ECCE TIL

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PROTOCOL TITLE

Phase II Study of Tumor Infiltrating Lymphocytes Generated with Engineered Cells for Costimulation Enhancement in Patients with Metastatic Melanoma Following Lymphodepletion.

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Précis:

Background:

- Tumor Infiltrating Lymphocyte (TIL) administration and high dose interleukin (IL)-2 following lymphodepletion can mediate durable complete responses in patients with refractory melanoma. Obstacles to administration of this therapy include failure to establish TIL in vitro for about 20% of patients, long delays between tumor resection and TIL establishment resulting in poor TIL attributes for therapy and patient ineligibility due to progression, and requirements for large numbers of "feeder" cells for TIL expansion to therapeutic numbers.
- The K562 cell line was engineered to express the 4-1BBL costimulatory molecule and CD64 (the high affinity Fc receptor for "loading" with antibodies such as OKT3). The K562.CD64.4-1BBL Engineered Cells with Costimulation Enhancement (ECCE) replaced up to 75% of feeder cells in large scale TIL expansions. ECCE added to tumor cell suspensions provided costimulation "in trans" resulting in rapid and reliable lymphocyte growth even from tumors with no TIL growth under standard conditions.
- A cloned K562.CD64.4-1BBL-7F11 ECCE line was produced under GMP conditions and a master cell bank has been generated. An optimized protocol was established to rapidly expand (REP) young TIL using minimum feeders and 7F11. These ECCE REPed TIL retained tumor recognition and some other attributes of standard TIL, but differed from standard TIL by containing fewer CD4+ cells and more natural killer cells.
  When 7F11ECCE were added directly to single cell tumor suspensions, young TIL cultures were reliably generated even from patients who otherwise would not have a standard young TIL culture for treatment.

Objectives:

Primary objectives:

- In cohort 1, to evaluate whether young TIL that are rapidly expanded using 7F11 ECCE to replace some feeder cells and administered with IL-2 in patients following a non -myeloablative conditioning regimen will result in clinical tumor regression in patients with refractory metastatic melanoma.
- In Cohort 2, to evaluate whether young TIL generated using "in trans" costimulation with 7F11 ECCE in patients for whom standard young TIL did not grow can mediate tumor regression after a nonmyeloablative conditioning with high dose IL-2 in patients with refractory metastatic melanoma.
- Determine the toxicity of ECCE young TIL in these treatment regimens

Eligibility:

Patients who are 18 years of age or older must have

- metastatic melanoma;
- ECOG performance status 0 or 1
- One or more lesions 2 cm or greater suitable for resection for TIL culture

Patients may not have:

- Concurrent major medical illnesses;
- Any form of immunodeficiency;
- Severe hypersensitivity to any of the agents used in this study;
- Contraindications for high dose IL-2 administration.

Design:

- Patients will undergo resection to obtain tumor for generation of autologous young TIL cultures.
- Parallel TIL cultures will be established using a) the standard technique with IL-2 only and b) the Engineered Cells with Costimulation Enhancement (ECCE) protocol using irradiated K562.CD64.4-1BBL-7F11 (7F11) cells.
- After 10 to 20 days cultures will undergo evaluation for TIL establishment. Standard TIL will be used preferentially and patients who have TIL established by standard methods will be assigned to "Cohort 1"
- Cohort 1:
    - TIL will undergo ECCE REP by exposure to OKT-3, IL-2, feeder cells and irradiated 7F11.
    - Patients will receive a non-myeloablative lymphocyte depleting preparative regimen of cyclophosphamide (60 mg/kg/day IV) on days -7 and -6 and fludarabine (25 mg/m^2/day IV) on days -5 through -1.
    - On day 0 patients will receive the infusion of autologous young TIL and then begin high-dose aldesleukin (720,000 IU/kg IV every 8 hours for up to 15 doses).
    - Clinical and immunologic response will be evaluated about 4-6 weeks after treatment.
    - Using an optimal two-stage Phase II design, initially 18 patients will be enrolled, and if three or more of the first 18 patients have a clinical response (PR or CR), accrual will continue to 35 patients, targeting a 30% goal for objective response.
- If standard young TIL fail to grow then ECCE young TIL will be evaluated and patients who have ECCE TIL available will be assigned to "Cohort 2".
- Cohort 2:
    - Cultures from patients in Cohort 2 will be evaluated for ECCE TIL establishment. If adequate ECCE TIL are available, TIL will undergo ECCE REP by exposure to OKT-3, IL-2, feeder cells and irradiated 7F11.
    - Patients will receive a non-myeloablative lymphocyte depleting preparative regimen of cyclophosphamide (60 mg/kg/day IV) on days -7 and -6 and fludarabine (25 mg/m^2/day IV) on days -5 through -1.
    - On day 0 patients will receive the infusion of autologous young TIL and then begin high-dose aldesleukin (720,000 IU/kg IV every 8 hours for up to 15 doses).
    - Clinical and immunologic response will be evaluated about 4-6 weeks after TIL infusion.
    - Using a small optimal two-stage Phase II design, initially 9 patients will be enrolled, and if one or more of the first 9 patients has a clinical response (PR or CR), accrual will continue to 24 patients, targeting a 25% goal for objective response.
- If TIL cultures were not established by either standard methods or ECCE young TIL protocols, patients will be eligible for re-resection and evaluation of TIL from a different site.

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1.0 Introduction

1.1. Study Objectives

1.1.1. Primary objectives:

- In cohort 1, to determine whether the administration of young TIL generated with the ECCE REP process in patients receiving a non myeloablative conditioning regimen plus IL-2 will result in clinical tumor regression in patients with metastatic melanoma.
- In Cohort 2, to evaluate whether ECCE young TIL from patients for whom standard young TIL did not grow can mediate tumor regression after a nonmyeloablative conditioning with high dose IL-2 in patients with metastatic melanoma.

1.1.2. Secondary objective:

- Determine the toxicity of young TIL expanded with the use of ECCE in these treatment regimens

1.2. Background and Rationale

1.2.1. Surgery Branch, NCI Studies of Tumor Infiltrating Lymphocytes for the Treatment of Patients with Metastatic Melanoma

Melanoma is the sixth leading cancer in both men and women[1]. Metastatic melanoma has a poor prognosis with five-year survival of less than 5%. FDA-approved treatments for metastatic melanoma include aldesleukin and dacarbazine chemotherapy. Aldesleukin has an objective clinical response rate of about 16% and a complete response rate of 6%[2]. It is the only approved treatment known to cure some patients with metastatic melanoma. Dacarbazine-based chemotherapy has a clinical response rate of up to 20% but few complete responders or long-term survivors[3]. With the incidence of melanoma increasing in the United States, more effective treatment for metastatic melanoma is needed. Previous studies in animal models demonstrated that the cellular arm of the immune system plays an important role in tumor surveillance and may be recruited to destroy tumor[4]. Therefore, most therapeutic strategies focusing on immunotherapy against metastatic melanoma have focused on the ability of effector T-cells to mediate tumor destruction.

The Surgery Branch of the National Cancer Institute has pioneered Adoptive Cell Therapy (ACT) for the treatment of patients with metastatic melanoma. We have reported the results of ACT therapy in 93 patients with metastatic melanoma who received autologous TIL following a lymphodepleting regimen plus aldesleukin administration[5]. Forty-three patients received a non-myeloablative chemotherapy consisting of 60 mg/kg cyclophosphamide qd x 2 and 25mg/m^2 fludarabine qd x 5 prior to cell transfer and aldesleukin administration. Twenty-five patients each also received the same chemotherapy agents in conjunction with either 200 or 1200 cGy whole body irradiation prior to cell infusion and aldesleukin administration. The overall objective response rate using RECIST criteria in these 93 patients was 56%.

The clinical results in these three trials are shown in Table 1, and the toxicities shown in Table 2 for the total body irradiation (TBI) studies (04-C-0288, 06-C-0136) and Table 3 for the initial study without TBI (99-C-0158). There was one treatment related death in these 93 patients which occurred in a patient who had an undetected diverticular abscess prior to beginning therapy. There appeared to be an increase in survival with increasing lymphodepletion although the consecutive nature of these non-randomized trials and possible selection bias of patients entering the trials cast doubt concerning the validity of this conclusion (Figure 1). Of the 52 responding patients in this trial, 42 were previously shown to be refractory to aldesleukin therapy and 21 were refractory to prior aldesleukin plus chemotherapy.

In recent studies we have simplified the techniques for the generation of human TIL from melanomas. In the initial report of the treatment of melanoma patients with adoptive transfer of TIL in the absence of lymphodepletion, we noted that patients treated with TIL from younger cultures had a higher frequency of clinical response to treatment than patients treated with TIL from older cultures ($p = 0.0001$)[6]. More recent studies in patients receiving rapidly expanded TIL after lymphodepletion support these studies [7]. Additional experiments examining the role of telomere length of the TIL cultures in the 93 patients who received selected TIL support this hypothesis. Differentiation and expansion of T cells is associated with telomere shortening and with altered and loss of cell functions, including proliferation. Zhou et. al. reported that TIL which were associated with objective clinical responses in treated patients had longer telomere lengths than TIL that were not associated with clinical responses ($p < 0.01$)[8]. TIL which persisted in vivo had longer telomere lengths than TIL which did not persist ($p < 0.001$).

The hypothesis that younger TIL have improved in vivo survival and anti-tumor activity was corroborated by studies demonstrating that younger T cells have phenotypes with more favorable characteristics for in vivo persistence and antitumor activity. CD27 and CD28 are associated with proliferation and survival of T cells and are often used as markers of less-differentiated T cells. Huang, et al reported that T-cell clonotypes with long-term in vivo persistence expressed higher levels of CD27 and CD28 than clonotypes with short-term persistence[9]. In a murine model of adoptive immunotherapy, Gattinoni et al demonstrated that the differentiation of effector T cells in culture had a negative effect on their capacity to function in vivo, and T cells expressing higher levels of CD27 had better in vivo antitumor activity than CD27-low cells[10].

We thus developed an approach to rapidly generate TIL for treatment that involved growth of the entire complement of infiltrating lymphocytes for about two weeks and then expanding them to numbers sufficient for cell treatment. We called these cells "young" TIL[11]. Matched pairs of young TIL (mean age = 14 days) and standard TIL (mean age = 31 days) were generated from 14 tumor specimens. Expression of CD27 and CD28 as well as CD3, CD4, and CD8 were measured in both sets of TIL by fluorescence-activated cell sorting (FACS). Young TIL had significantly higher expression of CD27 ($p < 0.0001$) and CD28 ($p < 0.003$) than standard TIL. In addition, FACS analysis of gated CD8+ cells from these TIL populations demonstrated a strong relationship between culture age and the expression of CD27 and CD28. These observations suggested that younger effector T

cells have phenotypic expression of CD27 and CD28 which may indicate better in vivo proliferation, survival, and antitumor activity compared to older effector T cells. An additional potential advantage of the use of this simpler method of producing TIL is that they were more heterogeneous in their reactivity than highly selected TIL which have grown for longer periods of time.

A clinical trial is being performed in the Surgery Branch, NCI utilizing these young TIL to treat patients with metastatic melanoma (07-C-0176). Twenty-four patients were treated in the initial arm with rapidly expanded young TIL following non-myeloablative lymphodepleting chemotherapy (cohort 1). Five patients in this cohort have had confirmed clinical responses, four partial responses of 35+, 13, 4, and 3 months , and one complete response of 26+ months duration. The toxicities observed in cohort 1 were mostly expected and related to chemotherapy and aldesleukin, but the incidence of serious (grade 3-4) adverse events was higher than expected with a total of 7 patients who were intubated and two patients who required CVVH support. The protocol was amended to investigate the hypothesis that the removal of CD4+ cells from the infusion would reduce the toxicity and improve the effectiveness of the therapy. CD8+ cell enrichment was performed and 39 patients have now been treated with CD8+ enriched young TIL cells following non-myeloablative (NMA) lymphodepletion (cohort 2), and an additional 23 patients have been treated with CD8+ enriched young TIL plus 600 cGy of total body irradiation as a preparative regimen (cohort 3). In cohort 2, three patients were intubated, and in cohort 3, two patients were intubated. One patient in each of these cohorts died, both from sepsis during the neutropenic period. In addition, one patient in cohort 2 experienced a subarachnoid bleed from a known brain metastasis and recovered without sequelae and is an ongoing responder, and one patient in cohort 3 experienced renal microangiopathy that resolved. In cohort 2, 18 patients have confirmed PRs, and 3 patients have confirmed CRs for an objective response rate in the 36 evaluable patients of 58% in this cohort. Two CRs (26+ and 22+) and 2 of the PRs (24+ and 22+) are currently ongoing. In Cohort 3, six patients have confirmed partial responses (one on-going at 17+ months), and four patients have confirmed complete responses (2 ongoing at 17+ and 16+ months) for an objective response rate in the 22 evaluable patients of 45 % in this cohort. It thus appears that the administration of CD8 enriched “young TIL” plus IL-2 following lymphodepleting chemotherapy represents a simplified safe and effective treatment for patients with metastatic melanoma

In order to definitively assess the role of CD8 enrichment in potentially reducing toxicities and increasing response rates without patient selection bias, in November 2009, an amendment was approved in which cohorts 1-3 were closed to accrual and cohorts 4 (identical to cohort 1- Young TIL) and 5 (identical to cohort 2- CD8 enhanced Young TIL) were opened with accrual to both cohorts occurring in a randomized fashion. To date, 25 patients have been enrolled in cohort 4 and 25 patients have been enrolled in cohort 5. It is still too early to evaluate clinical response in most patients treated in cohorts 4 and 5. At this time, in cohort 4, one patient has a confirmed complete response, five patients have confirmed partial responses and eleven patients were taken off study for disease progression. In cohort 5, two patient have ongoing confirmed complete responses, four patients have

confirmed partial responses and twelve patients were taken off study for disease progression. No patients in these two cohorts received CVVH and one patient in cohort 5 was intubated. The adverse events observed in patients in these two cohorts are comparable.

