Note that the above activity is previously expressed in International Units (1 million U/50mcg). This is equivalent to what was previously expressed as units (1.5 million U/50mcg).
Stability and storage: Vials of *ACTIMMUNE* should be kept at 2-8°C (36-48°F). DO NOT FREEZE. Avoid excessive or vigorous agitation. DO NOT SHAKE. An unentered vial of *ACTIMMUN*E should not be left at room temperature for a total time exceeding 12 hours prior to use. Vials exceeding this time period should not be returned to the refrigerator; such vials should be discarded. The formulation does not contain a preservative, therefore a vial of *ACTIMMUNE* is suitable for a single dose only. The unused portion of any vial should be discarded.
Drug procurement: Actimmune will be purchased for this study by the Cell Processing Section of the Department of Transfusion Medicine.
Administration: To be used in dendritic cell culture, not administered directly to patients. Cells will be extensively washed before administration.

8.7 KLH (INVESTIGATIONAL INTRACEL'S BCI-IMMUNEACTIVATOR)

Background: Intracel's BCI-ImmuneActivator™ is a potent form of clinical grade KLH. It is purified from the hemocyanin of the keyhole limpet. The denatured subunit of KLH is a glycoprotein with a molecular weight of 400-450,000 daltons. The native form of KLH is a dodecamer, which consists of twenty (20) subunits of KLH with a molecular weight of 8-9,000,000 daltons. In the hemocyanin, at least 50% of the KLH exists as a dodecamer and the remainder can be found as dodecamer aggregates. Intracel's BCI-ImmuneActivator™ is purified as a native molecule in which at least 50% of the KLH is a dodecamer and the remainder as dodecamer aggregates thus enhancing immune potency.

Formulation and Preparation: Intracel's BCI-ImmuneActivator™ is provided in a soluble form in a physiological buffer. It is provided by the manufacturer in 5 mg vials at a concentration of 5 mg/ml. It has been revialed by the Clinical Center Pharmacy Department into single use vials.
Drug Procurement: Intracel's BCI-ImmuneActivator™ will be purchased from Intracel Resources and then vialed by the Clinical Center Pharmacy Development Service. It will be dispensed by PDS to the DTM and to the nursing unit for use in dendritic cell culture and DTH studies respectively.
Administration: To be used in dendritic cell culture at a concentration of 50 mcg/ml. Cells will be extensively washed before administration. In addition, 50 mcg will be dispensed in tuberculin syringes for intradermal inoculation in 0.1 ml for DTH testing.

8.8 CliniMacs Plus Cell Separation Device (Commercial, Miltenyi Biotech)

Background: This instrument is used for the clinical scale depletion of CD25+ T cells labeled with CliniMACS CD25 MicroBeads. Leukapheresis product containing up to 40 x 10^9 total cells and up to 6 x 10^9 CD25+ cells are selected using CliniMACS CD25 MicroBeads, Order No. 274-01, CliniMACS ^{plus} Instrument, Miltenyi Biotec, e.g. Order No. 155-02, software version 2.3x, 1 CliniMACS Tubing Set, Miltenyi Biotec, e.g. Order No.162-01, 168-01 ,1 Pre-System Filter, Miltenyi Biotec, Order No.181-01. The enriched labeled CD25+ cells or the CD25 depleted fraction of unlabeled target cells is collected in the Cell Collection Bag. The Department of Transfusion Medicine has extensive experience with this device for cell selection applications.
Procurement: Already available in DTM. Disposable tubing and reagents for individual selections will be purchased from Miltenyi biotech.

8.9 Elutra Gambro Cell Separation System (Investigational, Gambro BCT)

Background: The Elutra Cell Separation System allows separation of cell populations into multiple fractions based on both size and density. The platform results in cell enrichment, depletion, concentration, and washing within a functionally-closed system. The Elutra Cell Separation System provides predictable monocyte enrichment direct from leukapheresis products without antibodies or preprocessing in less than one hour. Primarily intended for monocyte enrichment (ME) with optional RBC debulking, it comes with a default protocol designed to provide consistent monocyte enrichment. The Elutra Cell Separation System uses counter-flow centrifugal elutriation — fluid flowing through cell layers in a centrifugal field — to separate cell populations.
Procurement: This device is already available in the DTM. We will cross reference the Gambro masterfile as part of the IND in this trial.

8.10 Acetaminophen

(commercial: Tylenol): Will given as a pre-medication. It will be supplied by the Clinical Center Pharmacy. The most common side effect seen with high doses or chronic usage is hepatotoxicity. Rarely, sensitivity reactions can occur. See package insert for additional information.

8.11 DIPHENHYDRAMINE

(commercial: Benadryl): Will given as a pre-medication IV over 10-15 minutes. It will be supplied by the Clinical Center Pharmacy. The most common side effects are sleepiness, dizziness, restlessness, and irritability. See package insert for additional information.

