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Phase I trial of ECI301 in Combination with Radiation in Patients with Advanced or Metastatic Cancer

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PRECIS

BACKGROUND:

- Patients with metastatic or locally advanced cancer frequently require palliative radiotherapy to relieve symptoms; however, progression of disease is frequent in patients with extended survival
- Radiation results in tumor cell death which can result in increased dendritic cell activation and trafficking
- ECI301 is a derivative of Macrophage Inflammatory Protein-1α, a 70 amino acid chemokine that is a ligand for CCR1 and CCR5, the chemokine receptors of immature dendritic cells.
- ECI301 has been shown to enhance the effect of radiotherapy in animal models.

Objectives:

- The primary objective is to determine the maximum tolerated dose (MTD) of ECI301 delivered in combination with 30 Gy of external beam radiation to patients with metastatic or locally advanced cancer.
- The secondary objectives are:
    - To describe the safety and tolerability of ECI301 delivered in combination with 30 Gy of external beam radiation to patients with metastatic or locally advanced cancer
    - To evaluate the humoral and cellular immune responses by:
        - Measurement of circulating precursor dendritic cells before and after the completion of ECI301
        - Measurement of circulating MIP-α before and after the completion of ECI301
        - Assessment of T-lymphocyte quantitative and qualitative changes by flow cytometry and assays for IFNγ production
    - To define pharmacologic parameters following the intravenous dose of ECI301
    - To determine if neutralizing anti-EC301 antibodies occur after treatment
    - To describe the response at the radiated site and distant sites after radiation in combination with ECI301

Eligibility:

- Age >18 years.
- ECOG performance status <2.
- Life expectancy of greater than 3 months
- Histologically confirmed metastatic or locally advanced cancer for which radiotherapeutic management would be appropriate
- No recent history of myocardial infarction or unstable angina

Design:

- This is a Phase I trial to determine the maximum tolerated dose of ECI301 in combination with external beam radiation therapy in patients with locally advanced or metastatic solid tumors.
- Patients will be treated with radiation therapy in a standard manner for two weeks with ECI301 given daily following radiation during the second week. The dose of ECI301 will be escalated over the course of the trial to determine the MTD of daily ECI301 in combination with radiotherapy.
- We anticipate that accrual to this trial of 30 patients will take approximately 2 years.

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1 INTRODUCTION

Metastases are a common complication of the majority of solid tumors including malignancies of the breast, prostate, lung, head and neck, and gastrointestinal tract. Metastatic disease can cause local symptoms such as pain, mass effect (airway obstruction, spinal cord compression), and pathologic fracture. Therapy for metastatic disease is usually palliative with the goal of ameliorating symptoms. Often, a short course of radiation such as 250 cGy fractions to a total dose of 3750 cGy (three weeks) is delivered with the intention of providing palliation for these symptoms. Although these doses provide palliation in the majority of patients these are not considered curative or sterilizing doses. Patients diagnosed with locally advanced or metastatic cancer, with a few exceptions, have a poor prognosis regardless of the therapy they receive, with the vast majority of patients succumbing to their disease. This setting offers the unique opportunity to test an agent that may enhance local therapy or result in an abscopal effect due to immune activation.

Radiation is known to result in a number of changes in the tumor bed which may enhance the efficacy of immunotherapy, including tumor cell necrosis, upregulation of MHC class I molecules, expression of adhesion molecules on tumor vasculature, and expression of a variety of proinflammatory cytokines and chemokines.[1, 2] Chemokines play a major role in the recruitment of leukocytes to sites of infection and activation of these cells to enhance local inflammation [3-5]. One such chemokine MIP-1α, is produced by macrophages and binds to specific dendritic cell (DC) chemokine receptors (CCR1 and CCR5). Dendritic cells are potent antigen presenting cells that perform a vital role in the initiation and regulation of both innate and adaptive immunity including anti-tumor responses. Tissue dendritic cells internalize and process locally released antigens, migrate to draining lymph nodes, and present antigenic peptides in association with co-stimulatory molecules leading to the induction of helper and cytotoxic T cell responses. ECI301 or eMIP is a derivative of MIP-1α with favorable pharmacologic properties and an excellent safety profile in lower species.

We propose a phase I study of ECI301 in combination with concurrent RT directed at sites of locally advanced disease or metastases to define the maximum tolerated dose of this combination and to describe any toxicities observed. Correlative assays and optional biopsies will allow investigation of any biologic effect of ECI301 delivered in this setting. We hypothesize that the addition of ECI301 will enhance radiotherapeutic effect locally in a variety of histologies while increasing immune recognition of tumor at distant sites with acceptable toxicity.

1.1 STUDY OBJECTIVES:

- The primary objective is to determine the maximum tolerated dose (MTD) of ECI301 delivered in combination with 30 Gy of external beam radiation to patients with metastatic or locally advanced solid tumors.
- The secondary objectives are
  - To describe the safety and tolerability of ECI301 delivered in combination with 30 Gy of external beam radiation to patients with metastatic or locally advanced cancer.
  - To evaluate the humoral and cellular immune responses by
    - Measurement of circulating precursor dendritic cells before and after the completion of ECI301
    - Measurement of circulating MIP-α before and after the completion of ECI301

- Assessment of T-lymphocyte quantitative and qualitative changes by flow cytometry and assays for IFNγ production
- To define pharmacologic parameters following the intravenous dose of ECI301
- To determine if neutralizing anti-EC301 antibodies occur after treatment
- To describe the response at the radiated site and distant sites after radiation in combination with ECI301

1.2 BACKGROUND AND RATIONALE:

1.2.1 Radiotherapy for palliation of metastases

Metastatic disease is a common occurrence in patients with cancer. Common sites of metastasis include the lung, bone, brain, and liver. Symptoms of metastatic disease are dependent on the location of the metastasis, the rate of growth, and the proximity to critical structures. For example, bone metastases may lead to pain or an increased risk of fracture. Visceral metastases may lead to end organ dysfunction due to obstruction, mass effect, or replacement of parenchyma. The therapy chosen for metastatic disease often is dependent on the site of metastasis, the overall disease status, and the histology.

Radiation has been used extensively for the management of metastases at a variety of sites. One common indication for palliative radiotherapy includes treatment of bone metastases causing pain or bone destruction. Visceral and soft tissue metastases are also frequently treated with radiation to provide local symptom relief or prevent impending oncologic emergencies or urgencies such as cord compression from spinal metastases, superior vena cava syndrome from lung or mediastinal metastases, airway compromise from pulmonary or mediastinal metastases, obstructive jaundice or uncontrolled capsular pain from hepatic metastases, renal failure due to an obstructing lesion, deep venous thrombosis as a result of bulky malignant adenopathy, and a variety of other end-organ dysfunctions related to soft tissue or visceral metastases.

The goal of therapy usually depends on the presenting signs and symptoms, the extent of systemic disease, and the systemic therapeutic options. Frequently, a short course of hypofractionated radiation is delivered to maximize pain or symptom relief while minimizing the amount of time required for therapy. Unfortunately, re-treatment due to progression or increase in metastatic burden distant to the irradiation site occurs often in patients with relatively longer survivals. In one series of patients treated with radiation for prostate cancer bone metastases, as many as 58.6% required re-treatment at some point in their course with 22% of these patients requiring re-treatment of the spine [6]. In the majority of randomized trials evaluating various radiation fractionation schemes, re-treatment rates for bone metastases for pain or other indications approaches a disappointing 20-42% with most conventional doses [7]. Radiation has been studied extensively in this setting with local control frequently used as the primary endpoint in most series, even in the face of varying systemic therapies.