1.2.2. Rationale for the Use of Engineered Cells with Costimulatory Enhancement (ECCE) in TIL Generation.

A major problem for standardization and dissemination of TIL protocols and for individual patients is attrition from TIL protocols after surgery. Between 2002 and 2007, only 27% of the 386 patients who underwent resection of a metastatic melanoma in the Surgery Branch with intent to generate TIL were ultimately treated with their antigen-reactive TIL[12]. Careful retrospective review of all patient charts revealed that for 147 patients (38%) an appropriate TIL culture could not be obtained. Process improvements implemented with the "young TIL" protocol included more patients in the pool that were eligible for treatment. From 2007 to 2009, 122 consecutive patients were resected with intent to generate minimally cultured TIL, and 21 patients (17%) failed to generate adequate TIL for therapy[13]. Retrospective analysis of these samples demonstrated that patients whose TIL did not grow in vitro had a low level of lymphocytic infiltration in their tumors, with a median of only 8 % lymphocytes in the initial single cell suspension (Figure 2). Further improvement to protocol accrual based on generation of a TIL product for therapy will require new approaches that yield therapeutic lymphocyte cultures from tumors with low lymphocyte infiltrates.

Another source of attrition involves patients for whom TIL were obtained but therapy was not administered due to disease progression or protocol specific ineligibilities subsequent to tumor resection. This category included 62 of 386 patients (16%) set up for selected TIL through 2007, and 20 of the 122 patients (16%) patients resected for generation of minimally cultured TIL between 2007 and 2009. Reducing protocol attrition due to patient progression will require further protocol simplification and elimination of hurdles to rapid patient treatment. A significant delay in cell production arises due to securing adequate peripheral blood mononuclear cells (PBMC) for "feeders" in the rapid expansion protocol (REP). Large patient volumes made recruitment of adequate normal donors to support REPs difficult. Recent Surgery Branch efforts have focused on quantifying, standardizing, and comparing autologous and allogeneic feeder cells for clinical lymphocyte expansion. No contribution of the genotype of the feeder cells (autologous vs. allogeneic cells) toward either total expansion of the TIL or to the ultimate clinical response in the patient (Figure 3) was found. Further simplifications to autologous feeder collection could shorten the time between resection and treatment for individual patients, and help disseminate ACT clinical protocols using TIL.

To minimize the use of feeder cells in the rapid expansion protocol (REP), and maximize the establishment of TIL cultures from tumors with limited lymphocytic infiltration, we examined the requirements for optimal human CD8+ lymphocyte proliferation. Prior published research and experience in the Surgery Branch focused our efforts in two areas: the molecular requirements comprising "costimulation" and

a vehicle for presenting surface-bound signals. Costimulatory signals in T cell expansion and activation are critical; however, optimal signals for expanding CD8+ memory cells without driving them to exhaustion are not known. Good candidates include members of the B7 family (CD80, CD86, B7-H1) or TNF family (4-1BBL, OX40L, CD70). Several cellular and inert vehicles for presenting costimulation to lymphocytes usually referred to as "artificial antigen presenting cells" have been studied preclinically and clinically. These include inert magnetic beads[14]; insect cells[15]; mouse fibroblast cells[16]; and the human erythromyeloid cell line K562[17;18]. Literature review and empirical observations with all these costimulatory molecules and presentation vehicles led us to pursue 4-1BBL and the K562 cell line for clinical applications.

K562 is a cell line that grows in suspension and can be indefinitely propagated in culture, and is easy to manipulate genetically because it is readily transduced by retroviruses in vitro. The K562 cell is also well suited for use as an immune stimulator because it is deficient in HLA class I and II expression minimizing endogenous anti-K562 responses, but endogenously expresses high levels of the lymphocyte adhesion molecules ICAM-1 (CD54), and LFA-1 (CD58) so it optimizes engineered immune activity[19]. These K562 attributes have been exploited by many investigators for artificial antigen presentation in preclinical studies[17-20]. Additionally, several clinical studies have been approved using K562 derived cell lines as an accessory reagent for cellular therapy manufacturing (Butler, Zhang, unpublished communication) or directly injected into pateints as a GM-CSF secreting vaccine, GVAX[21;22]. These physical and immunological attributes and the extensive preclinical and clinical experience with the K562 cell line make it an ideal cellular vehicle for optimizing TIL proliferation.

T cell proliferation is dependent on T cell receptor (TCR) engagement, gamma chain cytokine signaling (e.g. interleukin-2), and optimal costimulation. In mouse, the CD28/CD80 interaction is optimal for immune activation and T cell function. However, the CD28 does not have a dominant impact on human CD8+ T cells. Recent studies have implicated 4-1BB to be a more potent stimulatory pathway for CD8 cells in vitro than CD28[23]. 4-1BBL expression has been reported "in trans", meaning that expression of 4-1BBL and antigen do not have to exist on the same cell for antigen-specific costimulation to occur[24]. The high activity and relative specificity of 4-1BBL for CD8+ lymphocytes made this costimulatory molecule a strong candidate for further investigation in promoting melanoma TIL expansion.

1.2.3. Construction of Engineered Cells with Costimulatory Enhancement (ECCE) using K562 with CD64 and 4-1BBL and Their Use in Establishing Young TIL Cultures

Engineered cells with costimulatory enhancement (ECCE) for young TIL cultures were based on K562 obtained from the American Type Culture Collection (ATCC). The K562 line was verified to be mycoplasma and microbial pathogen free, and was adapted to rapid growth in GMP approved media. K562 cells were first retrovirally transduced to express CD64 (the high affinity Fc receptor), then transduced with a retrovirus containing the 4-1BBL (CD137L) gene.

A bulk K562.CD64.4-1BBL line was initially tested for its ability to stimulate proliferation of lymphocytes from melanoma tumors with low levels of T cell infiltration (<20% lymphocytes) . Cryopreserved tumors with minimal lymphocyte infiltrates were selected, and then thawed TIL cultures were initiated in the absence or presence of the K562.CD64.4-1BBL cell line . Lymphocytes proliferated in six of 21 samples (29%) in standard conditions, and in 14 samples with costimulation enhancement. These results were confirmed using poorly infiltrated "fresh" melanoma tumors. Four of 12 samples cultured in standard conditions resulted in TIL proliferation, while all 12 matched samples demonstrated rapid lymphocyte proliferation with the K562.CD64.4-1BBL line. These experiments demonstrate a highly significant (p=0.0002) improvement in establishment of ECCE TIL (26 of 33 samples, 79%) compared to standard TIL (10 of 33 samples 30%, Table 4). 4-1BBL expression was essential, with 0/9 TIL established when K562 cells were added without 4-1BBL compared to 9/9 TIL established using K562 with 4-1BBL expression (p=0.0001, Table 4). These studies demonstrated the K562.CD64.4-1BBL line can be added to poorly infiltrated tumors to improve TIL generation. Experiments were performed with the 7F11 ECCE clone (see below) with similar results, indicating that ECCE young TIL cultures suitable for therapy may be generated for some patients using this approach who would otherwise be ineligible for treatment due to lack of a suitable lymphocyte culture.

To investigate whether ECCE young TIL may have different attributes or composition than standard TIL, six tumors were used to generate standard TIL and ECCE TIL in parallel cultures, and phenotype and function were compared. Time to generation of TIL was reduced in ECCE TIL cultures compared to standard TIL (Figure 4, p=0.004). The composition of standard TIL and matched ECCE TIL were investigated by FACS analysis, and ECCE TIL were found to contain a significantly increased fraction of CD8+ lymphocytes (p=0.03). Importantly, anti-tumor function exhibited by ECCE TIL in response to an autologous tumor cell lines was statistically similar to standard TIL (p=0.23, Table 5). These results suggest that CD8+ enhanced, functionally equivalent ECCE young TIL can be generated more quickly than standard TIL, even from well infiltrated tumors. The therapeutic efficacy of ECCE young TIL cultures cannot be predicted *a priori*, while a (preliminary) therapeutic response rate for standard young TIL cultures has been established.

1.2.4. Validating 7F11 Engineered Cells for Costimulation Enhancment (ECCE) Rapid Expansions of TIL at clinical scale

The number of cells infused for TIL treatments may be a critical determinant of patient response. In our studies, the number of CD8+ cells infused was correlated with objective response in prior TIL trials. Similarly, in a recent trial with young TIL following non-myeloablation in melanoma patients, cell number infused was correlated with objective response[24] The rapid expansion protocol (REP) using irradiated peripheral blood mononuclear cell (PBMC) "feeders," OKT3 and IL-2 is required for expansion of TIL to large numbers for patient infusion, but the REP is often limited by the number of feeder cells available. K562.CD64.4-1BBL cells were

investigated as a substitute for PBMC in the REP. Initial experiments demonstrated that TIL expanded poorly when K562.CD64.4-1BBL were loaded with OKT3 and used alone as stimulators. However, the K562.CD64.4-1BBL cells functioned well when added to PBMC to partially replace PBMC in REPS (Figure 5). The expression of 4-1BBL was required for optimal ECCE TIL REP ($p=0.03$, Figure 6).

To obtain a GMP compliant ECCE REP protocol, a K562.C64.4-1BBL master cell bank was established in the following way. Multiple clones from the K562.CD64.4-1BBL cell line were isolated and characterized. The clone 7F11 (K562.CD64.41BBL-7F11) expressed uniformly high levels of CD64 and 4-1BBL (Figure 7), and was further expanded for GMP production and generation of a master cell bank (MCB). Full characterization of the MCB including safety information will be submitted with an investigational new drug (IND) application.

The 7F11 ECCE line was tested at clinical scale for its ability to replace feeder cells in a REP. Optimal TIL expansion at clinical scale requires a 200:1 ratio of PBMC feeder cells:TIL responders. The addition of 7F11 reduced the requirement for PBMC feeders to an optimal ratio of 50:10:1 PBMC feeders:K7F11:TIL responders. These optimal ratios generated similar numbers of expanded TIL in clinical scale REPs (Figure 8). The composition of REPed cells was determined by FACS analysis, and the 7F11 and PBMC expanded TIL had a similar fraction of CD8+ cells, but ECCE REP TIL were comprised of significantly fewer CD4+ cells and more natural killer (NK) cells (CD3-CD56+, Figure 9). Although NK cells do not have an unambiguously defined role in ACT therapy, they have been implicated in reducing metastatic disease in lung metastases models in mice and in eliminating HLA loss tumor variants in vitro. Similarly, reducing CD4+ cells in TIL may improve the therapies, although the contribution of the CD4+ compartment is complex. The presence of NK cells at modest numbers and the reduction of CD4+ cells from TIL may improve the efficacy of the TIL product. Media composition and other culture conditions are currently being evaluated for differential expansion of T cells and NK cells during clinical scale TIL expansions with 7F11.

1.2.5. Safety Considerations

Two principal safety concerns arise from TIL cells expanded with the use of 7F11 accessory cells. First, the 7F11 may themselves survive the expansion process and cause toxicity when infused. Second, the TIL may acquire new attributes through exposure to the 7F11 cells including their 4-1BBL costimulation.

The K562 cell line, the progenitor to 7F11, is well characterized for clinical use. Irradiated K562 has been administered directly to patients in GVAX vaccine trials[21;22] and has also been used as an accessory component in the production of autologous cells that have been used for patient therapy (M Butler, Dana Farber Cancer Center, personal communications). These clinical experiences have not resulted in evidence of adverse reactions.