8.12 TUMOR LYSATE/KLH PULSED DENDRITIC CELL VACCINE

Availability: Dendritic cells will be pulsed with autologous tumor lysate and keyhole limpet hemocyanin (KLH: Intracel's BCI-ImmuneActivator™) and cryopreserved according to the Standard Operating Procedures of the Department of Transfusion Medicine of the Clinical Center, NIH.
Storage and Stability: Dendritic cells will be stored according to Standard Operating Procedures of the Department of Transfusion Medicine of the Clinical Center, NIH and used immediately upon thawing for administration to eligible patients, and as outlined in 3.2.4.e.
Administration: Each vaccination consists of three subcutaneous injections (1-5 x 10^7 cells total/site) and three intradermal injections (1-5 x 10^6 cells/site) using the ventromedial portion of the upper arm or anterior thigh. Subsequent vaccines will be administered approximately every 14 days for 6 doses. Sites may be prepared with anesthetic cream prior to injection to minimize discomfort.
Toxicity: Induration, erythema, and/or pain are expected; allergic reaction is possible.

8.13 TUMOR PURGED/CD25 DEPLETED LYMPHOCYTES

Availability: Autologous lymphocytes will be depleted of CD25 cells using the Miltenyi CLINICMACS® System and depleted of tumor contamination using the Elutra Gambro Cell Separation System by the Department of Transfusion Medicine of the Clinical Center, NIH. The product to be infused will be cryopreserved using Standard Operating Procedures of the Department of Transfusion Medicine of the Clinical Center, NIH.
Storage and Stability: Lymphocytes will be stored according to Standard Operating Procedures of the Department of Transfusion Medicine, and infused immediately upon thawing.
Administration: Lymphocytes collected by apheresis and processed as described in Section 3.2.1.b. will be thawed rapidly in a 37°C water bath and infused over 15-30 minutes. 90% of the product collected will be administered (10% for research) with a minimum infusion dose of 1 x 10^6 cells/kg and no maximal dose.
Toxicity: Potential adverse reactions include fever, allergic reactions, transient dyspnea, nausea or vomiting; autoimmune reactions are also possible.

8.14 RECOMBINANT HUMAN INTERLEUKIN-7, CYT107 (INVESTIGATIONAL; CROSS FILE CYTHERIS IND)

Availability: CYT107 will be provided by Cytheris Inc. to the NIH Clinical Center Pharmacy. The investigator, his/her designee, or a hospital pharmacist must maintain an adequate record of the receipt and distribution of all trial supplies using the Drug Accountability Form. These forms must be available for inspection at any time.

Toxicity: Grade 1/2 local induration, pruritis and erythema at the site of subcutaneous injection commonly occurs, Grade 1/2 constitutional symptoms are seen within 24 hours after injection. Transient reversible transaminases usually comprising Grade I/II

elevations in AST/ALT have been observed. In five patients treated thus far, transient grade 3 liver enzyme elevation has been observed but all resolve to baseline within 7 days. Reversible splenomegaly has been observed by CT scans and reversible bone marrow expansions of polyclonal CD19+ B cell progenitors has been observed. Asymptomatic grade one prolongation of the QTc was observed in two patients who were monitored with systematic EKGs; this was an asymptomatic finding. Transient grade 3 lymphopenia may occur on day 1 and 2 due to transient redistribution of lymphocytes from blood to tissues.

Formulation and Preparation: CYT107 Drug Product (DP) is supplied in boxes of 2cc vials as a colorless solution suitable for subcutaneous administration. CYT107 DP is formulated at a concentration of 4 mg/ml in 10mM Sodium acetate, 25 mM NaCl, 50 mM glutamic acid and 50 mM arginine, with 5 mM methionine, 2% mannitol, 0.9% Benzyl Alcohol and 0.01% Tween 20. The pH ranges from 4.8-5.2 and the osmolality ranges from 280-360 mOsm/kg. CTY107 DP is supplied as a sterile solution in closed vials.

Stability and storage: Vials should be stored in a refrigerator at 2-8 degrees Celsius. Stability studies are ongoing and will be continued throughout the clinical study. Stability information will be periodically communicated to the Clinical Center Pharmacy, to guarantee stability. Once opened, any unused CYT107 should be discarded the day of administration.

Administration procedure: CYT107 drug product is supplied for subcutaneous administration. Once CYT107 is drawn up into a syringe, CYT107 should be administered immediately. Do not shake vials before injection. CYT107 should be injected slowly and strictly in the subcutaneous space. A maximum of 0.45 mL can be collected from one vial. If 2 vials are needed (or any volume over 0.45 mL), divide the volume into two equal injections.

Dose modification in obese patients
– In obese patients, a corrected weight will be used to calculate the final dose the patient will receive. This corrected dose will be calculated and used if the patient's actual weight gives a Body Mass Index (BMI) at the upper limit of normal (i.e. BMI $\geq$ 35).

– BMI = weight (kg) / height (m2)
– Corrected weight (kg) = 35 x height (m2)

Incompatibilities: None known.