1.2.2 Modulating the effects of radiation

Radiation modifiers are agents that alter the effect of radiation when delivered in proximity to the radiation dose. A classic radiation sensitizer is a radiation modifier that has minimal toxicity and enhances radiation response selectively in tumor cells compared to normal tissue. A number of agents have been evaluated as radiation modifiers, but currently only cetuximab, a monoclonal antibody to the endothelial growth factor receptor (EGFR), is approved for clinical use as a radiation modifier. A number of chemotherapies are used to enhance the effects of radiation in

the clinic, including cisplatin and 5-fluorouracil. Although these agents are used to enhance radiation effects, they are also known to both potentiate radiation toxicity and to result in their own significant toxicities which preclude their use as radiation modifiers in the palliative setting.

Recently, interest has increased in identifying agents or strategies that can exploit the effects of radiation on the tumor and tumor microenvironment to increase the efficacy of immunotherapy for cancer. An understanding of the mechanisms by which radiation can alter the immune response is critical to the development of strategies combining radiation and immunomodulatory drugs. Importantly, the immune effects of irradiation are quite different depending on the volume irradiated (total body versus localized).

The possible mechanisms by which radiation can enhance immunotherapy are multiple. Total body irradiation has been used as a method of immune suppression for adoptive cell therapies.[8] This approach requires delivery of lethal doses of total body irradiation and intensive preparative regimens that prepare the host for reconstitution with infused cells, such as tumor infiltrating lymphocytes. A number of other immune modulating effects of total body irradiation have been elucidated that may contribute to the superior efficacy of total body irradiation compared to alternative preparative regimens.[9, 10] This therapy has been pioneered and developed by investigators at NCI.

Conversely, localized irradiation has been proposed as a mechanism to minimize the immunosuppressive effects of radiation by leaving the majority of host cells unirradiated while generating a more immunogenic tumor bed.[1, 2, 11] Radiation results in tumor cells necrosis and increased expression of MHC Class I receptors in the irradiated field.[12, 13] Additionally, the radiation induced expression of pro-inflammatory cytokines and adhesion molecules can result in the influx of antigen presenting cells, such as dendritic cells and macrophages.[2, 14] Recruited antigen presenting cells are then able to process antigens from necrotic tumor and mature into effector T cells, which may be more efficient in the irradiated field. Mature T-cells are able to travel distantly and affect immune responses at distant disease sites. Importantly, localized irradiation avoids delivery of sterilizing doses to the majority of the bone marrow, hence minimizing the immunosuppressive effects of therapy. The immune activating effects of localized irradiation have recently been exploited in studies of immune modulating drugs and in vaccine based therapies.[15-18]

1.2.3 ECI301

Chemokines play a major role in the recruitment of leukocytes to sites of infection and activation of these cells to enhance local inflammation [3-5]. One such chemokine MIP-1α, is produced by macrophages and binds to specific dendritic cell (DC) chemokine receptors (CCR1 and CCR5). Dendritic cells are potent antigen presenting cells that perform a vital role in the initiation and regulation of both innate and adaptive immunity including anti-tumor responses. Tissue dendritic cells internalize and process locally released antigens, migrate to draining lymph nodes, and present antigenic peptides in association with co-stimulatory molecules leading to the induction of helper and cytotoxic T cell responses. ECI301 or eMIP is a derivative of MIP-1α with favorable pharmacologic properties and an excellent safety profile in lower species (see below).

1.2.3.1 Pre-Clinical experience with ECI301

When administered to laboratory animals, it was found that MIP-1α and its functional derivatives have an ability to attract DCs to local sites with inflammation or local cancer and can recruit dendritic progenitor cells into the blood at higher magnitude.[19] And when MIP-1α was

induced at the local site of inflammation in mouse model, dendritic cells accumulated to induce antigen specific immune response.[20] As described above, radiation treatment of tumors is known to induce inflammation in the irradiated field and to recruit tumor specific T cells and dendritic cells, which seem to play an important role in remission of tumors.[1, 21]

It has been hypothesized that the proinflammatory properties of MIP-1α could be exploited to enhance systemic immunity in a number of clinical conditions. For the purpose of pre-clinical evaluations, Effector Cell Institute (Tokyo) re-engineered MIP-1α to generate a biologically active derivative with fewer tendencies to form aggregates at physiologic pH and ion concentrations. The derivative, ECI301 is a 69 amino acid variant of MIP1 α which has an Asp residue substituted for Ala at position 26 of MIP1 α. Recombinant ECI301 was produced in *S. pombe* and found to incorporate the properties of increased solubility and stability while retaining full biologic activity and was used in preclinical studies.[22]

No noticeable toxicity was found in mice as a result of the I.V. administration of ECI301.[22] There have been studies reported using the British Biotech product (BB-10010), the same active variant of human MIP-1α as ECI301. Recombinant BB-10010 was produced using a budding yeast expression system. The biological activity of this recombinant protein was confirmed in receptor binding, calcium mobilization and biological assays in vitro and in vivo. The concept in the development of BB-10010 was to use the agent as an inhibitor of hematopoetic stem cell proliferation at the time of delivery of systemic chemotherapy to minimize hematologic toxicity and to enhance the number of peripheral blood stem cells for transplantation. Its myelosuppressive effect was investigated in several clinical trials of patients receiving chemotherapy but the myeloprotective effect was therapeutically insufficient.[23-25]

In a phase I clinical study by E. Marshall et al, 9 patients with advanced breast cancer and 40 normal healthy volunteers received escalating doses of BB-10010 from 0.1- 300 μg/kg i.v or subcutaneous routes, and there was no significant toxicity observed at any dose level. Treatment was associated with a dose-related increase in monocyte count and no measurable effects were noted on other leukocyte subsets or on circulating progenitor cell numbers. Pharmacokinetic analysis revealed that serum concentrations of BB-10010 were detectable using doses of > or = 10 μg/kg i.v. or > or = 30 μg /kg S.C., and that a single S.C. injection resulted in sustained plasma levels over a 24 h period. In all cases, BB-10010 was extremely well tolerated with no significant toxicity observed at any dose level and a maximum tolerated dose was not defined.[26]

In another phase II study of BB-10010 conducted by Dr Bernstein et al., use of human MIP-α in patients with malignant lymphoma and breast cancer receiving high dose etoposide and cyclophosphamide, the compound was well tolerated by the patients. BB-10010 was extremely well tolerated without any notable toxicity related to the compound.[27]

1.2.3.2 Toxicology studies of ECI301

Single dosing studies were conducted to evaluate the acute toxicity of IV (bolus) administration of ECI301 in mice and to support further toxicity studies. A single dose of 0 (PBS; non-treated [NT], n=2), 1, 10, or 300 mg/kg ECI301 (n=3/dose) was administered to 5-week-old male ICR mice. All mice were fasted prior to dosing and were observed for 4 hours post dose for signs of general condition and mortality. Body weight measurements were taken on Days -1, 0, 1, and 2. Animals were sacrificed 2 days after dosing. Parameters evaluated included: hematology for albumin/globulin ratios, blood urea nitrogen, creatinine, lactic dehydrogenase, aspartate

aminotransaminase, alanine aminotransaminase, and inorganic phosphorus of the isolated plasma. Urine was analyzed for glucose, urobilinogen, and pH. Histological assessment of brain, lung, liver, kidney, thymus, intestine, and colon was conducted. No animals died following administration of single doses of ECI301 up to 300 mg/kg. There were no significant treatment-related differences in clinical signs, body weights, plasma examination, histology, or organ weights in any dose group. Protein levels in urine showed minor elevations in the 10-mg/kg and 300-mg/kg groups. There were no other observed changes. These results indicate that a single dose of ECI301 of up to 300 mg/kg did not cause any overt toxicity during observations for 2 days post-treatment. It was concluded that a single dose of ECI301, up to 300 mg/kg, was well tolerated, and therefore the NOAEL was 300 mg/kg for this study. [28]