The ECCE used as an ancillary manufacturing reagent are incapable of proliferation due to irradiation. To insure lack of ECCE proliferation, each production batch of 7F11 cells is examined for proliferation after 100Gy irradiation, the dose that is used in TIL manufacture. The proliferation of 7F11 following irradiation is assessed by tritiated thymidine incorporation (Figure 10). The

proliferation assay evaluates live 7F11 at various concentrations spiked into irradiated 7F11 cells. Live 7F11 can be reliably detected at concentrations down to and including 1 live cell per 10^5 irradiated cells (detection limit < 0.001%). 100Gy irradiated 7F11, the irradiation dose used for 7F11 in clinical TIL production, was sufficient to completely inhibit the proliferation of 7F11, and no viable 7F11 was detected with 100Gy irradiation. During the REP, 7F11 is eliminated from the final product in the same manner as allogeneic feeder cells, through non-specific killing mechanisms of highly activated lymphocytes. However, to insure that 7F11 are not infused into patients, additional safety testing will be undertaken. Each production batch of 7F11 will undergo extensive testing and characterization with a detailed, batch-specific COA prior to approval for use in ECCE TIL manufacturing protocols. The tritium incorporation assay will be one of the tests used in the lot release criteria for 7F11. 7F11 production lots will by irradiated and fully characterized prior to release for use in production. In addition, each batch of patient cells will include a batch specific release assay to evaluate the elimination of 7F11 cells from the final product. The assay will evaluate the expression of 4-1BBL (CD137) which is expressed by 7F11 but not by the infused TIL. Infused cells will have to demonstrate CD137 expression <0.5% prior to release for infusion.

TIL expanded with 7F11 could acquire attributes mediating toxicity upon transfer. This seems unlikely, as no phenotypic or functional characteristics of ECCE TIL were observed suggesting that they have superior inflammatory attributes compared to conventional TIL. Over 300 autologous TIL infusions have been safely administered in the Surgery Branch to doses as high as $3x10^{11}$ cells.

2.0 Eligibility Assessment and Enrollment

2.1. Eligibility Criteria

2.1.1. Inclusion Criteria

a. Measurable metastatic melanoma with at least one lesion that is resectable for TIL generation.
b. Patients with 3 or less brain metastases are eligible. Note: If lesions are symptomatic or greater than or equal to 1 cm each, these lesions must have been treated and stable for 3 months for the patient to be eligible.
c. Greater than or equal to 18 years of age.
d. Willing to sign a durable power of attorney
e. Able to understand and sign the Informed Consent Document
f. Clinical performance status of ECOG 0 or 1.
g. Life expectancy of greater than three months.
h. Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for up to four months after receiving the preparative regimen.
i. Serology:
    1. Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients

who are HIV seropositive can have decreased immune-competence and thus be less responsive to the experimental treatment and more susceptible to its toxicities.)

2. Seronegative for hepatitis B antigen, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then patient must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.
3. Women of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the preparative chemotherapy on the fetus.

j. Hematology:
1. Absolute neutrophil count greater than 1000/mm^3 without the support of filgrastim.
2. WBC (> 3000/mm^3).
3. Platelet count greater than 100,000/mm^3.
4. Hemoglobin greater than 8.0 g/dl.

k. Chemistry:
1. Serum ALT/AST less or equal to 2.5 times the upper limit of normal.
2. Serum creatinine less than or equal to 1.6 mg/dl.
3. Total bilirubin less than or equal to 1.5 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl.

l. More than four weeks must have elapsed since any prior systemic therapy at the time the patient receives the preparative regimen, and patients' toxicities must have recovered to a grade 1 or less (except for toxicities such as alopecia or vitiligo). Patients may have undergone minor surgical procedures within the past 3 weeks, as long as all toxicities have recovered to grade 1 or less or as specified in the eligibility criteria in Section 2.1.1.

m. Six weeks must have elapsed since any prior anti-CTLA4 antibody therapy to allow antibody levels to decline.

n. Patients who have previously received any anti-CTLA4 antibody and have documented GI toxicity must have a normal colonoscopy with normal colonic biopsies.

2.1.2. Exclusion Criteria

a. Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant.

b. Systemic steroid therapy required.

c. Active systemic infections, coagulation disorders or other major medical illnesses of the cardiovascular, respiratory or immune system, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease.

d. Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

e. Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who

have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities).

f. History of severe immediate hypersensitivity reaction to any of the agents used in this study.
g. History of coronary revascularization or ischemic symptoms
h. Any patient known to have an LVEF less than or equal to 45%.
i. Documented LVEF of less than or equal to 45% tested in patients with:
   - Clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block
   - Age ≥ 60 years old
j. Documented FEV1 less than or equal to 60% predicted tested in patients with:
   - A prolonged history of cigarette smoking
   - Symptoms of respiratory dysfunction

2.2. Research Eligibility Evaluation

Within **4 weeks** prior to starting the chemotherapy regimen:

a. Complete physical examination, including height, weight and vital signs, and eye exam noting in detail the exact size and location of any lesions that exist.
b. Chest x-ray
c. EKG
d. Baseline CT of the chest, abdomen and pelvis, and brain MRI to evaluate the status of disease. Additional scans and x-rays may be performed if clinically indicated based on patients' signs and symptoms.
e. Cardiac (MUGA or echocardiogram, stress thallium) and pulmonary evaluation (PFTs) if required as indicated in Section 2.1. **Note:** cardiac evaluation may be performed up to 6 months prior to treatment.
f. HIV antibody titer and HbsAG determination, anti HCV, anti CMV antibody titer, HSV serology, and EBV panel
g. Medical history (may be conducted at any point prior to this time).

Within **14 days** prior to starting the chemotherapy regimen:

h. Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
i. CBC with differential and platelet count
j. PT/PTT
k. Urinalysis and culture, if indicated

Within **7 days** prior to starting the chemotherapy regimen:

l. β-HCG pregnancy test on all women of child-bearing potential

2.3. Patient Registration

Patients will be registered on protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols) prior to tumor resection for TIL generation, by the clinical fellow or research nurse within 24 hours of the patient signing the consent by faxing a completed Eligibility Checklist to the Central Registration Office (CRO) at 301-480-0757. Patients will sign the consent document for this protocol prior to treatment and the Eligibility Checklist will be completed and faxed to the Central Registration Office at 301-480-0757 within 24 hours of the patient signing the consent.

3.0 Study Implementation

3.1. Study Design

3.1.1. Treatment Phase:

3.1.1.1. Cell Preparation

Patients with evaluable metastatic melanoma who have lesions suitable for initiating standard and ECCE TIL that can be resected with minimum morbidity will undergo resection of tumor. TIL will be obtained while enrolled on the Surgery Branch protocol 03-C-0277, "Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols". Separate tumor biopsies may be performed to obtain TIL for subsequent lymphocyte cultures. TIL will be grown and expanded for this trial according to standard operating procedures submitted in the IND. Cell preparation and patient cohort enrollment will follow the schematic shown in Figure 11. Briefly, a single cell suspension will be prepared from the resected tumor. "Standard" young TIL will be initiated in 3000 IU/ml interleukin (IL)-2 and "ECCE young TIL" will be initiated with 100Gy irradiated 7F11 cells plus 3000 IU/ml IL-2. TIL cultures will be monitored regularly. If standard TIL proliferate to at least $5x10^7$ lymphocytes within 20 days, then this standard TIL culture will be used preferentially, and the patient will be eligible for enrollment in Cohort 1. Clinical evaluation of patients in this cohort will establish for the first time the value of ECCE replacement of feeder cells in REPs for standard young TIL that have an established record of therapeutic benefit. If standard TIL fail to grow within 20 days then the ECCE young TIL will be evaluated for growth and patients who have ECCE young TIL available will be eligible for enrollment in Cohort 2. Clinical evaluation of patients in this cohort will establish for the first time whether ECCE young TIL are therapeutically beneficial.

All TIL will undergo ECCE REP using anti-CD3 (OKT3), IL-2, feeder cells and 100Gy irradiated 7F11. Five to seven days later the cells will be tested for potency by interferon-gamma release as specified in the Certificate of Analysis shown in Appendix 1. Volunteers will also undergo apheresis to obtain mononuclear cells to be used as feeder cells in cell culture. The procedures used are the same as those in all prior ACT protocols and are in routine use in the Department of Transfusion Medicine in the Clinical Center. Separate consents will be obtained from all blood and apheresis volunteers.

It is anticipated that TIL that meet the COA will not be achievable in approximately 5% of patients who undergo resection. These patients may undergo a second resection to grow TIL, if another suitable lesion exists, and be treated in the same cohort as initially assigned.

3.1.1.2. Cell Administration

Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the COA, (approximately 7 days after the REP procedure has been initiated) the patient will receive the lymphocyte depleting preparative regimen consisting of fludarabine and cyclophosphamide, followed by infusion of up to $1x10^{11}$ ECCE REP lymphocytes (minimum of $1x10^{9}$) and the administration of high-dose aldesleukin.

Approximately 4-6 weeks after completion of aldesleukin administration, patients will undergo a complete tumor evaluation and evaluation of toxicity and immunologic parameters. This will comprise one course of therapy. Patients will receive no other experimental agents while on this protocol. For both cohorts, cells will be administered in a volume of 100-200 ml.

3.1.2. Protocol Stopping Rules:

The study will be halted pending discussions with the FDA and NCI IRB if the following conditions are met:

- Two or more patients develop a grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen or IL-2 (or circumstances unrelated to this study).
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop grade 3 autoimmunity, that cannot be resolved to less than or equal to a grade 2 autoimmune toxicity within 10 days, or any grade 4 or greater autoimmune toxicity.

3.2. Drug Administration

(see table 3.2)

3.2.1. Preparative Regimen with Cyclophosphamide and Fludarabine

Day -7 and –6 (Times are offered as examples and may be changed as long as a similar time relationship between drug administration is maintained)

1 am

Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 2.6 ml/kg/hr (starting 11 hours pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion)

11 am

Ondansetron (0.15 mg/kg/dose [rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight] IV every 8 hours X 3 days) will be given for nausea.

Furosemide 10-20 mg iv.

12 pm (NOON)

Cyclophosphamide 60 mg/kg/day X 2 days IV in 250 ml D5W with mesna 15 mg/kg/day X 2 days over 1 hr. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 6.

1 pm

Begin to monitor potassium level every 12 hours until hydration is stopped. KCl will be adjusted to maintain serum potassium levels in the normal range.

1 pm

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section) over 23 hours after each cyclophosphamide dose. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 6.

Day -5:

Stop IV hydration (24 hours after last cyclophosphamide dose)
If urine output <1.5 ml/kg/hr give additional 20 mg furosemide iv.
If body weight >2 kg over pre cyclophosphamide value give additional furosemide 20 mg iv.

Day -5 to Day -1:

Fludarabine 25 mg/m2/day IVPB daily over 30 minutes for 5 days. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 6.

3.2.2. Cell Infusion and Aldesleukin Administration

Cells are delivered to the patient care unit by a staff member from the Cell Production Laboratory. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The cells are to be infused intravenously over 20-30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

Day 0 (one to four days after the last dose of fludarabine):

- Cells will be infused intravenously (i.v.) on the Patient Care Unit over 20 to 30 minutes (between one and four days after the last dose of fludarabine). Cells will be administered by an infusion of up to $1x10^{11}$ lymphocytes (minimum of $1x10^{9}$). For cell numbers less than 1×10^{10} cells, the volume will be 100 mls; and for cell numbers greater than or equal to 1×10^{10} cells, the volume will be 200 mls.
- Aldesleukin 720,000 IU/kg IV (based on total body weight) over 15 minute every eight hours (+/- 1hr*)* beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum of 15 doses.)

Day 1-4 (Day 0 is the day of cell infusion):

- Beginning on day 1 or 2, filgrastim may be administered subcutaneously at a dose of 5 mcg/kg/day (not to exceed 300 mcg/day). Filgrastim administration will continue daily until neutrophil count > 1.0×10^{9}/L X 3 days or > 5.0×10^{9}/L. Aldesleukin 720,000 IU/kg IV (based on total body weight) over 15 minute every eight hours for up to 5 days.

Table 3.2

Day	-7	-6	-5	-4	-3	-2	-1	0[1]	1	2	3	4
Therapy												
Cyclophosphamide 60 mg/kg	X	X										

Fludarabine 25 mg/m^2			X	X	X	X	X					
ECCE TIL Cells								X				
Aldesleukin								X[2]	X	X	X	X
Filgrastim[3] 5 mcg/kg/day									X	X	X	X
TMP/SMX[4] 160mg/800mg (example)		X			X				X			
Fluconazole[5] 400 mg po								X	X	X	X	X
Valacyclovir po or Acyclovir IV[6]								X	X	X	X	X

[1]One to four days after the last dose of fludarabine
[2]Initiate within 24 hours after cell infusion
[3]Continue until neutrophils count > 1X10^9/L for 3 consecutive days or > 5x10^9/L.
[4]The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 X 2
[5]Continue until ANC > 1000/mm^3
[6]In patients positive for HSV continue until ANC is greater than 1000/mm^3

3.2.3. Infection Prophylaxis

3.2.3.1. Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) P.O. daily three times a week on non-consecutive days, beginning on day -7.