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10 APPENDICES

Appendix I: ***ELIGIBILITY EVALUATION AND PRE-IMMUNOTHERAPY EVALUATION***

Appendix II: ***TREATMENT SCHEMA***

Appendix III: ***PERFORMANCE STATUS***

Appendix IV: ***SAMPLE COLLECTION, PROCESSING, AND STORAGE OF PLASMA FOR PK AND IMMUNOGENICITY STUDIES***

10.1 APPENDIX I: ELIGIBILITY EVALUATION AND PRE-IMMUNOTHERAPY EVALUATION

A PILOT STUDY OF TUMOR VACCINATION AND/OR R-hIL-7 FOLLOWING STANDARD MULTIMODALITY THERAPY IN PATIENTS WITH PEDIATRIC SOLID TUMORS

	Prior to enrollment AND Prior to immunotherapy
Documented measurable disease*	**X**
Documented signs and symptoms*	**X**
Ht/wt/BSA*	**X**
LABS:	**X**
CBC/diff*	**X**
(electrolytes, Mg, Ca, Phosphorous, uric acid, BUN, Cr)*	**X**
Hepatic panel	**X**
(PT/PTT)*	**X**
10 ml serum storage (red top) for cryopreservation*	**X**
UA	**X**
Urine pregnancy for females of childbearing potential	**X**
ECG	**X**
Radiographic Studies•	**X**
Bone marrow analysis^{o}	**See below ^{o}**
*To be performed w/in 1 week of initial enrollment	
• Of all sites of known disease including but not limited to CT or MRI of sites of known disease, chest CT, FDG-PET scan (radionuclide bone scan can be substituted at the prior to enrollment time point if FDG-PET is not available to avoid delay in therapy). Pre-immunotherapy radiographs should have been performed within 3 weeks of immunotherapy. *Scans may be performed at referring hospital (electronic or hard copies forwarded to NIH).*	
^{o} **If newly dx** with neuroblastoma, RMS or ESFT, a bone marrow asp/bx should be performed w/in 4 weeks of protocol enrollment. **If marrow is negative at dx.,** no bone marrow studies required prior to immunotherapy, unless clinically indicated**.** **If marrow is positive at dx**, bone marrow asp/bx _____ **For patients enrolled on the trial following a disease recurrence**, bone marrow analysis not required if no signs/symptoms of bone marrow involvement OR if pt. has no h/o bone marrow involvement. These patients do NOT require bone marrow analyses prior to initiation of immunotherapy unless clinically indicated. *Bone marrow asp/bx may be performed at referring hospital with copies of bone marrow report sent to NIH.*	

10.2 APPENDIX II: TREATMENT SCHEMA

Cohort 1 Completed and no longer enrolling with Amendment L):

ENROLLMENT AT EITHER INITIAL DX W/METASTATIC DISEASE OR AT TIME OF TUMOR RECCURENCE:
• Tumor biopsy or surgery
• Apheresis

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STANDARD THERAPY:
•If **newly diagnosed metastatic disease**, "standard frontline therapy" dictated and administered by patient's referring oncology team.
•If **recurrent disease**, therapy will be dictated by the primary oncology team caring for the patient.

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IMMUNOTHERAPY for all Patients:

Baseline:
• small volume apheresis (sent to Frederick laboratory)
• radiographic studies of all sites of previous disease including but not limited to CT scans or MRI, chest CT, and FDG PET imaging. These studies should be performed at the NIH.
• DTH testing:
•50 mcg KLH in 0.1ml and 50 mcg tumor lysate in 0.1 ml will be provided in separate prefilled tuberculin syringes
• intradermal injections of each on the volar aspect of the forearm
• read 48 hours later: inspect for induration and record result in medical record
• A positive response is induration which exceeds 5 mm
(cont'd)

IMMUNOTHERAPY: (cont'd):
Day 0 (Arm B only): CYT107 20 mcg/kg/dose SQ (approx 48 h prior to vaccine #1). Monitor in clinic for 6 hours post administration and ECG must be done pre and 3 hours post CYT107 administration.
Day 2:
• **DENDRITIC CELL VACCINE #1: (**to be administered on the same day as the Autologous Lymphocyte Infusion (ALI), at least one hour prior to administration of ALI): Administer three subcutaneous injections ($1\text{-}5x10^7$ cells total/site) and three intradermal injections ($1\text{-}5x10^6$ cells/site) using the ventromedial portion of the upper arm or anterior thigh. Sites may be prepared w/anesthetic cream prior to injection.
•Patients will be monitored at least one hour w/vital signs q 15 minutes.

•**ALI:**
•Pre-medicate with 0.5-1.0 mg/kg (max 50 mg) diphenhydramine IV and 10 mg/kg (max 1000 mg) acetaminophen po 30-60 min. prior to infusion.
• Infuse lymphocytes over 15-30 minutes. Supplemental O_2 should be available at the bedside.
• Post infusion, observe patient for one hour with vital signs recorded every 15 minutes.
• Any adverse reactions should prompt cessation of infusions and should be reported to the P.I. If there is an allergic reaction, studies appropriate for investigation of a transfusion reaction will be performed (cbc, ua, Coomb's test)

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IMMUNOTHERAPY Con't:
Day 14(± 7 days): CYT107(in Arm B only) and Dendritic cell vaccine #2 (as per section above) This should occur approximately 14 days (±7 days acceptable) following vaccine #1

Day 28(± 7 days): CYT107 (in Arm B only) and Dendritic cell vaccine #3 (as per Step 2, Week 2 section above). This should occur approximately 14 days (±7days acceptable) following vaccine #2.

Day 42(± 7 days):
•small volume apheresis (if feasible via indwelling catheter or peripheral IV, if not feasible draw peripheral blood 1 cc/kg in green top tube, max 50 cc to send to FCRDC for immune monitoring)
• lymphocyte immunophenotyping
• radiographic imaging (as per baseline)
• DTH testing (as per baseline)

• CYT107 (in Arm B only) and Dendritic cell vaccine #4 (as per above "dendritic cell vaccine administration" section). This should occur approximately 14 days (±7days acceptable) following vaccine #3.