A single dose study was conducted in accordance with GLP to evaluate the acute toxicity of IV administration of ECI301 in rats. A single dose of 0 (0.01% polysorbate 80 in PBS), 0.05, 0.5, or 5 mg/kg ECI301 was administered IV (bolus) to Crl:CD® (SD) IGS BR rats (5/sex/dose). All rats were observed daily for 14 days after dosing for general clinical condition, signs of toxicity, and mortality. All animals were subjected to necropsy and macroscopic investigation. An additional group of animals was administered a single dose of 0.05, 0.5, or 5 mg/kg ECI301 IV for toxicokinetic analysis (6/sex/dose). Animals were bled at 5, 15, and 30 minutes, and 1, 2, and 4 hours postdose for toxicokinetic analysis. There were no treatment-related deaths for this study. There were no significant treatment-related clinical signs or effects on body weight. Organ weights were not affected, and there were no treatment-related macroscopic findings. The NOAEL was determined to be greater than the 5-mg/kg ECI301 used in this study. The toxicokinetic results indicated that the rate and extent of systemic exposure of rats to ECI301 appeared to be characterized by nonlinear (dose-dependent) kinetics over the dose range. Increasing the dose of ECI301 above 0.05 mg/kg was considered likely to result in a higher systemic exposure than would be predicted from a linear relationship, which is consistent with the possibility of a capacity-limited process for ECI301 elimination. There were no differences between the sexes for systemic exposure. It was concluded that a single dose of ECI301 at 5 mg/kg did not cause any toxicological effects. [28]

Repeated dosing studies of ECI301 in 5- to 7-week-old ICR mice were conducted as a non-GLP investigation to evaluate the potential toxicity of repeat IV administration. ECI301 was administered IV at 0 (PBS; 2/dose), 1, 10, or 100 mg/kg/day (3/dose) for two 5-day dosing cycles, split by a 3-day off-dose period. An additional group receiving bovine serum albumin (BSA; 100 mg/kg) was added to serve as an additional control group for using a different protein. Animals were observed for 4 hours post-dose on Day 3 and Day 13 for signs of general condition and mortality. Animals were autopsied 24 hours following the last dose. Parameters evaluated included daily body weights, urinalysis, and hematology. Organ weights and histology of brain, lung, liver, kidney, thymus gland, and colon tissue were conducted. There was no treatment-related mortality. There were no treatment-related effects on body weights or any of the hematological parameters measured. The protein in urine increased slightly in 1 of 3 animals in the 10-mg/kg group and 1 of 3 animals in the 100-mg/kg group. At 100 mg/kg ECI301, there was an increase in lung and spleen weights. Histology of the kidney showed an increase in the adhesion of glomerular basement membrane and renal glomeruli, and disruption of the glomerulus structure in all animals in the group receiving ECI301 at 100 mg/kg, and 1 of 3 animals in the 10-mg/kg group. It is unclear if these results are due to administration of ECI301 or to immune complex deposition. In spleen samples from both BSA and ECI301 (100 mg/kg), the boundary between the red and white pulp was unclear. There was structural damage to the

white pulp, and the central artery was unclear. There were no treatment-related changes in any of the other organs or tissues. This experiment demonstrated that repeated doses of ECI301, to a maximum total dose of 1000 mg/kg (1000 times the effective dosage), had no critical effect on the kidney, but abnormal tissue images and urine protein elevations were observed that indicated some renal damage. Further study is required to determine if this damage is attributable to ECI301 or another protein. Data from this non-GLP study supported further study of repeat doses over 2 to 4 weeks.[28]

A separate study was conducted in accordance with GLP to evaluate the systemic toxicity and toxicokinetics of IV administration of ECI301 alone or in combination with docetaxel in rats for three 5-day dosing cycles. A single dose of 0 (0.01% polysorbate 80 in PBS), 0.05, 0.15, or 1.5 mg/kg/day ECI301, 12.5 mg/kg docetaxel, or a 0.05-/12.5- mg/kg/day combination of ECI301 and docetaxel, was administered IV (bolus) to Crl: CD® (SD) IGS BR rats daily for 5 days. After 51 days, 10 animals/sex/dose were sacrificed and an additional 5 animals/sex/dose were sacrificed following a 10-day recovery period. All rats were observed daily after dosing for general clinical condition, signs of toxicity, and mortality. Additionally, food consumption, ophthalmology, hematology, blood chemistry, urinalysis, anti-ECI301 antibody assessments, peripheral blood immunophenotyping, and organ weights were analyzed. All animals were subject to necropsy with macroscopic and microscopic pathology. An additional group of animals for each group (6/sex/dose) except control (3/sex/dose) was bled for toxicokineticanalysis of ECI301 and docetaxel plasma levels. Animals were bled at 5, 15, and 30 minutes, and 1, 2, and 4 hours post dose for toxicokinetic analysis. There were no deaths in this study attributable to ECI301 treatment alone. Six animals died following docetaxel treatment. There were no treatment-related clinical signs or any effect upon bodyweight and food consumption. Similarly, there were no treatment-related ophthalmoscopic findings or any effect upon the composition of the urine. The hematological examination indicated a slight increase of lymphocyte count after the first treatment cycle, resulting in an increased total leukocyte count, in males given 0.15 or 1.5 mg/kg, with similar trends also occurring at all doses in females after Cycle 3. This was not evident in the males after Cycle 3 or in the females at the end of the recovery period. Biochemical changes in the blood plasma were confined to a reduction of glucose concentrations after Cycles 1 and 3 in males given 1.5 mg/kg and after Cycle 3 in males given 0.15 mg/kg and a small increase of plasma urea after Cycle 3 in males given 1.5 mg/kg. An analysis of organ weights after the completion of the third dosing cycle indicated a small reduction of spleen and thymus weight in males given 0.05 mg/kg, which was attributed to treatment since this dose was at the maximum biological effect level for ECI301. There were no treatment-related macroscopic or histopathological findings. The plasma samples taken from the control animals were below the limit of quantification in all cases, demonstrating that there was no quantifiable contamination with ECI301 in these animals.[28]

The toxicokinetic investigations indicated that the extent of systemic exposure of rats to ECI301 appeared to be characterized by non-linear (dose-dependent) kinetics over the dose range 0.05 to 1.5 mg/kg/day on Days 1 and 47. Increasing the dose of ECI301 above 0.05 mg/kg/day resulted in a higher systemic exposure than would be predicted from a linear relationship, indicative of limited elimination capacity. In general, there were no differences in the systemic exposure of males and females to ECI301 and there was no accumulation after repeated doses. One female given 0.05 mg/kg ECI301 showed a positive anti-ECI301 antibody response. All other animals showed negative responses. Immunophenotyping after completion of Cycle 3 indicated, in animals given 1.5 mg/kg, an increased percentage of CD4+ T cells and the males showed a

corresponding decrease in the percentage of CD8+ T cells, although the total numbers for all lymphocyte subsets studied were not affected. The numbers of NK lymphocytes were reduced in females given 1.5 mg/kg. Following recovery, males given 1.5 mg/kg still showed a reduced percentage of CD4+ lymphocytes and an increased percentage of CD8+ lymphocytes. It was concluded that IV (bolus) administration of ECI301 to CD rats at doses up to 1.5 mg/kg during three 5-day cycles of treatment, each separated by 16 days, was well tolerated, was not immunogenic, and caused no toxicologically significant findings. Docetaxel treatment 2 days after each cycle in which animals were given ECI301 at 0.05 mg/kg (a pharmacologically active dose) at 12.5 mg/kg in the first cycle in both sexes and the second cycle in males, subsequently reduced to 6.25 mg/kg for the remaining cycles, caused a range of findings that were attributable to its anti-mitotic activity. Compared with animals given docetaxel on the same study days, but with no ECI301 treatment, all differences in the response to docetaxel were attributed to normal biological variation in response to treatment and, consequently, there was no evidence that ECI301 modulated the effects of docetaxel treatment.[28]