Pentamidine will be substituted for TMP/SMX-DS in patients with sulfa allergies. It will be administered aerosolized at 300 mg per nebulizer within one week prior to admission and monthly for at least 6 months and until the CD4 count is greater than 200 for 2 consecutive measurements.

3.2.3.2. Herpes Virus Prophylaxis

Patients with positive HSV serology will be given valacyclovir orally at a dose of 500 mg daily the day after chemotherapy ends, or acyclovir, 250 mg/m^2 IV q 12 hrs if the patient is not able to take medication by mouth which is continued until absolute neutrophil count is greater than 1000/mm^3. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

3.2.3.3. Fungal Prophylaxis (Fluconazole)

Patients will start Fluconazole 400 mg p.o. the day after chemotherapy concludes and continue until the absolute neutrophil count is greater than 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

3.2.3.4. Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fever of 38.3^{o}C once or two temperatures of 38.0^{o}C

or above at least one hour apart, AND an ANC <500/mm^3. Aminoglycosides should be avoided unless clear evidence of sepsis. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

3.2.4. Blood Product Support

Using daily CBC's as a guide, the patient will receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hb >8.0 gm/dl, and plts >20,000/mm3. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

3.2.5 Aldesleukin: Intravenous Administration

Aldesleukin will be administered at a dose of 720,000 IU/kg (based on total body weight) as an intravenous bolus over a 15 minute period every eight hours *(+/-* 1hr*)* beginning on the day of cell infusion and continuing for up to 5 days (maximum 15 doses). Doses may be delayed or skipped depending on patient tolerance if this is determined to be clinically indicated in the best clinical judgment of the attending physician. Doses will be skipped if patients reach Grade 3 or 4 toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 2. Toxicities will be managed as outlined in Appendix 3. If these toxicities can be easily reversed within 24 hours by supportive measures then additional doses may be given. Additional instances may arise when in the clinical judgment of the attending physician, based on the extensive clinical experience in the Surgery Branch with aldesleukin, when doses of aldesleukin may be skipped. If greater than 2 doses of aldesleukin are skipped, aldesleukin administration will be stopped. Aldesleukin will be administered as an inpatient and will be purchased by the NIH Clinical Pharmacy Department from commercial sources.

Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney will be signed by the patient to identify a surrogate to make decisions if a patient becomes unable to make decisions.

3.3. On-Study Evaluation

3.3.1. Prior to starting the preparative regimen

- Apheresis
- Within 14 days prior to starting the preparative regimen, patients will have a complete blood count, serum chemistries performed including electrolytes, BUN, creatinine, and liver function tests. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.3.2. During the preparative regimen: DAILY

- Complete Blood Count
- Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase,

ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid)
- Urinalysis
- Review of systems and physical exam at least 3 times/week
- Vital signs as per routine

3.3.3. <u>After Cell Infusion:</u>

- Vital signs will be monitored hourly for 3 hours, and then routinely (every 4 hours) unless otherwise clinically indicated.
- CMV PCR assay will be assessed if clinically indicated (e.g. unexplained fevers, pulmonary changes).

3.3.4 <u>During and after Aldesleukin administration (until hospital discharge)– Every 1-2 days</u>

- Complete Blood Count
- Chem 20: [Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid]
- Review of systems and physical exam at least 3 times/week
- Vital signs as per routine

3.3.5. <u>Additional research evaluations:</u>

3.3.5.1 <u>Post cell infusion evaluations:</u>

- The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.
- Once total lymphocyte count is greater than 200/mm^3, the following samples will be drawn and sent to the TIL lab on Monday, Wednesday and Friday (while the patient is hospitalized):
    - 5 CPT tubes (10 ml each)
    - 1 SST tube (10 ml)
- At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be 1) cryopreserved for immunological monitoring of cell function.

3.3.6.2. <u>Biopsies</u>

- Biopsies of tumor tissue or lymph nodes may be performed but are not required during the course of therapy. A maximum of three biopsies will be performed. These biopsies will only be performed if they are superficial and extracavitary. Biopsy tissue will be processed in the Surgery Branch Cell Production Facility in the presence of a Pathology Laboratory pathologist and part of all biopsy tissue will go to the Laboratory of Pathology. Studies will be performed to evaluate the antigen expression by the tumor and to evaluate the reactivity of lymphocytes grown from these biopsies.

3.3.6.3. Immunological Testing:

- Apheresis will be performed, prior to and 4-6 weeks after the treatment. At other time points, patient peripheral blood lymphocytes (PBL) will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be cryopreserved for immunological monitoring of cell function.
- Lymphocytes will be tested directly and following in vitro culture using some or all of the following tests. Direct immunological monitoring will consist of quantifying T cells reactive with targets FACS analysis using tetramer staining. Ex vivo immunological assays will consist of cytokine release by bulk PBL (+/- peptide stimulation) and by other experimental studies such as cytolysis if sufficient cells are available. If cell numbers are limiting, preference will be given to the direct analysis of immunological activity. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the ECCE TIL cryopreserved at the time of infusion. In general, differences of 2 to 3 fold in these assays are indicative of true biologic differences. Foxp3 levels will be analyzed by semiquantitative RT-PCR to evaluate for mRNA on PBL samples obtained prior to cell infusion and at the follow up time point.

3.4. Post Study Evaluation (Follow-up)

Patients will be evaluated 4 to 6 weeks after the initial treatment regimen (defined as the last day of aldesleukin administration).

- Physical examination
- Chem 20: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO^2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid), complete blood count and thyroid panel
- Toxicity assessment, including a review of systems.
- CT of the chest, abdomen and pelvis. This end of course evaluation will be used to determine tumor response. If clinically indicated, other scans or x-rays may be performed, e.g. brain MRI, bone scan.
- Visual symptoms will be evaluated and if changes have occurred from baseline, i.e. changes in visual acuity, an ophthalmologic consult will be performed.
- A 5 liter apheresis will be performed. Subsequently, 60 ml of blood will be obtained at follow up visits (approximately monthly) for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed
- If the patient has SD or tumor shrinkage, repeat complete evaluations will be performed monthly for approximately 3-4 months, and then every 3-4 months until off study criteria are met.

3.5. Off Treatment Criteria

Patients will be taken off treatment (and followed until progression of disease) for the following

- Grade 3 or greater autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).
- If a patient experiences a grade 3 or greater toxicity due to cell infusion (reaction to cellular product or infusion reaction) the patient will receive no further treatment.

3.6. Off Study Criteria

Patients will be taken off study for the following:

- The patient voluntarily withdraws
- There is significant patient noncompliance
- Progressive disease, unless:
  - the patient is being followed for a serious adverse event related to the research. The event must resolve to less than or equal to grade 2 or baseline prior to removing from the study.
- The patient develops a second malignancy.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment on this study in the judgment of the investigator.

4.0 Supportive Care

Concomitant medications to control side effects of therapy will be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg g6h) and ranitidine (150 mg g12h). If patients require steroid therapy they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5.0 Data Collection and Evaluation

5.1. Response Criteria

Clinical response will be determined using RECIST version 1.0

5.1.1. Evaluation of target lesions[1]

- Complete Response (CR): Disappearance of all target lesions
- Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions taking as reference the baseline sum LD.

- Progression (PD): At least a 20% increase in the sum of LD of target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD.

5.1.2. Evaluation of non-target lesions[2]

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.
- Non-Complete Response: Persistence of one or more non-target lesions
- Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions

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[1] All measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

[2] All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent."

5.1.3. Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

5.1.4. Confirmatory Measurement/Duration of Response Confirmation

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed at least 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must

have met the SD criteria at least once after study entry at a minimum interval of 6-8 weeks.

Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of Stable Disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

5.2. Toxicity Criteria

This study will utilize the CTCAE version 3.0 for toxicity and adverse event reporting. A copy of the CTCAE v3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov).

Over 150 patients have been treated in the Surgery Branch, NCI with tumor infiltrating lymphocytes. Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to aldesleukin administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of cells can include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this treatment as profound myelosuppression occurs in all patients. In the 68 patients treated with young TIL using the non-myeloablative chemotherapy regimen, there was one treatment related death due to overwhelming sepsis.

The standard approach to the administration of high-dose aldesleukin in all studies is to continue dosing until grade 3 or 4 events occur. The most commonly seen grade 4 events are pulmonary, and renal impairment, and mental status changes. These toxicities may sometimes require intubation for protection of the patient's airway. It is important to note that although these patients require significant supportive measures during this period, all toxicities are reversible and the overwhelming majority of patients have suffered no long term sequelae following this treatment regimen. However, fatal complications are possible and it is therefore only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer. Toxicities seen on protocols using this non-myeloablative regimen and aldesleukin, that occur during the follow up period are rare but have included EBV lymphoma following prolonged lymphopenia, herpes zoster infection, and sensory neuropathy likely related to fludarabine. High-dose aldesleukin: a variety of side effects have been associated with high-dose aldesleukin administration in our

experience and a listing of these side effects in 652 patients who received 1,039 treatment courses are listed in Appendix 2.

To ensure safety using this treatment, the NCI SB will review safety data on all protocols semi-annually at the time of continuing review. Data will be presented for both the recent 6 month period and for the entire length of time the protocol has been open. The toxicity data for review will include all toxicities captured on the protocol and will be presented in individual tables as follows:

- all toxicities attributed to the cells,
- all incidences of intubation including the duration of and reason for intubation,
- all grade 2 unexpected adverse events, and all grade 3 or greater events regardless of attribution.

The major discomforts of the research are those of nausea, mucositis, anorexia, diarrhea, fever and malaise. Side effects of common drugs used in this nonmyeloablative regimen include: *Cyclophosphamide:* Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, SIADH. *Fludarabine*: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity including sensory neuropathies and blindness, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. *Antimicrobials in general:* Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression.

5.3. Statistical Section

The primary objective of this study is to determine if the transfer of ECCE young TIL in conjunction with aldesleukin after lymphodepletion will be associated with a modest response rate (CR+PR) in patients with metastatic melanoma.

Cohort 1

In cohort 1, success with this regimen would allow more rapid and reliable treatment of individual patients, and improve procedures for manufacturing TIL so they are more easily adopted in other centers. In an ongoing clinical trial (07-C-0176), two cohorts with standard young TIL expanded using conventional feeder cells demonstrated response rates of 21% (5 of 24 patients) and 35% (6 of 17 patients) for an overall response with unselected young TIL in patients who received nonmyeloablative conditioning of 27 % (11 of 41 patients). Accrual to this cohort will take place using an optimal two-stage phase II trial design (Simon R, Controlled Clinical Trials 10:1-10, 1989), in order to rule out an unacceptably low 10% clinical response rate (PR+CR; p0=0.10) in favor of a modest response rate of 30% (p1=0.30). With alpha=0.05 (probability of accepting a poor treatment=0.05) and beta = 0.10 (probability of rejecting a good treatment=0.10), cohort 1 will initially enroll 18 evaluable patients and if 0-2 of the 18 have a clinical response, then no further patients will be accrued. If 3 or more the first 18 have a response, then accrual would continue until a total of 35 patients have enrolled. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause of up to 6 months in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 3-6 responses in 35 patients, this would be an uninterestingly low response rate, while if there were 7 or more responses in 35 patients, then this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (10% response rate), the probability of early termination is 73%.

Cohort 2

Patients assigned to Cohort 2 would not have any other option for TIL therapy. For these patients, a modest response rate in a relatively limited number of patients would warrant subsequent study. Accrual to this cohort will take place using an optimal two-stage phase II trial design, in order to rule out an unacceptably low 5% clinical response rate (PR+CR; p0=0.05) in favor of a modest response rate of 25% (p1=0.25). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta = 0.10 (probability of rejecting a good treatment=0.10), cohort 2 will initially enroll 9 evaluable patients and if 0 of the 9 have a clinical response, then no further patients will be accrued. If 1 or more of the first 9 patients has a response, then accrual would continue until a total of 24 patients have been enrolled. As it may take several weeks to determine if a patient has experienced a clinical response, a temporary pause in the accrual to the trial may be necessary to ensure that enrollment to the second stage is warranted. If there are 1-2 responses in 24 patients in this cohort, this would be an uninterestingly low response rate, while if there were 3 or more responses in 24 patients, then this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% response rate), the probability of early termination in the trial is 63%.