Day 56(± 7 days): Dendritic cell vaccine #5 (as per above "dendritic cell vaccine administration" section). This should occur approximately 14 days (±7days acceptable) following vaccine #4. Perform ECG to assess QTc interval.

Day 70(± 7 days): Dendritic cell vaccine #6 (as per above "dendritic cell vaccine administration" section). This should occur approximately 14 days (±7days acceptable) following vaccine #5.

Day 84(± 7 days):
• small volume apheresis (if feasible via indwelling catheter or peripheral IV, if not feasible draw peripheral blood 1 cc/kg in green top tube, max 50 cc to send to FCRDC for immune monitoring)
• lymphocyte immunophenotyping
• DTH testing (as per baseline)

Day 126(± 7 days):
• small volume apheresis
• lymphocyte immunophenotyping
• radiographic imaging (as per baseline)
• DTH testing (as per baseline)
• Perform ECG to assess QTc interval.

Cohort 2:

Enrollment:
•Patients metastatic Ewing sarcoma following standard frontline therapy.
•Patients who have received subsequent salvage regimens due to progressive, unresponsive or recurrent disease are not eligible.
•Patients must be in a state of "no evidence of disease" at the time of enrollment as best determined using standard imaging and clinical studies.
•Patient must enroll within 8 week of completing standard frontline therapy.

Protocol Directed Studies and Immunotherapy:
•no more than three weeks prior to enrollment, patients should have radiographic studies of all sites of previous disease including but not limited to CT scans or MRI, chest CT, and FDG PET imaging. These studies may be performed at their home institution.
•within 7 days after enrollment:
--small volume apheresis if feasible via indwelling catheter or peripheral IV, if not feasible draw peripheral blood 1 mL/kg in green top tube, max 50 mL to send to FCRDC for immune monitoring
Spleen ultrasound prior to 1st dose of CYT107 and 2 weeks after last dose.

COHORT 2 Study Calendar

IMMUNOTHERAPY
Day 0:
- History and Physical exam including lymph nodes and spleen
- Lab evaluation*, and 10mL serum storage, urine pregnancy test for females of childbearing potential
- PK blood draw, and Plasma Storage for PK and anti-IL-7 Ab, specific IL-7 binding via ELISA
- EKG
- Vital signs

CYT107 20 mcg/kg/dose SQ
1 hour (± 10 min**) post CYT107:**
- Vital signs

2 hours (± 10 min**) post CYT107**:
- PK
- Liver Panel

3 hours (± 15 min**) post CYT107**:
- Vital signs
- EKG

4 hours (± 15 min**) post CYT107**
- PK

6 hours (± 15 min**) post CYT107**
- Vital signs
- PK blood draw, and Plasma Storage for PK and anti-IL-7 Ab
- Liver Panel

Approx. 24 hours and 48 hours
- PK

Day 14 (± 7 days):
- History and Physical exam including lymph nodes and spleen
- Lab evaluation*, and 10mL serum storage, urine pregnancy test for females of childbearing potential
- PK blood draw, and Plasma Storage for PK and anti-IL-7 Ab
- EKG
- Vital signs

CYT107 20 mcg/kg/dose SQ
1 hour (± 10 min**) post CYT107:**
- Vital signs

2 hours (± 10 min**) post CYT107**:
- Liver Panel

3 hours (± 15 min**) post CYT107**:
- Vital signs
- EKG

6 hours (± 15 min**) post CYT107**
- Vital signs
- Plasma Storage for PK and anti-IL-7 Ab
- Liver Panel

Day 28 (± 7 days**)**:
- History and Physical exam including lymph nodes and spleen
- Lab evaluation*, and 10mL serum storage, urine pregnancy test for females of childbearing potential
- PK blood draw, and Plasma Storage for PK and anti-IL-7 Ab
- EKG
- Vital signs

CYT107 20 mcg/kg/dose SQ

1 hour (± 10 min**) post CYT107:**
- Vital signs

2 hours (± 10 min**) post CYT107**:
- Liver Panel

3 hours (± 15 min**) post CYT107**:
- Vital signs
- EKG

6 hours (± 15 min**) post CYT107**
- Vital signs
- Plasma Storage for PK and anti-IL-7 Ab
- Liver Panel

Day 35 (± 7 days**)**:
- Draw specific IL-7 binding via ELISA

Day 42 (± 7 days**)**:
- History and Physical exam including lymph nodes and spleen
- Lab evaluation*, and 10mL serum storage, urine pregnancy test for females of childbearing potential
- PK blood draw, and Plasma Storage for PK and anti-IL-7 Ab
- EKG
- Vital signs
- --small volume apheresis if feasible via indwelling catheter or peripheral IV, if not feasible draw peripheral blood 1 mL/kg in green top tube, max 50 mL to send to FCRDC for immune monitoring
- --lymphocyte immunophenotyping
- --radiographic imaging as per baseline

CYT107 20 mcg/kg/dose SQ

1 hour (± 10 min**) post CYT107:**
- Vital signs

2 hours (± 10 min**) post CYT107**:
- Liver Panel

3 hours (± 15 min**) post CYT107**:
- Vital signs
- EKG

6 hours (± 15 min**) post CYT107**
- Vital signs
- Plasma Storage for PK and anti-IL-7 Ab
- Liver Panel