A separate study was conducted to evaluate the potential for systemic toxicity of 3 different doses of ECI301, when administered IV to common marmosets for a 5-day dosing period with an 18-day observation period, to confirm doses to be used in the main toxicity study. Common marmosets (*Callithrix jacchus*) received 0.05, 0.15, or 1.5 mg/kg ECI301 (1/sex/group) IV bolus, once a day, for 5 days. Animals were observed for 18 days for clinical condition, injection-site reactions, and body weight. Hematology, blood chemistries, organ weight, and macroscopic pathology investigations were undertaken. There were no treatment-related deaths during this study. There were no treatment-related clinical signs of toxicity, and body weights were unaffected. There was no effect of treatment on organ weights or macropathology. All animals had reduced hematocrit, hemoglobin concentration, and/or erythrocyte count on Day 9 and/or Day 22, often accompanied by increased reticulocyte count. Mean cell volume was increased in the male receiving 0.05 mg/kg, the female receiving 0.5 mg/kg, and both animals receiving 1.5 mg/kg. Mean cell hemoglobin concentration was decreased in the male receiving 0.05 mg/kg and the female receiving 1.5 mg/kg. Anisocytosis was reported on the blood film for all females. There was no strong trend with dosage, and it is possible that these changes may have been caused by repeated blood sampling. However, with the absence of control animals on this study, the possibility that these changes were due to treatment with ECI301 cannot be excluded. An increase in monocyte count occurred on Day 9 in the female marmosets receiving 0.05 mg/kg or 0.15 mg/kg. These values normalized by Day 22. Minor blood chemistry changes occurred on Day 9 and Day 23, but these changes were small and were not dose related in degree. All animals showed reduced alkaline phosphatase activity on Day 23. Creatinine concentrations were reduced on Day 23 in the males given 0.05 mg/kg and in animals given 0.15 mg/kg or 1.5 mg/kg. Total triglyceride concentrations were high on Day 9 and Day 23 for all animals except males given 1.5 mg/kg. In the animals given 0.05 mg/kg and the females given 0.15 mg/kg, there was a reduction of total cholesterol level. All animals showed an increase of sodium concentration on Day 9 and, at 0.15 and 15 mg/kg, this was associated with increased chloride concentration. Potassium concentrations were low on Day 9 in all animals, and in the males, this persisted to Day 23. Total protein concentrations were low on Day 23 in animals given 0.05 mg/kg or 0.15 mg/kg, but not in those given 1.5 mg/kg, and most showed an increase in the albumin to globulin ratio. It was concluded that the IV administration of ECI301 to common marmosets for a single 5-day dosing period was well tolerated up to doses of 1.5 mg/kg. There were no toxicologically

significant findings, and therefore it was concluded that 1.5 mg/kg was a suitable dose for future studies in marmosets.[28]

Study EFC0010 was conducted to evaluate the systemic toxicity and toxicokinetics of IV administration of ECI301 to *Callithrix jacchus*, the common marmoset for three 5-day cycles. A single dose of 0 (0.01% polysorbate 80 in PBS), 0.05, 0.15, or 1.5 mg/kg ECI301 was administered IV (bolus) to marmosets (3/sex/dose). Radiation with chemotherapy is standard of care regimen for treatment of cancer. Docetaxel is one of the common chemotherapy drugs; however, significant AEs such as suppression of bone marrow function are associated with the administration of docetaxel. In phase 1 clinical study, ECI301 is expected to be administered to cancer patients in combination with radiation treatment. However, it may be necessary for docetaxel to be administered to patients in addition to ECI301. Therefore, toxicity data of ECI301 in combination with docetaxel were found to be necessary. The design of the study was as indicated below and also included 2 groups that were dosed with docetaxel, one in combination with ECI301. All animals were observed daily throughout the study period for general clinical condition, injection-site observations, body weight, ophthalmology, electrocardiography, blood pressure, hematology, blood chemistry, urinalysis, peripheral blood immunophenotyping, organ weights, macroscopic and microscopic pathology, and mortality. An additional group of animals for each group except control (2/sex/dose) were bled for toxicokinetic analysis. Animals were bled at 5, 15, and 30 minutes, and 1, 2, and 4 hours postdose for toxicokinetic analysis of plasma concentrations of ECI301 or docetaxel, and anti-ECI301antibody assessment. Two animals were sacrificed prematurely during this study. Neither death was attributed to ECI301 treatment, but 1 of them was determined to be possibly due to docetaxel treatment. There were no clinical signs that were attributable to treatment, and there was no effect upon bodyweight. One male receiving 0.15 mg/kg was killed on Day 59 due to gradual decline in its general clinical condition. The macroscopic and histopathological examinations did not identify any finding that would account for the ill health of this animal and, consequently, this death was not attributed to treatment. There were no treatment-related ophthalmoscopic findings and no effect upon the electrophysiology of the heart or blood pressure. There were no hematological findings or changes in blood plasma or urinary composition that were attributable to treatment. There were no toxicologically significant organ weight differences after 3 cycles of dosing with ECI301 and no macroscopic or histopathological findings that were due to treatment.[28]

The plasma samples taken from the control animals were below the limit of quantification in all cases, demonstrating that there was no quantifiable contamination with ECI301 in these animals. Mean plasma concentrations (Cmax) and ECI301 concentration-time curves estimated up to 4 hours post dose (AUC4) on Day 1 and 47 are also shown. The toxicokinetic investigations indicated that the extent of systemic exposure of marmosets to ECI301 appeared to be characterized by non-linear (dose-dependent) kinetics over the dose range 0.05 to 1.5 mg/kg on Days 1 and 47. Increasing the dose of ECI301 above 0.15 mg/kg was considered likely to result in a higher systemic exposure than would be predicted from a linear relationship, which is consistent with the possibility of a capacity limited process for the elimination of ECI301. In general, there were no differences in the systemic exposure of male and female marmosets to ECI301, and there was no accumulation after repeated doses. All animals showed negative anti-ECI301 antibody responses. Immunophenotyping after completion of Cycle 3 indicated no significant change at any dose.[28]

The toxicokinetic investigations indicated that the co-administration of ECI301 with docetaxel generally appeared to have little effect on the systemic exposure of male and female marmosets to docetaxel. The co-administration of ECI301 with docetaxel did not elicit an anti-ECI301 antibody response in any animal. Peripheral blood immunophenotyping indicated a reduction in the number of CD3+ T lymphocytes in males and females given docetaxel alone and a reduction in CD20+ lymphocytes in the males. These males also showed an increase in the percentage of CD3+ lymphocytes and a corresponding decrease in the percentage of CD20+ B lymphocytes. This T/B cell ratio change was not seen in the females. A reduction in the number and percentage of CD14+ monocytes was also seen in the males, but not in the females. It was concluded that IV (bolus) administration of ECI301 to common marmosets at doses up to 1.5 mg/kg during three 5-day cycles of treatment, each separated by 16 days, was well tolerated, was not immunogenic, and caused no toxicologically significant findings. Docetaxel treatment at 3 mg/kg, 2 days after each cycle in which animals were given ECI301 at 0.05 mg/kg (a pharmacologically active dose), caused several findings that were attributable to its anti-mitotic activity. There was no evidence in this study that ECI301 modulated adversely the effect of docetaxel treatment, but ECI301 coadministration did appear protective with regard to the effect upon lymphocyte populations in the peripheral blood.[28]

1.2.3.3 Preclinical experience with ECI301 as a radiation modifier

K. Shiraishi et al used murine tumor models to demonstrate that administration of the MIP-1α derivative ECI301 after radiation of a primary subcutaneous tumor nodule enhanced not only the anti-tumor efficacy of radiation at this site, but also promoted anti-tumor immunity at distant sites, referred to as an abscopal effect (figure 1, figure2).[22] To determine this, mice were implanted with colon 26 adenocarcinoma cells in the right flank or both flanks and when the tumor size reached 10 mm, local irradiation with 6 Gy was delivered to the right flank tumor. After 20 hrs, 2 μg per mouse of ECI301 was administered for 3- 5 consecutive days. This study showed that a combination of radiotherapy and ECI301 I.V. administration effectively prevented tumor growth at the irradiated site and also promoted the abscopal effect (prevention of tumor growth at the non-irradiated site). Prevention of tumor growth was observed with various mouse tumors including Lewis lung cancer, Meth A fibrosarcoma and Colon 26 adenocarcinoma suggesting that the effect was not restricted to a specific tumor type. Perhaps most importantly, rechallenge with the same tumor type in mice exhibiting a complete response to ECI301 and irradiation resulted in no tumor growth.