Secondary objectives of this trial include evaluating the safety of TIL that have been manufactured using ECCE accessory cells with enhanced costimulation, and the persistence of these ECCE TIL. Accrual of a substantial number of patients in each cohort will also assist in achieving these secondary aims.

It is anticipated that 2-5 eligible patients may enroll on this trial in a month. Thus, with a requirement of up to 59 patients, this study should be able to complete its accrual within two to three years. In order to allow for the possibility of a limited number of inevaluable patients, the accrual ceiling will be set at 63 patients.

5.4. Clinical Trial Monitoring Plan

A detailed description of the clinical trial monitoring plan has been included in the initial IND submission as required. Briefly, Harris Technical Services Corporation provides study auditing/monitoring services under contract with the Center for Cancer Research, NCI. The number of patient records monitored is based on actual accrual. Below is a tabular summary of monitoring evaluations:

Time of year	Evaluation	# of records monitore	%data verification
1st quarter (Jan.- March)	Compare CRFs to source documentation, verify subject registration with Drug Accountability Records	3-4 study patients	100%
2nd quarter (April – June)	Compare CRFs to source documentation, verify subject registration with Drug Accountability Records	3-4 study patients	100%
3rd quarter (July – Sept.)	Compare CRFs to source documentation, verify subject registration with Drug Accountability Records	3-4 study patients	100%
4th quarter (Oct. – Dec.)	Compare CRFs to source documentation, verify subject registration with Drug Accountability Records	3-4 study patients	100%

*Based on expected accrual, 25% of enrolled patients will be monitored.

5.5. Data and Safety Monitoring Plan

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of cell infusions will be performed. Due to the nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor reactive T-cell proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly according to plan specified in Section 3.3.5.

The principal investigator will review all serious adverse events and will monitor the data and toxicities to identify trends monthly. The principal investigator will be responsible for revising the protocol as needed to maintain safety. The NCI IRB will review submitted adverse events monthly to also evaluate trends and will require a follow up plan from the principal investigator whenever a trend is identified. A CCR Safety Monitoring Committee will monitor toxicity trends on this study on at least an annual basis and report any trends to the NCI IRB and Principal Investigator.

5.6. Handling of Tissue Specimens for Research Purposes

Blood or Tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the Tumor Immunology Cell Processing Laboratory. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW Surgery Branch Laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the Clinical Pharmacology Program (CPP) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services (Fisher Bioservices) in Frederick, MD. Data is entered and stored securely in the Patient Sample Data Management System (PSDMS) utilized by the CPP, and data will be updated to the Surgery Branch central computer database weekly. All samples (serum,blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

At the conclusion of this protocol, if additional studies are to be performed on any samples 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.

6.0 Human Subjects Protections

6.1. Rationale for Patient Selection

The patients to be entered in this protocol have metastatic melanoma which is refractory to standard therapy, and limited life expectancies.

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts

will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

6.2. Participation of Children

The use of the nonmyeloablative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening metastatic cancer. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic cancer, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

6.3. Evaluation of Benefits and Risks

The experimental treatment has a chance to provide clinical benefit though this is unknown. The risks in this treatment are detailed in section 5.2. The goal of this study is to improve upon the number of patients who may benefit from adoptive cell therapy by using ECCE TILs in patients who do not have standard TIL available for treatment. The success of this effort cannot be predicted at this time. Because all patients in this protocol have melanoma cancer and limited life expectancies the potential benefit is thought to outweigh the potential risks.

6.4. Consent Document

If the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The Informed Consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The research nurse, principal investigator, associate investigator, or clinical associate is responsible for obtaining written consent from the patient.

7.0 Data Reporting

This study will utilize the CTCAE 3.0 for toxicity and Adverse Event reporting. A copy of the CTCAE 3.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov). All appropriate treatment areas should have access to a copy of the CTCAE 3.0.
This study will be monitored by the Internal Monitoring System conducted by Harris Technical Services Corporation, with reporting to the QA specialist of the CCR, NCI.

7.1 Definitions

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. 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 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.

7.1.5 Disability

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

7.1.6 Life threatening Adverse Drug Reaction

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.

7.1.7 Protocol Deviation (NIH Definition)

A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the investigator's control and that has not been approved by the IRB.

7.1.8 Protocol Violation (NIH Definition)

Any change, divergence, or departure from the study procedures in an IRB-approved research protocol that has a major impact on the subject's rights, safety, or well-being and/or the completeness, accuracy or reliability of the study data.

7.1.9 Unanticipated Problems

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**

- 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 Routine Data Reporting:

All information surrounding patient screening, diagnosis, tumor harvest, treatment, and follow up will be recorded in the patient medical record. Data will be reported via the NCI SB Immunotherapy reporting system.

7.3 Expected Adverse Events and Protocol-Specific Expedited Adverse Event Reporting Exclusions:

Since the patient is receiving multiple agents which include commercially available agents either alone or in combination with investigational agents (aldesleukin, fludarabine, cyclophosphamide and supportive medications), Grade 1 and 2 adverse events 'unrelated' or 'unlikely related' to the investigational agents, and 'possibly', 'probably' or 'definitely' related to the commercially available agents as specified in the package inserts, or Appendix 2 or 3, do not require reporting/recording. For additional information about expected toxicities, refer to Section 8.

7.3.1 Aldesleukin:

Expected toxicities of aldesleukin are listed in the product label and in Appendix 3 and 4. No expedited reporting of expected grade 3 toxicities or grade 4 laboratory toxicities clearly related to aldesleukin, expected, easily managed, and reversed to less than grade 3 within 48 hours is required. Aldesleukin toxicities that must be reported in an expedited fashion include: need for endotracheal intubation, renal dialysis, coma, myocardial infarction, myocarditis, bowel perforation, sustained ventricular tachycardia, death or any grade 4 toxicity that is not listed in Appendix 2 or 3.

7.3.2 Chemotherapy Preparative Regimen:

Expected toxicities of cyclophosphamide and fludarabine are listed in the product label and will not be reported via expedited reporting system, including Grade 4 myelosuppression.

7.3.3 Adoptive Cell therapy:

Expected toxicities that may occur with cell infusion include fever, chills, headaches, malaise, shortness of breath (10% of patients have required intubation either for airway protection or dyspnea), renal toxicity and transient rash. It is possible that patients may develop autoimmune reactions after receiving cells (e.g. vitiligo or uveitis).

7.4 NCI IRB Reporting Requirements

7.4.1 NCI-IRB Expedited Reporting of Adverse Events, Unanticipated Problems and Deaths:

The Protocol PI will report to the NCI-IRB:

- All unexpected serious adverse events that are possibly, probably, or definitely related to the research
- All deaths, except deaths due to progressive disease
- All Protocol Violations or Deviations
- All Unanticipated Problems

Reports must be received by the NCI-IRB within 7 days of notification of the event. Follow-up reports can be submitted to the IRB after the NCI-IRB review. Information may be sent via the NCI iRIS Application: **https://iris.nci.nih.gov/iMedris/**

7.4.2 NCI-IRB Requirements for PI Reporting of Adverse Events at Continuing review

The protocol PI will report to the NCI-IRB:

- 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.

7.4.3 NCI-IRB Reporting of IND Safety Reports:

Only IND Safety Reports that require a sponsor recommended change to the protocol or the consent form or in the opinion of the PI increases risks to study participants will need to be reported to the NCI IRB.

7.5 Serious Adverse Event Reporting to the FDA:

During the duration of this trial, the principal investigator will assume responsibility for reporting any serious and unexpected suspected adverse reaction to the U.S. Food and Drug Administration (FDA) with appropriate deadlines defined by the regulations.

FDA will be informed by phone or fax of any unexpected fatal or life threatening suspected adverse reaction as soon as possible but in no event, later than 7 calendar days after the sponsor's initial receipt of the information.

FDA will be informed of any other unexpected and serious adverse event, by written notification within 15 calendar days after the sponsor's initial receipt of the information. All adverse events, including serious adverse events, will be reported in the annual report to the FDA as indicated in 21CFR312.33.

8.0 Pharmaceutical Information

8.1 Interleukin-2 (Aldesleukin, Proleukin, Recombinant Human Interleukin 2)

How Supplied: Interleukin-2 (aldesleukin) is manufactured by the Chiron Corporation, Emeryville, CA, and will be purchased by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Aldesleukin, NSC #373364, is provided as single-use vials containing 22 million IU (~1.3 mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/ml or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

Storage: Intact vials are stored in the refrigerator (2^0 - 8^0C) protected from light. Each vial bears an expiration date.

Dilution/Stability: Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent prior to the addition of RIL-2. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 2^0 – 30^0C.

Administration: The dosage will be calculated based on total body weight. The final dilution of aldesleukin will be infused over 15 minutes. Aldesleukin will be administered as an inpatient.

Toxicities: Expected toxicities of aldesleukin are listed in the product label and in Appendix 5 and 6. Grade III toxicities common to aldesleukin include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 2. Additional grade III and IV toxicities seen with aldesleukin are detailed in Appendix 3.

8.2 Fludarabine

Description: (Please refer to package insert for complete product Information) Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

How Supplied: It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

Stability: Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/ml, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8^{0}C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribonucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Storage: Intact vials should be stored refrigerated (2-8°C).

Administration: Fludarabine is administered as an IV infusion in 100 ml 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 6.

Toxicities: At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of

CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

8.3 Cyclophosphamide

(Refer to FDA-approved package insert for complete product information)

Description: Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

How Supplied: Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8^{0}C.

Administration: It will be diluted in 250 ml D5W and infused over one hour. The dose will be based on the patient's body weight. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 6.

Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

8.4 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)
(Please refer to the FDA-approved package insert for complete product information)

Description: Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/ml solution.

Storage: Intact ampoules are stored at room temperature.

Stability: Diluted solutions (1 to 20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% NaCl.

Administration: Dilute to concentrations less than or equal to 20 mg mesna/ml fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 6. Toxicities include nausea, vomiting and diarrhea.

8.5 Filgrastim (Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen)

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 ug/ml and 480 ug/1.6 ml vials. G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken. It is generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe. G-CSF will be given as a daily subcutaneous injection. The side effects of G-CSF are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

8.6 Trimethoprim and Sulfamethoxazole Double Strength (TMP / SMX DS)

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO daily three times a week (on NON-consecutive days) beginning on day -7 and continuing for at least 6 months and until the CD4 count is greater than 200 on 2 consecutive lab studies. Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever eight to fourteen days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

8.6.1. Aerosolized Pentamidine in Place of TMP/SMX DS:

Patients with sulfa allergies will receive aerosolized Pentamidine 300 mg per nebulizer within one week prior to admission and continued monthly until the CD4 count is above 200 on two consecutive follow up lab studies and for at least 6 months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

8.7 Herpes Virus Prophylaxis

8.7.1. Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valacyclovir will be started the day after the last dose of fludarabine at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

8.7.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7mg/mL or less and infused over 1 hour to avoid renal damage. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

8.8 Fluconazole

Fluconazole will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prophylax against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage which may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 MG/ML solution for injection, and prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr.

8.9 Cell Preparation

The procedure for growing and expanding the autologous young TIL and the Certificate of Analysis are similar to those approved by the Food and Drug Administration and used at the NCI in protocols 04-C-0181, 04-C-0251, and 07-C-0003 with slight changes to reflect improved techniques, including a potency assay consisting of cytokine release (IFN > 200 pg/mL) following OKT3 stimulation. This product will be provided for investigational use only under the investigator-sponsor IND 14265. The Certificate of Analysis is in Appendix 1 and the Standard Operating Procedures for the growth of TIL and TIL expansion in the REP procedures are included in IND 14265.

8.10 OKT3

OKT3 will be obtained by the Surgery Branch Laboratory from commercial sources.

Formulation: Muromonab-CD3 (Ortho), NSC #618843, is provided as a sterile, clear, colorless solution at a concentration of 1 mg/ml in 5 ml ampoules. The solution may contain a few fine, translucent protein particles. The antibody is dissolved in a buffered solution at pH of 6.5 to 7.5. The solution contains 2.25 mg of monobasic sodium phosphate, 9 mg of dibasic sodium phosphate, 43 mg of sodium chloride and 1 mg of polysorbate 80 per 5 ml of water for injection.

Storage/Stability: Ampules should be stored in a refrigerator at 2-8° C. Solution should not be frozen or shaken. Each ampule bears an expiration date.

8.11 Support Medications

Ondansetron hydrochloride

Ondansetron hydrochloride will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention. Consult the package insert for specific dosing instructions.

Furosemide

Furosemide will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritis. Consult the package insert for a complete list of all side effects.