Day 49 (± 7 days**)**:
- Draw specific IL-7 binding via ELISA

Day 84(± 7 days):
• small volume apheresis (if feasible via indwelling catheter or peripheral IV, if not feasible draw peripheral blood 1 mL/kg in green top tube, max 50 mL to send to FCRDC for immune monitoring)
• Lymphocyte immunophenotyping

- Draw specific IL-7 binding via ELISA

Day 126(± 7 days):
• small volume apheresis (if feasible via indwelling catheter or peripheral IV, if not feasible draw peripheral blood 1 mL/kg in green top tube, max 50 mL to send to FCRDC for immune monitoring)
- lymphocyte immunophenotyping
- Draw specific IL-7 binding via ELISA
• radiographic imaging (as per baseline

*Laboratory evaluations include:

- CBC with differential
- Electrolytes (Na, K, Cl, CO_2, Mg, Ca, phosphorus, uric acid, BUN, Cr)
- liver panel (bilirubin (including conjugated bilirubin), alkaline phosphatase, AST/ALT)
- urinalysis
- thyroid function tests including anti-thyroid antibodies

10.3 APPENDIX III: PERFORMANCE STATUS

ECOG Performance Status

Score	Clinical Status
0	Asymptomatic
1	Symptomatic, fully ambulatory
2	Symptomatic, in bed < 50% of the day
3	Symptomatic, in bed > 50% of the day but not bedridden
4	Bedridden

Modified Lansky Score (Score as 0-100) – For children < 10 years

A. Normal Range
100 = Fully active
90 = Minor restrictions in physically strenuous play
80 = Restricted in strenuous play, tires more easily, otherwise active

B. Mild to moderate restrictions
70 = Both greater restrictions of and less time spent in active play
60 = Ambulatory up to 50% of time, limited active play with assistance /supervision
50 = Considerable assistance required for any active play; fully able to engage in quiet play

C. Moderate to severe restrictions
40 = Able to initiate quiet activities
30 = Needs considerable assistance for quiet activity
20 = Limited to very passive activity initiated by others, e.g. TV
10 = Completely disabled, not even passive play
0 = Unresponsive, coma

10.4 APPENDIX IV: SAMPLE COLLECTION, PROCESSING, AND STORAGE OF PLASMA FOR PK AND IMMUNOGENICITY STUDIES

1. Pharmacokinetics (PK)

1.1 Blood collection

- Collect 1 mL of blood in 2 or 3 mL paediatric EDTA tubes identified with labels.
- Mix the contents of the tube by slowly inverting several times (5 to 6 times) immediately after the blood draw.

1.2 Preparation and storage of plasma

- Within 5 min maximum after sampling, immediately centrifuge the sample at 2500 rpm for 7 minutes
- Using a transfer pipette, carefully draw off the plasma leaving the white and/or red cells at the RBC/WBC interface untouched. It is acceptable to leave a thin layer of plasma covering the red and white cells. If there is accidental mixing of red and white cells into the plasma, spin the sample again as above.
- Each plasma sample is to be split into aliquots (cryovials), each of them containing 200 µL (except the last one which will contain the remaining quantity).
- Prepare the appropriate labels from the Nurse Study File by entering the data with a cryopen.
- Label the cryovials with the labels provided by Cytheris.

Investigator study 07-C-0206
Subject ID number:
Date of collection:
Visit:
Time of collection:
PK

- Freeze the cryovials containing plasma samples at -80°C.
- Keep the samples in the freezer at -80°C until their shipment.

2. Immunogenicity assays

2.1 Blood collection

- According to the calendar, collect 1 mL of blood in 2 or 3 mL paediatric Li Heparinate tubes identified with labels.
- Mix the contents of the tube by slowly inverting several times (5 to 6 times) immediately after the blood draw.

2.2 Preparation and storage of plasma

- Within 5 min maximum after sampling, immediately centrifuge the sample at 2500 rpm for 7 minutes.
- Using a transfer pipette, carefully draw off the plasma leaving the white and/or red cells at the rbc/wbc interface untouched. It is acceptable to leave a thin layer of plasma covering the red and white cells. If there is accidental mixing of red and white cells into the plasma, spin the sample again as above.
- Each plasma sample is to be split into aliquots (cryovials), each of them containing 200 µL (except the last one who will contain the remaining quantity).
- Prepare the appropriate labels from the Nurse Study File by entering the data with a cryopen.
- Label the cryovials with the labels:

Investigator study 07-C-0206 Subject ID number: Date of collection: Visit: Time of collection: Ab IL7

- Freeze the cryovials containing plasma samples at -80°C.
- Keep the samples in the freezer at -80°C until their shipment.

3. DOCUMENTATION

Please document the appropriate information on the Study forms listed below:

- *Link form for laboratory blood samples* each time a sample is sent to the laboratory.
- *Plasma/Cells storage form each time cryovials are stored*
- *Link form for cryovials* each time a shipment happens.

INSTITUTE: National Cancer Institute

STUDY NUMBER: 07-C-0206 PRINCIPAL INVESTIGATOR: John Glod M.D.

STUDY TITLE: A Pilot Study of Tumor Vaccination and/or r-hIL-7 Following Standard Multimodality Therapy in Patients with High Risk Pediatric Solid Tumors

Continuing Review Approved by the IRB on 07/27/15
Amendment Approved by the IRB on 05/11/16 (N) Date Posted to Web: 05/19/16

Standard – Cohort 2

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary**.**

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

If you are signing for a minor child, "you" refers to "your child" throughout the consent document.