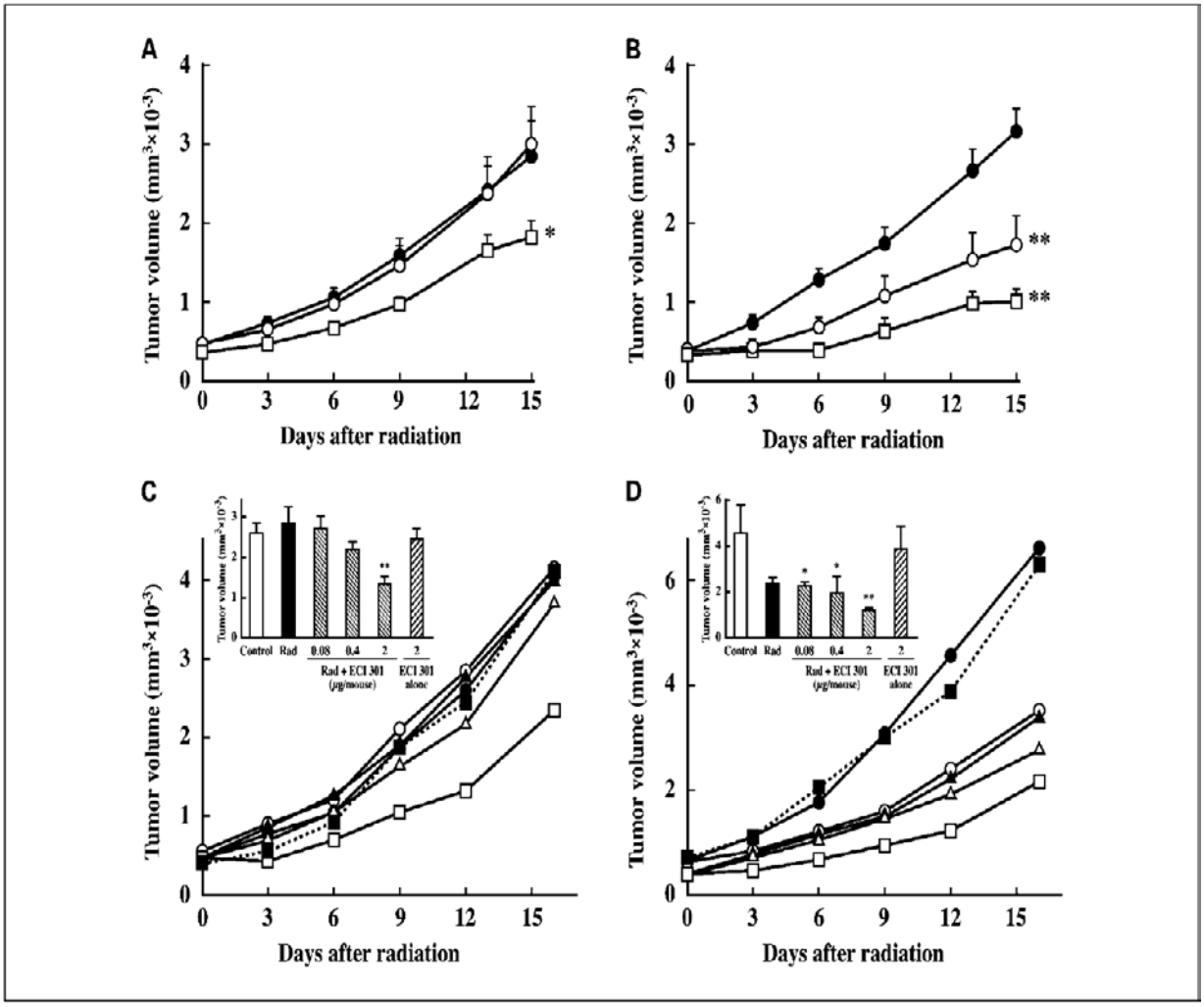

Figure 1. Effect of ECI301administration on Colon26 or LLC tumor growth at the irradiated (B and D) and nonirradiated sites (A and C). A and B, time course of tumor growth. Colon26 cells were inoculated in the left (1 x10^5 cells; A) or the right flanks (2 x 10^5 cells; B) of BALB/c mice and 18 d after inoculation; only the right side was exposed to radiation. ECI301was given at 1, 8, and 15 d starting 20 h after irradiation (□). Mice with radiation treatment only (O) or without treatment (●) served controls. Significant differences from the control group: *, P < 0.05; **, P < 0.01 (ANOVA). C and D, time course of tumor growth. LLC cells were inoculated in the right (4 _105 cells; D) and left flanks (2 x 10^5 cells; C) of C57BL/6 mice and 18 d after inoculation, only the right side was exposed to radiation. ECI301 (E, 0.08; 4, 0.4; 5 2 μg per mouse) was given at1, 8, and 15 d starting 20 h after irradiation. Results of mice with radiation treatment only (O), without treatment (●), or ECI301administration (2 μg per mouse) without irradiation (■) were used as controls. Points, mean tumor volume; bars, SE (n = 8). Inset, columns, mean tumor volumes of eight mice 12 d after irradiation; bars, SE. Significant differences from control group: *, P < 0.05; **, P < 0.01 (ANOVA). ***Reproduced from Shiraishi et al. [22].***