9.0 References

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10.0 Tables, Figures, and Appendices

Table 1
Cell Transfer Therapy (updated 8/1/10)

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (82+, 81+, 79+, 78+, 64+)	21 (49%)
200 TBI	25	8 (14, 9, 6, 6, 5, 4, 3, 3)	5 (68+, 64+, 60+, 57+, 54+)	13 (52%)
1200TBI	25	8 (21, 13, 7, 6, 6, 5, 3, 2)	10 (48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, 37+, 19)	18(72%)

(52 responding patients: 43 had prior IL-2; 23 had prior IL-2 + chemotherapy)
(20 complete responses: 19 ongoing at 37 to 82 months)

Table 2: Transfusions and grade 3 and 4 non-hematologic toxicities associated with NMA plus TBI lymphodepleting preparative regimens.

	200cGy TBI	1200cGy TBI
Total patients	**25**	**25**
Transfusions administered (+SD)		
Platelets (6-10 units per transfusion)	3.8 (±3.4)	8.1 (± 4.4)
Packed RBCs	4.0 (±3.7)	6.2 (± 4.0)
Infection related toxicities		
CMV infection	1	1
Herpes zoster	1	2
Positive blood cultures	2	4
Other toxicities		
Intubated for somnolence	1	4
Pulmonary hypertension	1	0
Febrile neutropenia	12	16
Jugular venous thrombosis	1	0
Autoimmune uveitis and hearing loss (transient)	0	1
Thrombotic microangiopathy	0	4
Death (bowel-perforation sepsis)	1	0

Table 3: Time in Hospital and Non-hematological Grade 3 and 4 Toxicities Related to Lymphodepleting Chemotherapy and Cell Transfer

Attribute measured	Duration, Number or Type	Number of Patients (%)
Days in Hospital[1]	6-10	6 (17%)
	11-15	18 (51%)
	16-20	4 (11%)
	21-25	7 (20%)
pRBC Transfusions	0	2 (6%)
	1-5	18 (51%)
	6-10	13 (37%)
	11-15	2(6%)
Platelet Transfusions	0	6 (17%)
	1-5	21 (60%)
	6-10	5 (14%)
	11-15	2 (6%)
	16-20	1 (3%)
Autoimmunity	Uveitis	5 (14%)
	Vitiligo	13 (37%)
Opportunistic Infections	Herpes zoster	3 (9%)
	Pneumocystis pneumonia	2 (6%)
	EBV-B cell lymphoma	1 (3%)
	RSV pneumonia	1 (3%)
Other	Febrile neutropenia	13 (37%)
	Intubated for dyspnea	3 (9%)
	Cortical blindness	1 (3%)

[1]Measured from the day of cell administration to discharge

Table 4. Establishment of TIL is improved with ECCE and dependent on 4-1BBL.
A) Standard TIL expansion from single cell enzymatic tumor cell suspensions was compared with Engineered Cellular Costimulation Enhanced (ECCE) TIL growth in 33 samples.
B) Subsequently, ECCE TIL growth using 4-1BBL or without 4-1BBL was compared in 9 samples. .

		Samples evaluated	Standard TIL growth	ECCE TIL growth	P value*
A	**Cryopreserved**	21	6	14	0.03
	Fresh	12	4	12	0.001
	Total	33	10 (30%)	26 (79%)	
		Samples	**No 4-1BBL**	**With 4-1BBL**	
B	**Total**	9	0 (0%)	9 (100%)	.0001

*Fisher's exact test

Table 5. TIL and ECCE TIL both demonstrate specific tumor recognition. Lymphocyte cultures derived from the tumors using standard methods (TIL) or with addition of K562 expressing 4-1BBL (ECCE TIL) were tested for specific tumor recognition by interferon-gamma (IFN-g) secretion after overnight coculture.

IFN-g (pg/ml)		**Allogeneic tumor**		**Autologous tumor**	
Patient	**HLA**	**TIL**	**ECCE TIL**	**TIL**	**ECCE TIL**
3018-1a	02,33	26	35	217	502
3289	29,32	55	58	789	410
3282-1,2,3	02,24	7	19	341	363
3084	02,24	0	12	1760	6040
3281	03,11	0	0	522	2290

TABLE 6: Modification of Dose Calculations* in patients whose BMI is greater than 35

Unless otherwise specified in this protocol, actual body weight is used for dose calculations of treatment agents. In patients who are determined to be obese (BMI > 35), the **practical weight** (see 3 below) will be used.

1. BMI Determination:

   BMI = weight (kg) / [height (m)]2

2. Calculation of ideal body weight

   Male = 50 kg + 2.3 (number of inches over 60 inches)
   Example: ideal body weight of 5'10'' male
   50 + 2.3 (10) = 73 kg

   Female = 45.5 kg + 2.3 (number of inches over 60 inches)
   Example: ideal body weight of 5'3'' female
   45.5 + 2.3 (3)
   = 57 kg

3. Calculation of "practical weight"

   Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

*Practical weight will NOT be used in the calculation of dose for aldesleukin.

Figure 1

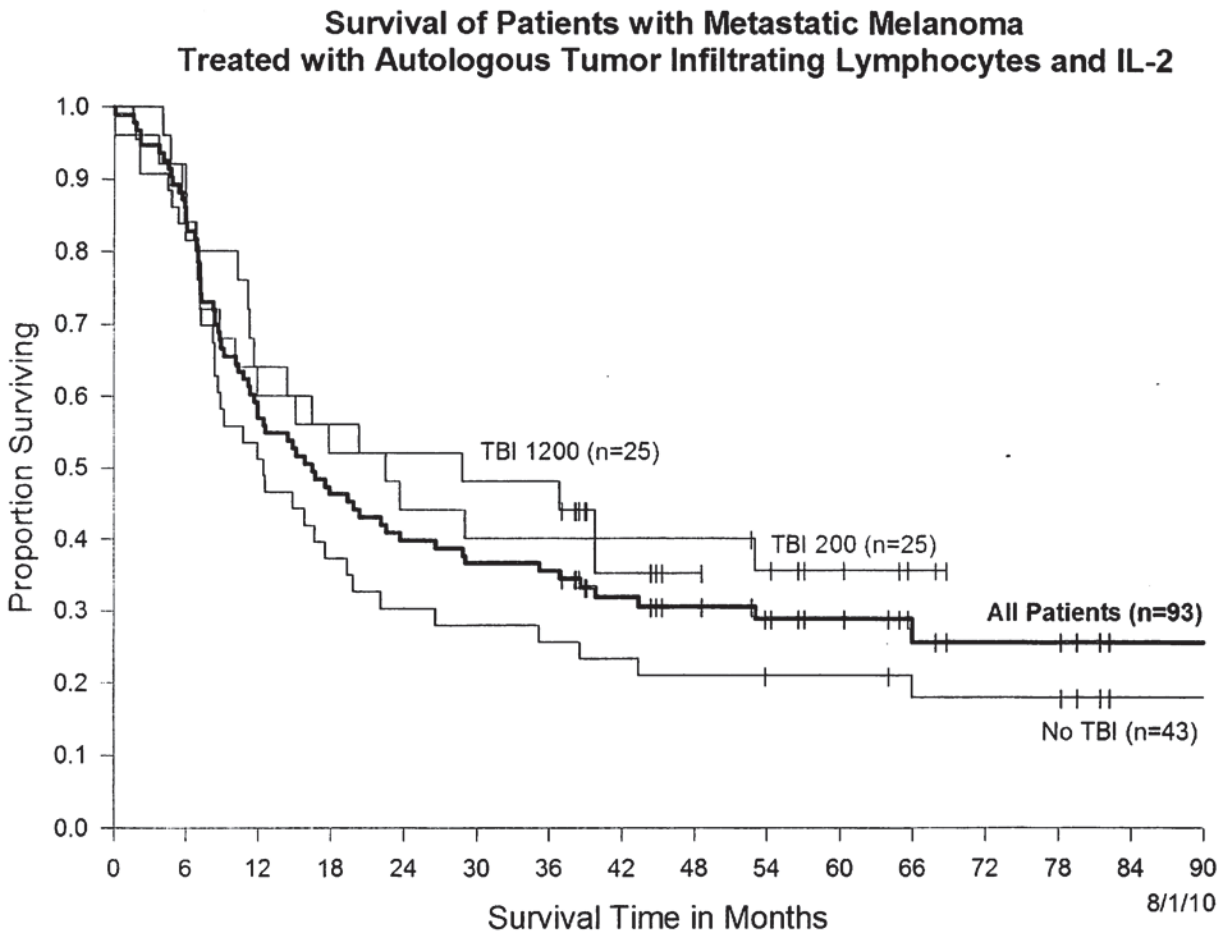
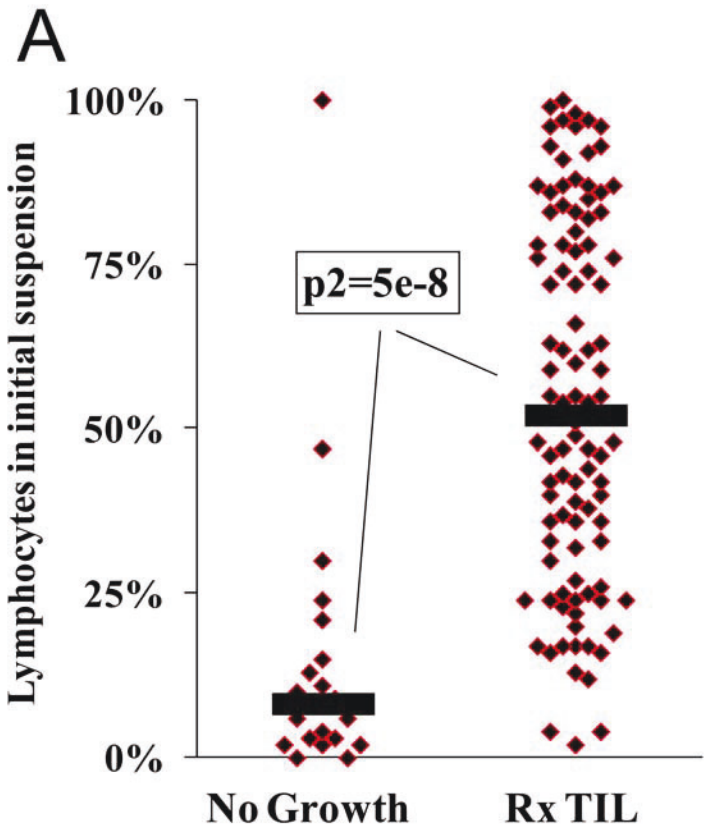
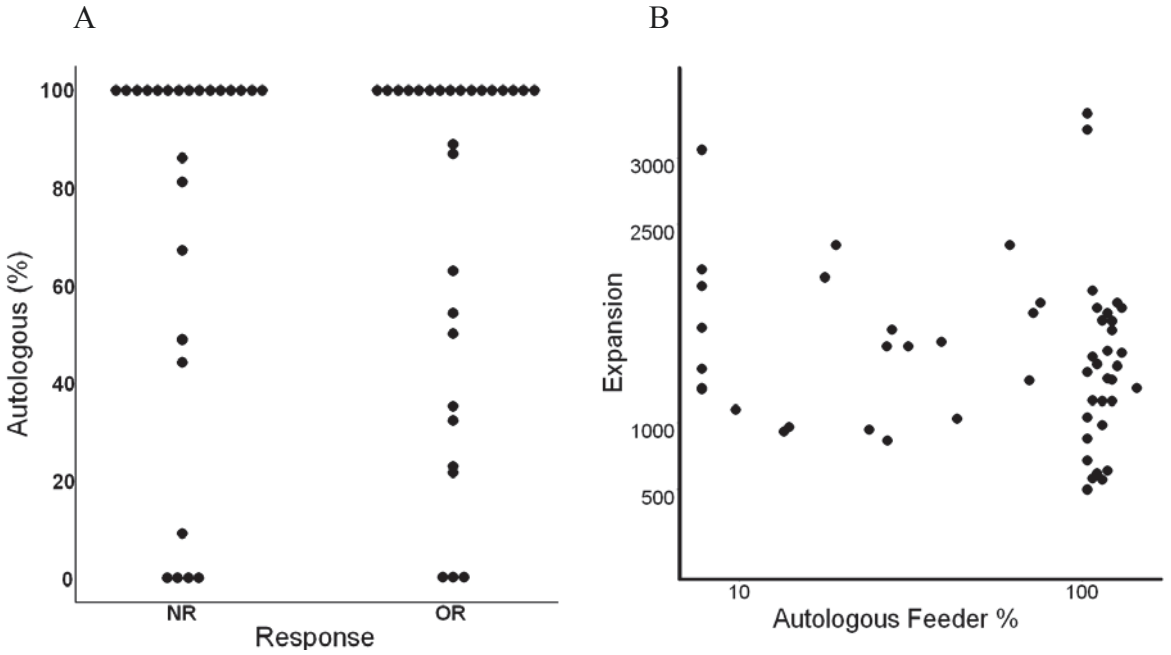