Why is this study being done?

It has been explained to you that you have a tumor diagnosed as Ewing sarcoma. Your tumor is often difficult to cure with standard treatment. Because of this, we are trying to develop new treatments for these tumors. This is a research study designed to answer whether immune therapy given after completion of successful sarcoma treatment can help your body develop immunity toward your tumor, to prevent the sarcoma from returning.

You have already completed standard therapy for your tumor which involves chemotherapy, potentially surgery and/or radiation therapy. These treatments are generally very good at shrinking the tumor, and can sometimes cure patients with your tumor. However they also cause damage to your immune system, which could increase the risk for recurrence of your tumor. In this study we will give you a cytokine, or experimental agent called CYT107, which has been shown to help your immune system recover more quickly from cancer treatments, which could help to delay or prevent tumors from re-growing. Up to 15 patients may receive the cytokine CYT107 on this Cohort of this study. You will first be tested for any problems in the vital organs (liver, kidney, bone marrow). We will also check to see if you have any tumor and perform special studies to check your immune system. After this, if your vital organs test normal, you will be eligible to receive the CYT107, described in more detail below.

CYT107 (recombinant human IL-7 or r-hIL-7): R-hIL-7 belongs to a class of molecules called cytokines which are important in the normal development of lymphocytes in your blood. Lymphocytes are important for the normal function of your immune system. R-hIL-7 is intended to increase the number of lymphocytes in your blood that may in turn help your immune system fight your tumor. In this study, r-hIL7 is manufactured by Cytheris, Inc., and is called CYT107. CYT107 is an experimental agent, and it will be given as shots (injections) under the skin of your thighs, abdomen or arms. CYT107 has been given to less than 200 people before, and a similar form of IL-7, CYT 99 007, also manufactured by Cytheris, was previously given to over 100 patients. CYT 99 007 was shown to increase the number of lymphocytes in the blood at doses safe for humans. Some side effects were noted, but they were rarely serious and did not interfere with the ability to give the CYT 99 007. The side effects will be discussed later in this document. No long term or debilitating side effects were seen with CYT 99 007 or CYT107.

The first dose of CYT107 will be given to you within one week of enrollment to try and increase the recovery of your immune cells. Because one person had a reversible increase in liver tests after receiving r-hIL-7, possibly connected to drinking alcohol or taking Tylenol, we ask that you avoid drinking any alcoholic beverages or taking Tylenol (acetaminophen) for 48 hours before and after every dose of CYT107. We will ask you to stay in the clinic for about 6 hours to watch you closely for any untoward effects. After each dose of CYT107 we will also do blood tests to check your liver function tests every 2-3 days for 1 week, and if your results are elevated, the tests will be repeated every day until the results return to normal. If you do not have any unacceptable ill effects from the first dose, three subsequent doses will be given to you and you will be asked to stay in the clinic for observation for about 6 hours after each dose of CYT107, followed by one week of liver function tests. If you have mild side effects, we will give you some medications to help prevent those side effects for future doses. We will watch you closely before or after each dose of CYT107 with the following tests and procedures:

Study Chart

CYT107	
DAY	**WHAT YOU DO**
Before starting CYT107	**Come into the clinic and do the following:** • **Get a physical exam by your doctor (including blood pressure, heart rate, weight, temperature)** • **Get an ultrasound of your spleen (uses sound waves to see your spleen)** • **Get routine blood tests** • **Have an apheresis procedure to get cells for immune tests** • **Have scans of previous tumor sites**
Day 0	**Come into the clinic, you will be watched in clinic for 6 hours:** • **Get a physical exam by your doctor, check blood pressure** • **Get routine blood tests** • **Get a pregnancy test (if you are a female able to get pregnant)** • **Get an ECG, to measure heart activity** • **Get research blood samples taken for PK and immune tests** • **CYT107 is given as 1-2 shots under the skin of arm and / or thigh** • **1, 3 and 6 hours later: Check blood pressure, heart rate, breathing** • **3 hours later: Get an ECG** • **2-3 and 5-6 hours later: check routine blood test of liver** • **2, 4, 6, 24 and 48 hours later: a research blood sample will be taken for PK**
Day 14, 28 and 42 (approximately)	**Come into the clinic, you will be watched in clinic for 6 hours:** • **Get a physical exam by your doctor, check blood pressure** • **Get routine blood tests** • **Get a pregnancy test (if you are a female able to get pregnant)** • **Get an ECG, to measure heart activity** • **Get research blood samples taken for PK and immune tests** • **CYT107 is given as 1-2 shots under the skin of arm and / or thigh** • **1, 3 and 6 hours later: Check blood pressure, heart rate, breathing** • **3 hours later: Get an ECG** • **2-3 and 5-6 hours later: check routine blood test of liver** • **6 hours later: a research blood sample will be taken for immune tests**

Day 35 (approximately)	• **A research blood sample will be taken for immune tests**
Day 42 (approximately)	• **Also get before CYT107 dose:** • **Have an apheresis procedure to get cells for immune tests** • **Have scans of previous tumor sites**
Day 49 (approximately)	• **A research blood sample will be taken for immune tests**
Day 84 and Day 126 (approximately)	**Come into the clinic:** • **Get a physical exam by your doctor, check blood pressure** • **Get routine blood tests** • **Research blood samples will be taken for immune tests** • **Day 126 only: Have scans of previous tumor sites**

At the following times, we will also take blood samples for routine blood tests to study the effects of this treatment on your body and make sure you are not having any bad effects. The days for routine blood tests are: before starting treatment, Day 0, 14, 28, 42, 56, 70, 84 and 126. If you are a woman who can have children, we will also do a pregnancy test.