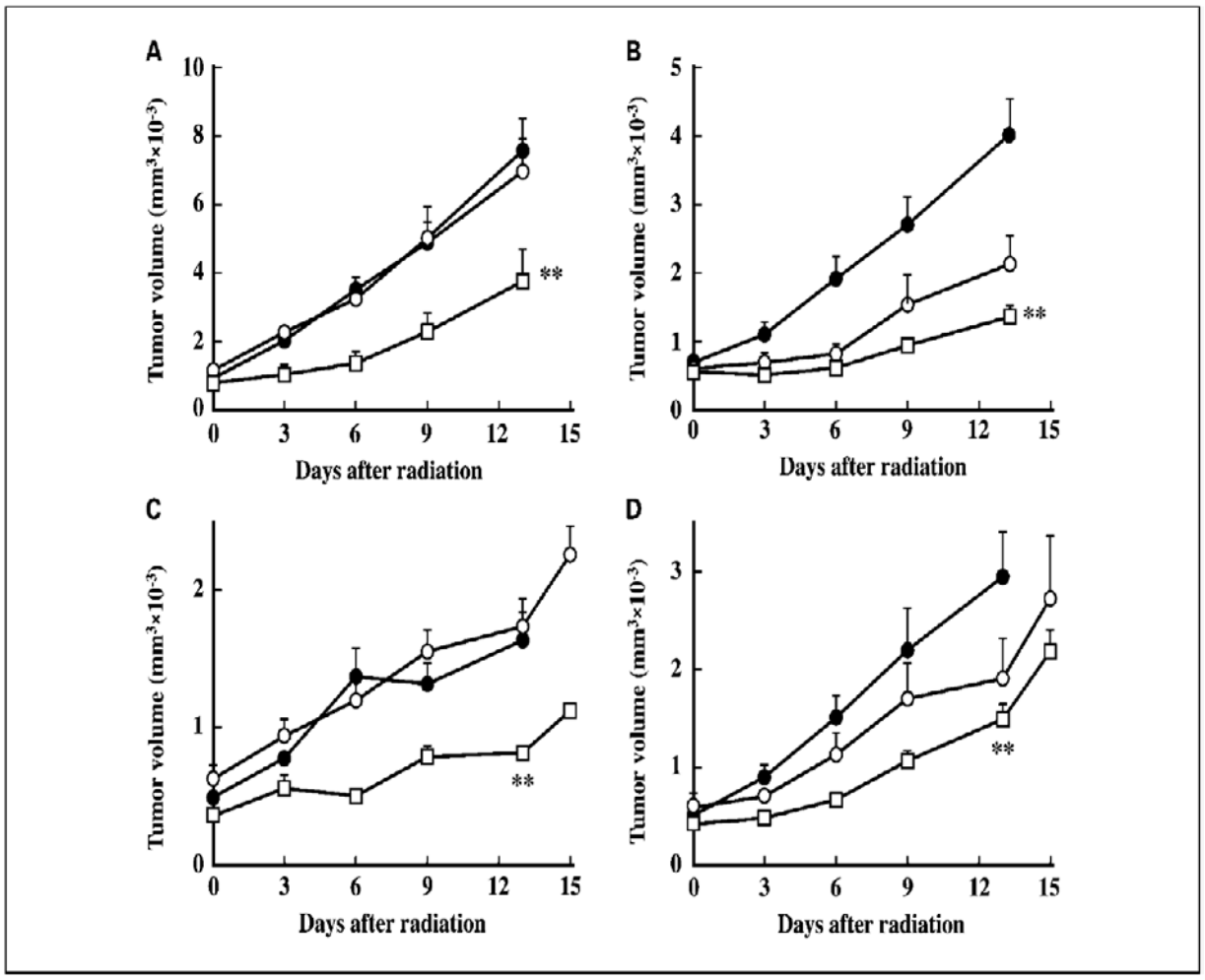

Figure 2. Effect of daily ECI301 administration on tumor growth. Effect of ECI301administration on different tumors grown at the irradiated (B and D) and non-irradiated sites (A and C). A and B, time course of tumor growth in the left flank (MethA, non-irradiated site; A) and the right flank (Colon26, irradiated site; B). Inoculum sizes of MethA and Colon26 cells were $1x10^5$ and $2 x 10^5$, respectively. ECI301 (2 μg per mouse) was given weekly starting from day 1after irradiation (□).Mice with radiation treatment only (O) and without treatment (●). Mean tumor volume in eight mice are presented. Significant differences from control group: **, $P < 0.01$ (ANOVA). A similar time course of tumor growth of MethA cells without treatment (●) was observed after ECI301administration without irradiation. C and D, time course of tumor growth in the left flank (Colon38, non-irradiated site; C) and the right flank (LLC, irradiated site; D). Inoculum size of LLC cells was 4 $x10^5$, and Colon38 was 2 mm3. ECI301(2 μg per mouse) was given weekly starting from day 1after irradiation (□). Mice with radiation treatment only (O) and without treatment (●). Significant differences from control groups: **, $P < 0.01$ (ANOVA). ***Reproduced from Shiraishi et al. [22].***

The enhancement in tumor response to irradiation was accompanied by an increase in infiltration of CD4+ and CD8+ lymphocytes into tumor sites at assessed by immunohistochemistry. Additional mechanistic studies completed in the radiation in combination with ECI301 studies included specific immune cell depletion via systemic delivery of CD4, CD8, and NK1.1 antibodies. Depletion of CD8+ cells reduced the benefit of ECI301 with radiation at both the irradiated site and at the unirradiated site (non-significant at the unirradiated site). Depletion of CD4+ or NK1.1 cells decreased the effect of combination therapy at the unirradiated site. Suppression of Natural Killer Cells did not alter the efficacy of the combined treatment.

Additional unpublished studies of ECI301 in combination with radiation have been completed with fractionated radiation and repetitive dosing to determine if delivering ECI301 concurrently with irradiation results in similar responses locally. The theoretical concern is that radiating the tumor bed repeatedly during dosing of ECI301 could impair the immune activation. Studies in BALB/c mice found that delivery of ECI301 during a portion of a fractionated treatment schedule in fact resulting in similar efficacy as that seen in the unfractionated model (Figure 3).

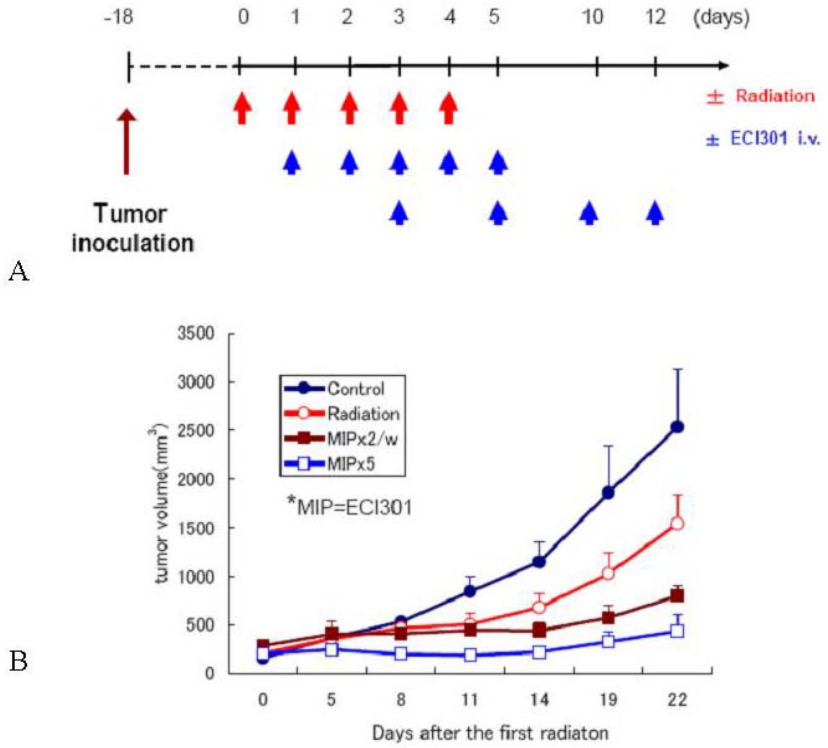

Figure 3. Effects of ECI301 administered concurrent to fractionated irradiation. BALB/c mice were implanted with colon26 cells in the right flank. A) On the day of treatment animals were randomized to no treatment, radiation alone, or fractionated radiation with the combination of ECI301 in one of two schedules. B) Tumor growth was inhibited in the groups receiving twice weekly and five times weekly dosing of ECI301. Improved efficacy was observed in the five times weekly dosing compared to the twice weekly dosing regimen. Confidential data provided by ECI. Inc.

1.2.3.4 Dose and Schedule Selection

The starting dose in this protocol is based on the results of toxicology studies conducted in rats and marmosets which established the no observable adverse effect level (NOAEL) for ECI301 at 0.15 mg/kg. The conversion factor from these species to humans is 0.16 which makes the human equivalent dose (HED) 0.024 mg/kg. The standard "safety factor" of 10 establishes the MRSD at 0.0024 mg/kg.

Preclinical studies demonstrated that a dose between 2μg and 3μg/mouse in combination with radiation therapy inhibited tumor growth. Given that the body weight of mice is 30g, the dosage between 2μg and 3μg is approximately 70μg/kg to 100μg/kg. Therefore it is predicted that the optimal dosage for obtaining the anti-cancer effect would be lower than 100μg/kg in mice. Thus it is proposed that 25 μg /kg, 50 μg /kg, 82.5 μg /kg, 125 μg /kg or 175 μg/kg will be intravenously administered for 5 consecutive days for one week to the patients in this phase I

study to assess the effect and safety of the combination treatment with radiation using dose-escalating method.