Survival of Patients with Metastatic Melanoma
Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2
Proportion Surviving
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TBI 1200 (n=25)
TBI 200 (n=25)
All Patients (n=93)
No TBI (n=43)
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
Survival Time in Months
8/1/10

Figure 2



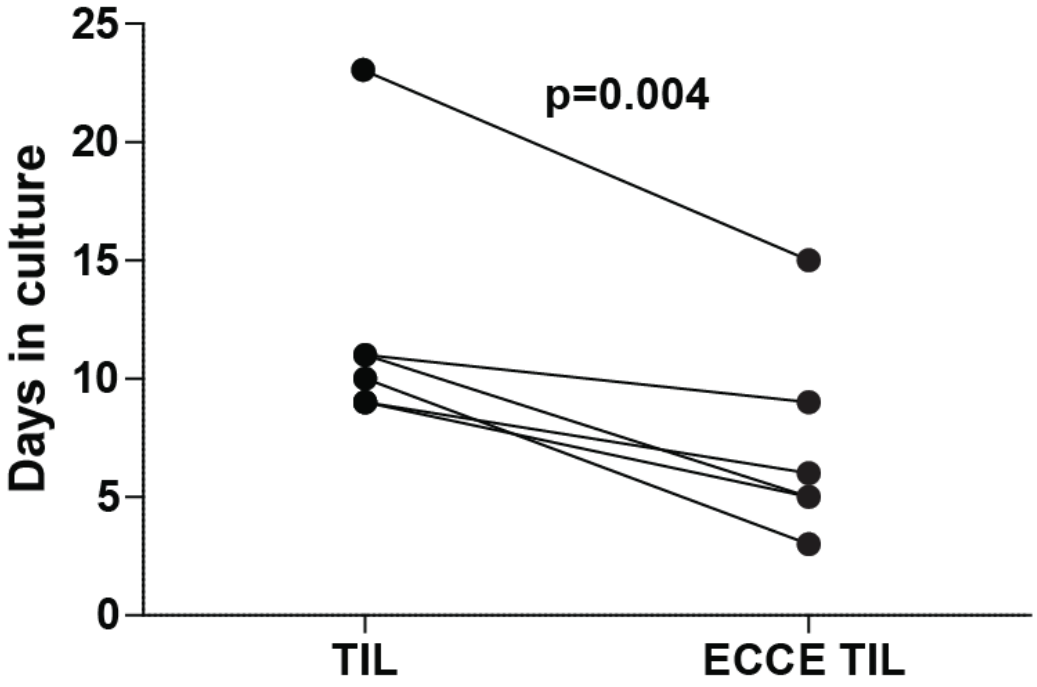
The fraction of lymphocytes in an initial single cell suspension of disaggregated tumor predicts TIL growth. Single cell disaggregated tumor samples from 121 sequential patients were evaluated by trypan blue staining and microscopic evaluation for the presence of lymphocytes immediately after processing. The cell suspensions were plated for initiation of TIL cultures and subsequently scored for "no growth" or establishment of TIL suitable for treatment ("Rx TIL"). Poorly infiltrated tumors rarely resulted in the growth of TIL cultures suitable for therapy.

Figure 3



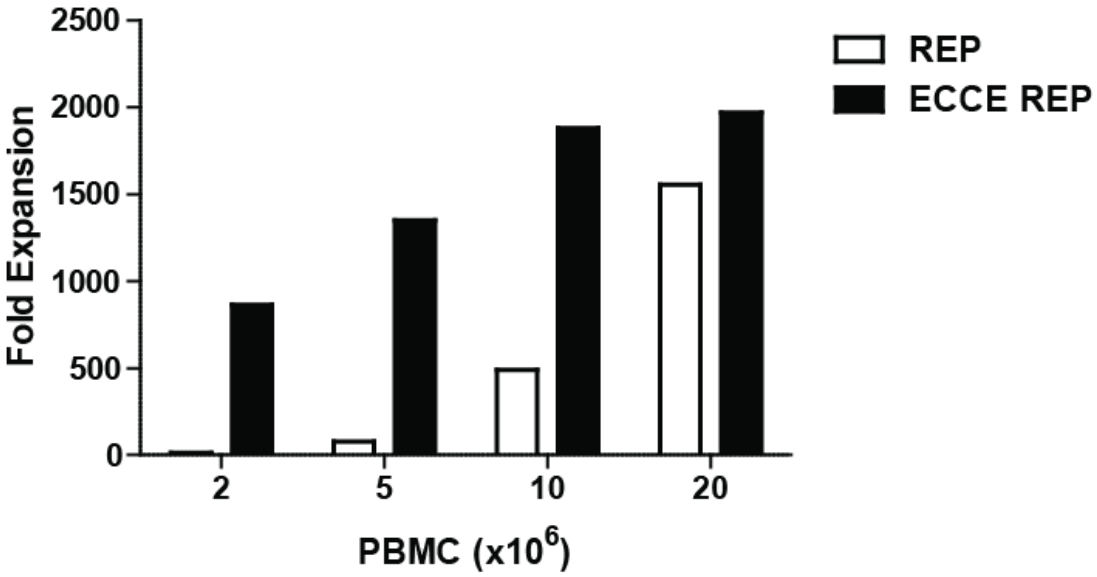
No impact of genotype (autologous vs. allogeneic) of PBMC on response or expansion. Patients treated with CD8+ enriched young TIL were evaluated. A) The fraction of autologous PBMC used as feeder cells in the REP is plotted compared to whether the product mediated an objective clinical response (OR) or no response (NR). There was no difference in clinical outcome compared to genotype of the feeder cells. B) Expansion of TIL in the REPis plotted compared to the fraction of autologous PBMC used as feeder cells. (some REPS with 100% autologous feeders were jiggered above the 100% mark for visibility). There was no correlation between genotype of feeders and proliferation in the REP

Figure 4



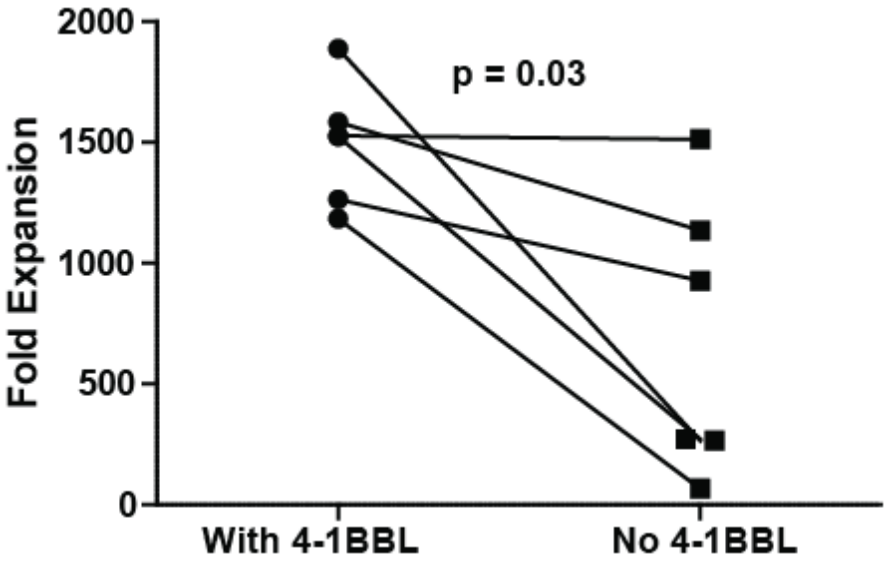
TIL cultures are rapidly established with inclusion of Engineered Cellular Costimulation Enhancement (ECCE). Single cell tumor suspensions were plated in the absence (TIL) or presence (ECCE TIL) of K562 expressing 4-1BBL. Lymphocyte growth was monitored daily and the day noted when confluent growth was first reached.

Figure 5



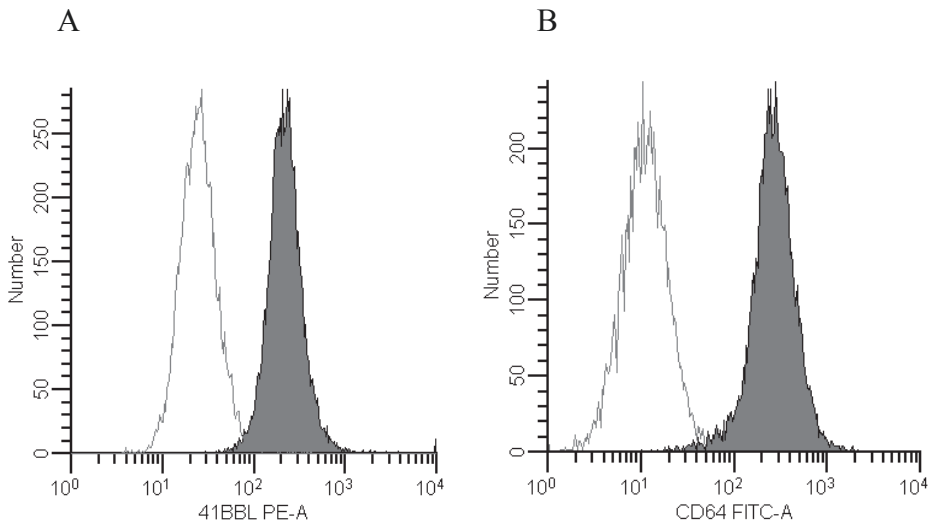
Expansion of TIL is directly dependent on feeder cell number in REP. $1x10^5$ responding TIL were stimulated with OKT3 and IL-2 in the presence of different numbers of PBMC without (REP) or with (ECCE REP) $1x10^6$ Engineered Cellular Costimulation Enhancement. Calculated fold expansion was determined 14 days later.

Figure 6



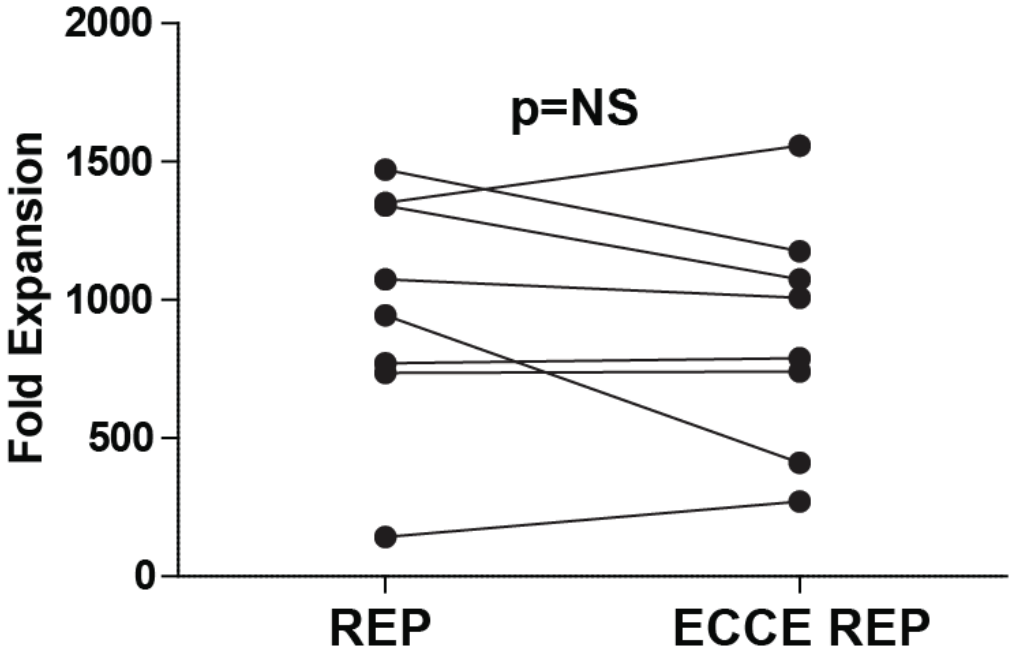
ECCE REP with K562 cells is dependent on expression of 4-1BBL. TIL were expanded by ECCE REP with OKT3, IL-2, reduced feeders cells, and K562 that were transduced to express 4-1BBL (With 4-1BBL) or were not transduced with 4-1BBL (No 4-1BBL). The calculated cell number after 14 days of expansion is plotted and the two tailed p value for a paired T test is shown (p=0.03).

Figure 7.