At the same time, we will take 10 mL of blood (2 teaspoons) to study the effect of this treatment on your body. We will also draw a 10 mL blood sample (2 teaspoons) 6 hours after every dose of CYT107 (Day 0, 15, 28 and 42) to study the effects on your body. These samples are for research tests.

As part of this study, we will test you for infection with the human immunodeficiency virus (HIV), the virus that causes AIDS. If you are infected with HIV you will not be able to participate in this study. We will tell you what the results mean, how to find care, how to avoid infecting others, how we report newly diagnosed HIV infection, and the importance of informing your partners at possible risk because of your HIV infection.

After you complete the immunotherapy portion of this study on Day 126, you may start having your follow up evaluations done by your local doctor and have him/her send us the following information at 6 months after your treatment, then about every 3 months for the first year, every 6 months for the second year and every year for as long as you remain on this study or for up to 5 years. At each of these visits you will be asked to have the following tests and procedures, and the results and blood samples will be sent to NIH:

- Physical exam and ask you questions about how you are feeling,
- Standard blood and urine tests to check your body's functions
- CT or MRI of your chest and any area of suspected disease
- PET scan

- Research blood samples (8 mL (about 1 ½ teaspoons) + 1 mL per every kg of your weight to a maximum of 3 1/3 teaspoons) to study the impact of this treatment on your immune system.

USE OF SAMPLES FOR RESEARCH PURPOSES

We will be drawing blood to see how your body handles the CYT107. The test is called pharmacokinetic testing or PKs, and blood will be drawn a total of nine times at the following times: before your first dose of CYT107, then 2, 4, 6, 24, and 48 hours after your first dose, then prior to the second dose of CYT107 (approximately Day 14), prior to the 3rd dose (approximately Day 28) and finally prior to the 4th dose (approximately Day 42). At each time we will take about 1 mL (1/5 of a teaspoon) of blood, for a total of 9 mL or 2 teaspoons.

In addition, to make sure your body is not making immune antibodies against the CYT107 we will draw 1 mL (1/5 teaspoons) of blood at the following times: before your first dose of CYT107, then approximately Day 35, and finally 1 week after the last dose of CYT107, on Day 84 and 126, for a total of a teaspoon. If the results of these tests are positive, we will continue to ask that samples be sent (1 mL) at your scheduled doctor's visits (listed above), until you are not longer making immune antibodies against the CYT107. Several patients participating in this study have developed antibodies against CYT107. Thus far there have been no ill effects seen as a result of this laboratory finding, but because there is the potential for these antibodies to adversely affect the activity of your "natural" IL-7, we will follow the levels of these antibodies closely and monitor your immune recovery.

The maximum amount of blood taken from you for research testing is based on your age and will not be more than the strict blood volume limit set for research by the NIH. In adults that limit is 37 tablespoons in an 8 week period. If you are under age 18, we will not draw more than1 teaspoon of blood for every kilogram (2.2 pounds) of your body weight in a single day and not more than 2 teaspoons of blood for every kilogram (2.2 pounds) of your body weight in an 8 week period. For example, if you weigh 85 pounds (weight of some 12 year olds), your weight in kilograms is about 38 kg; therefore we would not draw more 38 teaspoons (12.5 tablespoons) in one day and not more than 76 teaspoons (or 25 tablespoons) of blood in an 8 week period.

One important component of research such as this is the ability to obtain cells from patients with rare tumors, which allows the researchers to learn more about the immune responses and the inner workings of the tumor cells. For this reason, we will collect samples for immune monitoring (apheresis if possible by using your central line or intravenous lines in your arm, or if not possible, just a blood sample) for research purposes which may include but are not limited to studies of immune cell function, cytokine levels in the bloodstream, studies of immune cell targets and gene expression within the tumor cells. Genetic studies which seek to identify a risk for future cancers will not be performed.
We would like to keep some of your specimens and data that we collect and use them for future research and share them with other researchers. We will not contact you to ask about each of

these future uses. These specimens and data will be identified by a number and not your name. Your specimens and data will be used for research purposes only and will not benefit you. Researchers use specimens and data stored in scientific databases to advance science and learn about health and disease. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you decide now that your specimens and data can be kept for research and shared, you can change your mind at any time. Just contact us and let us know that you do not want us to use your specimens and/or data. Then any specimens that have not already been used or shared will be destroyed and your data will not be used for future research.

Please read the sentence below and think about your choice. After reading the sentence, circle and initial the answer that is right for you. No matter what you decide to do, it will not affect your care.

My specimens and data may be kept and shared for use in research to learn about, prevent, or treat cancer or other health problems.

Yes No Initials_____

Alternative Approaches or Treatments

What other choices do I have if I do not take part in this study?

Since the immunotherapy takes place after you receive standard therapy for your disease, the option of pursuing no further therapy at that point remains a possibility.