Daily dosing of ECI301 has been selected based on the preclinical data above that suggests optimal efficacy with daily dosing compared to less frequent dosing regimens. Repeated dosing experiments with irradiation lasted for one week. A theoretical concern of combining radiation with an immune adjuvant is that effector T cells could migrate into the radiation field and be lethally irradiated. Based on the available literature relating to vaccines and antigen dependent T-cell proliferation, a minimum of a 5-8 day window after is likely to be required for the development and expansion of effector T-cells.[29, 30] Therefore, we have designed the study to include one week of concurrent therapy with ECI301 during the final week of radiation delivery. Finally, animal models have confirmed that up to five daily doses of ECI301 following irradiation can result in measurable immune responses and abscopal effect.

1.2.4 Summary

In this planned clinical trial, we will attempt to establish the safety and tolerability of intravenous ECI301 (which has the same amino acid sequence as BB-10010) in combination with radiation treatment in patients with solid malignancies for whom radiation therapy alone would constitute standard of care. In correlative translational studies we will perform pharmacokinetic (PK) analysis of MIP-1α serum levels, determine precursor dendritic cell (DC) frequencies before and after ECI301 treatment, and measure levels of proinflammatory chemokines, in vitro studies of T cell functions and its consequences after completion of ECI301.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Age ≥18 years.

2.1.1.2 ECOG performance status ≤2.

2.1.1.3 Life expectancy of greater than 3 months

2.1.1.4 Histologically confirmed cancer

2.1.1.5 Extracranial metastatic cancer or locally advanced cancer for which palliative radiotherapeutic management would be appropriate (no more than two sites will be treated on this trial)

2.1.1.6 Patients must have measurable or evaluable disease at the site(s) requiring radiation

2.1.1.7 Adequate marrow and organ function defined as

- absolute neutrophil count (ANC) > 1.5 x 10^9/L,
- platelet count > 100 x 10^9,
- hemoglobin >9 g/L.
- creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal
- serum bilirubin <1.5 x upper limit reference range (ULRR),
- alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase (ALP) <2.5 x the ULRR (<5 x the ULRR in the presence of liver metastases)

2.1.1.8 Female patients of child bearing potential must either be surgically sterile to prevent pregnancy, be at least 1-year post-menopausal, or have had no menses for 12 months, or agree to use reliable methods of contraception (oral contraceptives, barrier methods, approved contraceptive implant, long-term injectable contraception, copper banded intrauterine device, tubal ligation or abstinence) from time of screening until 4 weeks after discontinuing study treatment. It is not known whether ECI301 has the capacity to induce hepatic enzymes so hormonal contraceptives should be combined with a barrier method of contraception.

2.1.1.9 Male patients must agree to use barrier contraception (i.e. condoms) and refrain from donating sperm from the start of dosing until 16 weeks after discontinuing study treatment. If male patients wish to father children they should be advised to arrange for freezing of sperm prior to the start of study treatment.

2.1.2 Exclusion Criteria

2.1.2.1 Pregnant or lactating females

2.1.2.2 Contraindications to radiotherapy (i.e. prior radiotherapy to the intended treatment site)

2.1.2.3 Untreated or previously treated but progressive intracranial metastases (Patients with previously treated intracranial metastases should have no clinical evidence of progression and be at least 4 weeks from therapy for intracranial metastases)

2.1.2.4 Need for emergent radiotherapy (defined as need for radiotherapy within 24 hours of consultation at the judgment of the treating radiation oncologist)

2.1.2.5 Active treatment with immunosuppressive therapy and subjects taking systemic corticosteroid therapy for any reason including replacement therapy for hypoadrenalism

2.1.2.6 Chemotherapy, radiation therapy, Tamoxifen or investigational therapy during the 4 weeks prior to initiation of protocol therapy

2.1.2.7 History of rheumatoid arthritis, systemic lupus erythematosus, Sjögrens disease, sarcoidosis, vasculitis, polymyositis, temporal arteritis or any other autoimmune disease

2.1.2.8 History of organ transplant

2.1.2.9 HIV, Hepatitis B, or Hepatitis C positivity

2.1.2.10 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements

2.1.2.11 Use of excluded immune modulating medications within 4 weeks prior to protocol therapy, or requirement for concurrent use (Appendix III).

2.2 SCREENING EVALUATION

2.2.1 Clinical Evaluation

- A complete history and physical examination will be completed by the PI or an associate investigator. This will include a detailed history including comorbidities and prior therapy received, measurements of measurable disease to be targeted with

radiation, signs, and symptoms caused by the cancer, determination of ECOG performance status.
- Vital signs: Including height and weight
- ECG within one week of study entry

2.2.2 Laboratory Evaluation

Pre- treatment blood tests should be performed within one week of study entry.
- Hematology: complete blood count, differential, platelet count, PT, PTT
- Chemistries: LDH, SGOT, SGPT, alkaline phosphatase, bilirubin (total and direct), BUN, serum creatinine, serum electrolytes, calcium, magnesium, phosphorus, uric acid, albumin.
- 24 hour urine for measurement of creatinine clearance and urine protein
- Urinalysis
- Urine Pregnancy Test: required for females of childbearing potential
- Viral serologies: HIV screening; Hepatitis B and C screening within three weeks of study entry

2.2.3 Radiographic Evaluation

- CT will be the preferred imaging modality for metastatic sites of non-bone location. CT of the metastatic site to be treated within 3 weeks of entry into the protocol can be used as the baseline evaluation. If scans were obtained from another institution, copies should be produced and maintained on file with the PI.
- For bone lesions with or without soft tissue extension, MRI and plain films of the site to be treated will be obtained as the baseline study.
- Additional complementary imaging studies may be obtained for baseline measurements if CT or MRI is felt to be insufficient (i.e. Ultrasound).

2.2.4 Pathologic Review**:**

Outside report confirming metastatic malignancy will be acceptable for study entry. In certain cases, for example a patient with pathologically documented prostate cancer and elevated PSA after definitive local therapy with radiographic evidence of metastases, pathologic confirmation of metastasis disease is not required. NIH review of pathologic material will be completed in all patients enrolled.

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://camp.nci.nih.gov/ccr/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN:

This is a Phase I trial of ECI301 delivered concurrently with palliative radiotherapy. Radiation will be delivered at a dose of 3 Gy per daily fraction, Monday through Friday, to a total dose of 30 Gy delivered in two weeks. As many as two extracranial sites may be treated with radiotherapy simultaneously on this protocol. ECI301 will be delivered as an IV infusion during week 2 on the day of irradiation approximately one to two hours after the radiation treatment. Escalation will begin at the 25 μg/kg dose level and will proceed in cohorts of 3-6 patients. The MTD cohort will be expanded to six patients. Optional tumor biopsies will be obtained prior to the start of therapy and at one week after completion of radiation. Patients will be followed until 6 months after completion of treatment to evaluate response.

3.1.1 Dose Limiting Toxicity

- Toxicity for the purposes of dose escalation will be assessed during the active treatment phase and for two weeks after the completion of therapy. Toxicity will be graded according to the National Cancer Institute CTCAE (version 4.0) and the RTOG acute toxicity scoring scale.
- Dose-limiting toxicity (DLT) will be defined as follows:
    - Any grade 3 or greater non-hematologic toxicity is a DLT
    - Any grade 3 neutropenia or thrombocytopenia is a DLT
    - Any grade 4 anemia is a DLT
    - Nausea, vomiting, diarrhea, tumor pain, or pre-existing hyponatremia, dyselectrolytemia, or orthostatic hypotension has been optimally treated with anti-emetics, anti-diarrheal, analgesics, or hydration and which persists for over 48 hours despite maximal medical therapy is considered a DLT
    - Toxicity requiring a cumulative radiation treatment delay of 4 or more days will be considered a DLT.
    - In addition, grade 3 or higher acute RTOG toxicity will be considered a dose-limiting toxicity (Appendix I).
- Grade 3 or higher RTOG toxicities attributable to disease progression or pre-existing medical condition will not be considered a DLT.
- No additional study drug will be delivered after a DLT.
- Toxicity will be recorded with the RTOG Scoring Scale in patients undergoing follow up at time points of weeks 3, 4, 6 and 12 weeks (acute scoring scale – Appendix I) and 6 months (late scoring scale - Appendix II).