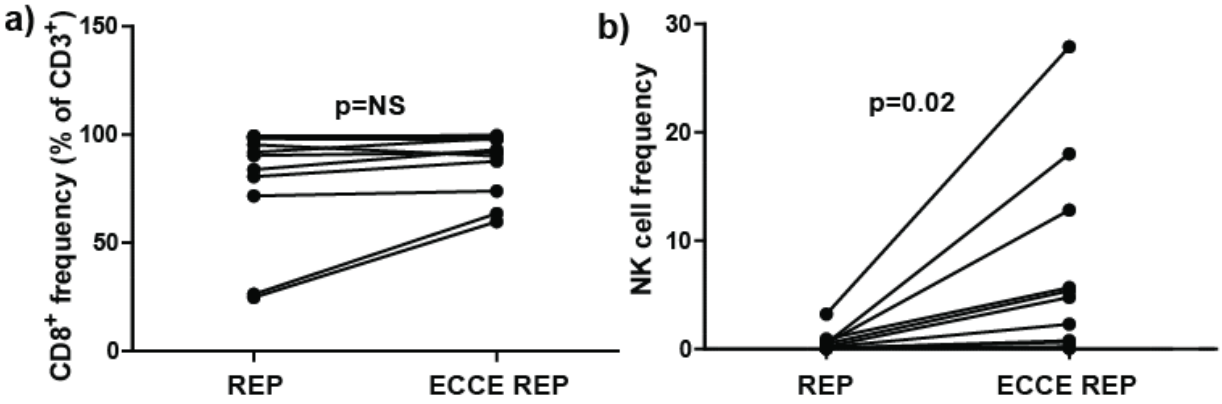
Clone 7F11 was generated for GMP production of a master cell bank suitable for ECCE TIL manufacture. K562 cells were transduced with a retrovirus encoding the CD64 gene then with the 4-1BBL gene. Cells were cloned by limiting dilution. Clone 7F11 was expanded and evaluated by FACS for surface expression of the transduced genes. A) 4-1BBL expression by K562 (open histogram) and 7F11 (filled histogram). B) CD64 expression by K562 (open histogram) and 7F11 (filled histogram).

Figure 8



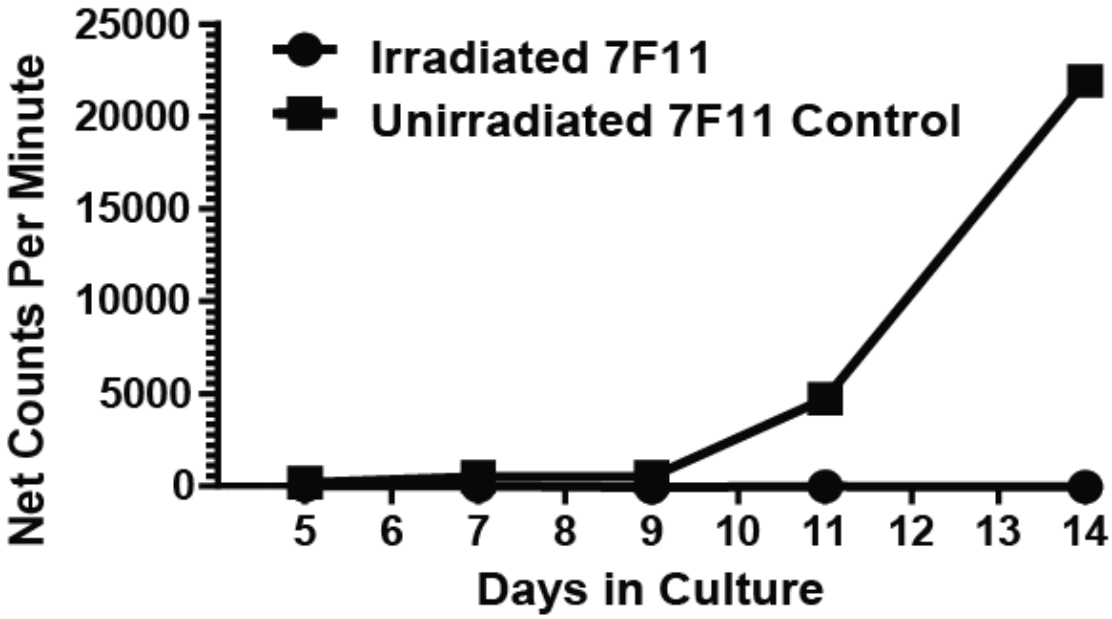
TIL showed similar proliferation when expanded by conventional REP and ECCE REP at clinical scale. Eight different TIL samples were expanded at clinical scale using the standard REP set up with OKT3, IL-2 and 200 fold excess PBMC feeder cells. Each REP was compared to an ECCE REP that was expanded with OKT3, IL-2, 50 fold excess PBMC feeder cells, and 10fold excess 100Gy irradiated 7F11. The expansions were not significantly different on day 14 (paired T test).

Figure 9.



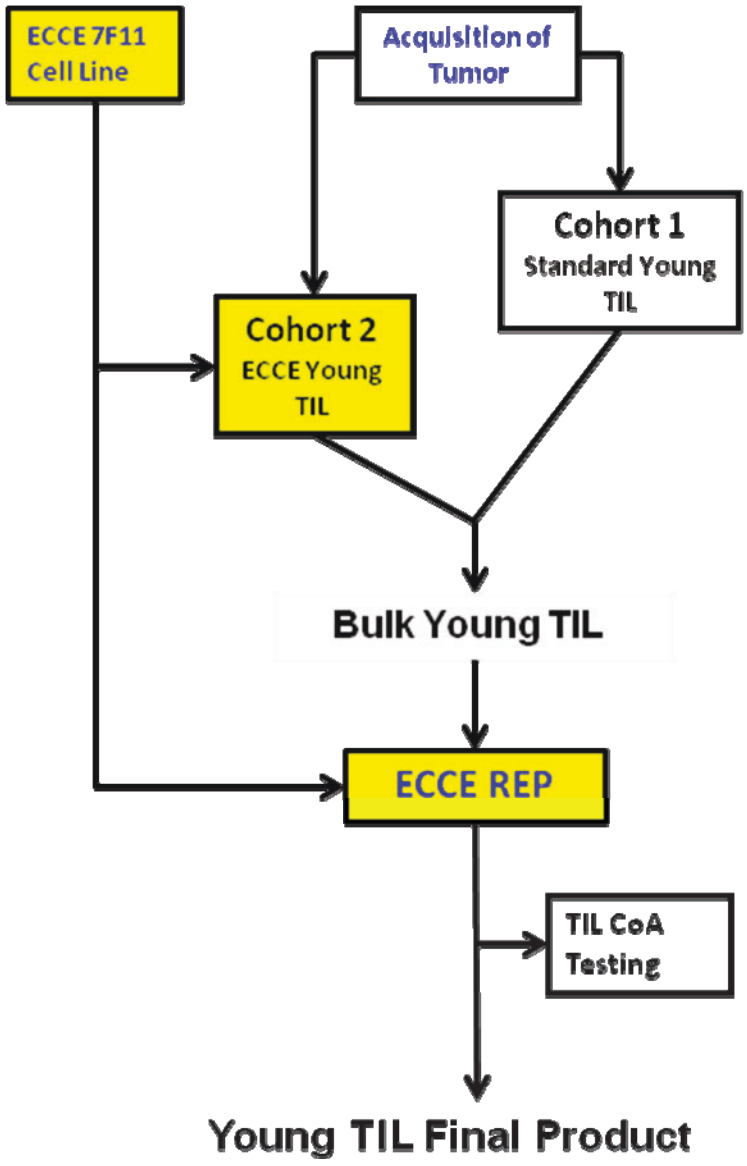
ECCE REP generates similar CD8+ cell frequency to REP, but significantly more NK cells in expanded TIL. ECCE REP TIL generated using 7F11 was compared to REP TIL generated with PBMC only. A) CD8+ cell frequency was measured by FACS and found to be similar (NS= not significant). B) NK cell frequency (%CD3-CD56+) was significantly higher in the ECCE REP samples.

Figure 10.



Safety of irradiated 7F11 Engineered Cells for Costimulatory Enhancement by demonstrating lack of proliferation. $1x10^5$ 7F11 cells (100gy gamma-irradiated) were plated per well in triplicate wells (circles) in 96 well plates. In parallel, non-irradiated 7F11 was added at an average of one cell per $1x10^5$ 100gy irradiated 7F11 cells prior to plating. At the indicated times, samples were labeled with tritiated thymidine and proliferation was measured by thymidine incorporation. Live cell controls were clearly detected at a sensitivity of 1 cell per $1x10^5$ total cells. No viable cells were detected in 7F11 ECCE cells during 14 days comprising the time of a clinical REP.

Figure 11: Schematic of Young TIL manufactured with ECCE 7F11



Appendix 1: **Certificate of Analysis:**
Engineered Cells for Costimulation Enhanced (ECCE) TIL

Date of preparation of final product:
Patient:

Allogeneic PBMC
Donor Name:
Pheresis date:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Initials/ Date*
Cell viability[1]	trypan blue exclusion	>70%		
Total viable cell number[1]	visual microscopic count	Between 1 x 10^9 and 1 x 10^{11} cells		
Identity[4]	FACS	> 70 % CD3+ after ECCE REP		
Absence of ECCE[4]	FACS	<0.5% CD137L+ after ECCE REP		
Activity[2]	IFNgamma release	>200 pg/ml, and greater than 2x background		
Microbiological studies	gram stain[1,3,]	no micro-organisms seen		
	aerobic culture[3,4]	no growth		
	fungal culture[3,4]	no growth		
	anaerobic culture[3,4]	no growth		
	mycoplasma test[5]	negative		
Endotoxin	limulus assay[1]	<5 E.U./kg		
Presence of tumor cells[2]	Cytopathology	No tumor cells per 200 cells examined		

[1] Performed on sample of the final product immediately prior to infusion. Results are available at the time of infusion.
[2] Performed 2-10 days prior to infusion. Results are available at the time of infusion.
[3] Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient.
[4] Performed 2-4 days prior to infusion. Results are available at the time of infusion but may not be definitive.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____
James C. Yang, M.D. or designee

Appendix 2:

ADVERSE EVENTS OCCURRING IN ≥10% OF PATIENTS TREATED WITH ALDESLEUKIN (n=525)[1]

Body System	% Patients
Body as a Whole	
Chills	52
Fever	29
Malaise	27
Asthenia	23
Infection	13
Pain	12
Abdominal pain	11
Abdomen enlarged	10
Cardiovascular	
Hypotension	71
Tachycardia	23
Vasodilation	13
Supraventricular tachycardia	12
Cardiovascular disorder[a]	11
Arrhythmia	10
Digestive	
Diarrhea	67
Vomiting	50
Nausea	35
Stomatitis	22
Anorexia	20
Nausea and vomiting	19
Hemic and Lymphatic	
Thrombocytopenia	37
Anemia	29
Leukopenia	16

Body System	% Patients
Metabolic and Nutritional Disorders	
Bilirubinemia	40
Creatinine increase	33
Peripheral edema	28
SGOT increase	23
Weight gain	16
Edema	15
Acidosis	12
Hypomagnesemia	12
Hypocalcemia	11
Alkaline phosphatase incr	10
Nervous	
Confusion	34
Somnolence	22
Anxiety	12
Dizziness	11
Respiratory	
Dyspnea	43
Lung disorder[b]	24
Respiratory disorder[c]	11
Cough increase	11
Rhinitis	10
Skin and Appendages	
Rash	42
Pruritus	24
Exfoliative dermatitis	18
Urogenital	
Oliguria	63

a Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.
b Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.
c Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.
[1]Source: Proleukin® Prescribing Information – June 2007

Appendix 3

Expected IL-2 Toxicities and their Management

Expected toxicity	Expected grade	Supportive Measures	Stop Cycle*	Stop Treatment **
Chills	3	IV Meperidine 25-50 mg, IV q1h, prn,	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethicin 50-75 mg, po, q8h	No	No
Pruritis	3	Hydroxyzine HCL 10-20 mg po q6h, prn; Diphenhydramine HCL25-50 mg, po, q4h, prn	No	No
Nausea/ Vomiting/ Anorexia	3	Ondansetron 10 mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg pr, prn or 10 mg IV q6h prn	No	No
Diarrhea	3	Loperamide 2mg, po, q3h, prn; Diphenoxylate HCl 2.5 mg and atropine sulfate 25 mcg, po, q3h, prn; codeine sulfate 30-60 mg, po, q4h, prn	If uncontrolled after 24 hours despite all supportive measures	No
Malaise	3 or 4	Bedrest	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation Vasopressor support	If uncontrolled despite all	No

			supportive measures	
Dyspnea	3 or 4	Oxygen or ventilatory support	If requires ventilatory support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased creatinine	3 or 4	Observation	Yes (grade 4)	No
Renal failure	3 or 4	Dialysis	Yes	Yes
Pleural effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose
Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated transaminases	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Hyperbilirubinemia	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours

** Unless the toxicity is not reversed to grade 2 or less by next treatment.