Risks or Discomforts of Participation

What side effects or risks can I expect from being in this study?

Autoimmunity as a Result of the Immunotherapy:

The risk of immune therapy for cancer is the development of immune reactions directed toward normal tissues. These could range from mild effects without symptoms to severe life-threatening reactions. The most likely spot for involvement would be the bowel, but other areas of the body could also be involved including (but not limited to) skin, liver, lungs, eyes, brain, etc. You will be watched closely for the development of autoimmunity and if it develops, you will be treated with immunosuppressive drugs to attempt to stop the reaction. In other cases where autoimmunity has developed following immunotherapy for cancer, the autoimmune reactions have been successfully stopped using immune suppressant drugs.

Risks of CYT107

The most common reactions in patients receiving rhIL7 were at the injection sites (redness, swelling) that gradually disappeared without requiring treatment. The discomforts observed in

patients receiving CYT107 to date include injection site reactions (pain, redness, itching), temporary increase in liver function blood tests (1 patient), and fever (1 patient). In addition, one patient had an allergic reaction after the CYT107 injection that caused difficulty breathing. It was treated with standard medications and resolved within 30-60 minutes but the risk of allergic reaction is one reason we require you to be observed closely after CYT107 treatment.

Some patients also developed antibodies that bound to the IL-7 and in one case, this appear to interfere with the ability of IL-7 to increase the number of lymphocytes in the blood. However this patient had no clinical symptoms related to the antibodies.

CYT107 has not been given to many people, and not all side effects are known at this time. Therefore, there is a risk that CYT107 could cause permanent harm to you and your immune system, and could potentially result in death, although this has not been seen to date. No deaths resulting from treatment with CYT 99 007, however, were reported in previous clinical trials.

Side effects from the previous form of IL-7, CYT 99 007, as mentioned above, included increases in certain enzymes in the liver of several patients at the higher doses, and flu like symptoms (chills, fever, fatigue) several hours after the CYT 99 007 were experienced by several patients, that were treated by common medications such as Tylenol. In a few patients, changes in some lymphocytes in the bone marrow were seen, but the bone marrow gradually returned to normal after treatment was stopped. Some patients experienced no side effects. On CT-scan, increases in the size of the spleen and some lymph nodes were observed at the higher doses of CYT 99 007, but they returned to normal size after treatment was stopped. Mild changes in the ECG pattern of 2 patients who received CYT 99 007 were observed. We will be doing ECGs before and after giving CYT107 to patients in Arm B to watch for any changes. Rarely patients experienced mild diarrhea, headache, nausea, and vomiting in the previous studies. In general, no long term or debilitating side effects were seen with CYT 99 007.

Risks associated with apheresis:

DO NOT take any medications without discussing them with your doctor first.

The procedure for obtaining blood cells through apheresis is a very common procedure that is done routinely here in the Clinical Center with very few risks. You may have some tingling in your face and lips due to the medicine used to keep your blood from clotting during the procedure. The nurses may give you a calcium containing antacid, like TUMS to chew that takes away this tingling. Rarely, people may experience lightheadedness or dizziness. We ask that you eat prior to the procedure to prevent this. Rare complications of this procedure are lowered blood pressure or bleeding.

The physicians will be checking closely to see if any of these side effects are occurring. Routine physical exams, blood and urine tests and biochemical studies of the liver and kidney function will be done to monitor the effects of the treatment. Side effects usually disappear after the treatment is stopped. In the meantime, the doctor may prescribe medication to keep these side effects under control.

There is a small risk of pain and bruising associated with blood being taken from a vein, and a small chance of infection of the central venous catheter if blood is taken from it.

Because of potential effects of these drugs on a fetus, all women of child-bearing age will be asked to have a negative serum pregnancy test prior to entry onto the study. We encourage patients to utilize one of the more effective birth control methods during treatment and for six months after treatment is stopped. You must notify the doctor if pregnancy occurs during the course of the study.

Potential Benefits of Participation

Are there benefits to taking part in this study?

We cannot guarantee that you will benefit from enrollment on this study, but it is possible that CYT107 will allow your immune system to eliminate any residual tumor cells before that can grow into a tumor.

Research Subject's Rights

Joining this research study is voluntary. You may ask the doctors and nurses any questions about this treatment. If you decide at any time that you do not want to receive this treatment any more, then tell us and we will stop it.

What are the costs of taking part in this study?

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.
- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Cancer Institute Institutional Review Board
- Qualified representatives from Harris Technical Services, the company who monitors this study for NCI.

A description of this clinical trial will be available on http://www.Clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

Stopping Therapy

Your doctor may decide to stop your therapy for the following reasons:

- if he/she believes that it is in your best interest.
- if your disease requires a different treatment, such as radiation therapy, surgery or chemotherapy during the immunotherapy portion of this study,
- if you have side effects from the treatment that your doctor thinks are too severe you will stop therapy and be watched closely,
- if new information shows that another treatment would be better for you.

In this case, you will be informed of the reason therapy is being stopped.

You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to Dr. Glod or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases can**not** be recalled and destroyed.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research

team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, John Glod, M.D., Building 10, Room 1-3940, Telephone: 301-451-0391. You may also call the Clinical Center Patient Representative at 301-496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 301-496-4251.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/ Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM JULY 27, 2015 THROUGH JULY 26, 2016.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____