3.1.2 Dose Escalation

Radiation will begin on day 1 for all dose regimens. ECI301 will be delivered daily approximately one to two hours after irradiation (week 2). Every effort will be made to have the time of day of these treatments be consistent.

Dose escalation will begin at 25 μg/kg daily.

Dose Escalation Schedule	
Dose Level	Daily Dose of ECI301
Level 1	25 μg/kg
Level 2	50 μg/kg
Level 3	82.5 μg/kg
Level 4	125 μg/kg
Level 5	175 μg/kg

Dose escalation will proceed in cohorts of 3–6 patients based on the rules outlined in the table below. DLT will be evaluated during the active treatment phase and in the first three weeks after treatment. Dose escalation will not proceed until all patients have concluded 2 weeks of follow up in the prior dose level to allow complete documentation of DLTs. The MTD or recommended Phase II dose is the highest dose level at which no more than 1 of 6 patients experience DLT during treatment or the first three weeks after treatment, and the dose below that at which at least 2 (of ≤6) patients have DLT as a result of the drug. If a patient did not experience DLT and did not finish treatment, he or she will not be evaluable for toxicity and will be replaced in the study.

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	Enter 3 patients at the next dose level
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

3.2 STUDY DRUG ADMINISTRATION:

ECI301 will be prepared by the Clinical Center Pharmacy and provided to the Day Hospital or inpatient unit. The pharmacy will provide a single syringe of ECI301 appropriate for intravenous bolus infusion. ECI301 will be delivered as a daily infusion approximately one to two hours after irradiation during week 2, Monday through Friday. The infusion will be delivered in the Clinical Center Day Hospital or inpatient unit. The infusion will be delivered after completion of radiation. Every effort will be made to have the time of day of these treatments and sample collections be consistent. The bolus infusion will be administered at a rate of approximately 1 mL/second. Vital signs (temperature, heart rate, blood pressure, respirations and pulse oximetry) will be assessed on Week 2 Day 1 prior to infusion and at 15 minutes, 30 minutes, 1, 2, 4, 6, and 8 hours following the dose. On subsequent days, Week 2, Days 2-5, vital signs will be assessed prior to ECI301 infusion and an hour following.

There is no required or recommended premedication prior to infusion.

3.3 DOSE MODIFICATION FOR TOXICITIES:

There will be no dose modifications for toxicity. If a patient experiences a DLT they will be removed from the study drug but will continue to receive radiotherapy as deemed appropriate by the PI.

3.4 PROTOCOL THERAPY AND EVALUATION

3.4.1 Pretreatment studies (Baseline)

(to be obtained within one week of initiation of protocol therapy unless otherwise noted)

- Clinical evaluation, vital signs
- Laboratory: if done at screening, repeat only if greater than a week has elapsed prior to start of treatment.
- CBC with differential, lymphocyte phenotype TBNK, serum chemistry with electrolytes, BUN, Creatinine, liver function tests, PT, PTT, Urinalysis
- Adverse Event/Toxicity assessment
- Optional biopsy
- Research laboratory studies. Every effort will be made to have the time of day of these collections be consistent.
  - 6 mL plasma EDTA tube
  - three 8 mL CPT tubes

3.4.2 On treatment studies and therapy

- Clinical evaluation, vital signs, assessment of adverse events weekly (CTC and acute RTOG)
- Laboratory: CBC with differential, lymphocyte phenotype TBNK, serum chemistry with electrolytes, BUN, Creatinine, liver function tests, PT, PTT, (Week 1Day 4 or 5; Week 2: Day 1 and 5)
- Urinalysis Week 2 Day 1 and 5
- Electrocardiogram Week 2 Day1 and Day 5 approximately 45 minutes after ECI301
- Radiotherapy: Radiotherapy will be delivered M-F week 1 and week 2, as described in section 3.6.

- ECI301 will be delivered as a bolus infusion daily, approximately one to two hours after irradiation during week 2, as described in 3.2.
- Vital signs (temperature, heart rate, blood pressure, respirations, pulse oximetry) will be assessed on Week 2 Day 1 prior to ECI301 infusion and at 15 minutes, 30 minutes, 1, 2, 4, 6, and 8 hours following the dose. On subsequent days, Week 2, Days 2-5, vital signs will be assessed prior to ECI301 infusion and an hour following.
- Research laboratory studies (Week 1: Day 4 or 5; Week 2 Day 1 and Day 5). Every effort will be made to have the time of day of these collections be consistent.
  - 6 mL plasma EDTA tube
  - three 8 mL CPT tubes (NOT needed on Week 2 Day 1)
- Pharmacokinetics: Week 2, Day 1and Day 4 at time 0, 15 minutes, 30 minutes, 1, 2, 4, 6, 8, and 24 hours after the last dose of ECI301. Plasma will be drawn in 3 mL EDTA tubes Follow-up studies

3.4.3 Follow-up studies

- Week 3 and 4 (week 1 and 2 after completion of therapy)
  - Clinical evaluation and vital signs, assessment of adverse events(CTC and acute RTOG)
  - Laboratory: CBC with differential, lymphocyte phenotype TBNK, serum chemistry with electrolytes, BUN, Creatinine, liver function tests , PT, PTT, urinalysis
  - Optional biopsy (week 3 only)
  - Research laboratory. Every effort will be made to have the time of day of these collections be consistent:
    - 6 mL plasma EDTA tube
    - three 8 mL CPT tubes (Not needed on Week 4).
- Week 6, week 12, and 6 months
  - Clinical evaluation and vital signs, RTOG toxicity scoring (acute scoring at 6 weeks and 12 weeks, late scoring at 6 months
  - Laboratory: CBC with differential, serum chemistry with electrolytes, BUN, Creatinine, liver function tests
  - Research labs (at week 6 only). Every effort will be made to have the time of day of these collections be consistent.
    - 6 mL plasma EDTA tube
  - 24 hour urine collection for creatinine clearance and protein (week 6 only).
  - Radiographic evaluation of the treated site as appropriate for response assessment, (CT or MR the same modality as used for pretreatment measurements)

3.5 STUDY CALENDAR

Procedure	***Screening/ Baseline***	***Active Treatment***		***Post Therapy Follow-up***				
		Week 1	***Week 2***	***Week 3***	***Week 4***	***Week 6***	***Week 12***	***Month 6***
History and PE	*X*[1]							
Clinical evaluation and Vital signs	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Performance Score	*X*							
Pathology Review	*X*[1]							
Radiation		*X*	*X*					
ECI301			*X*					
Radiological/ Response Assessments	*X*[1]					*X*	*X*	*X*
Adverse Events/Toxicity Assessment[5]	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Concomitant Medications	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
ECG	*X*		*X*[3] *X*[3]					
Clinical Labs: *CBC with diff, Acute Care, Hepatic & Mineral Panels, LDH, uric acid, PT/PTT*	*X*	*X*[2]	*X*[2] *X*[2]	*X*	*X*	*X*	*X*	*X*
lymphocyte phenotype TBNK	*X*	*X*[2]	*X*[2]*X*[2]	*X*	*X*			
urine HcG	*X*[1]							
24 hour urine creatinine/protein	*X*[1]					*X*		
Urinalysis	*X*		*X*	*X*	*X*			
HIV, Hep B, Hep C screening	*X*[1]							
Research Labs: *three 8mL CPT tubes* *6 mL EDTA tube*	 *X* *X*	 *X* *X*	 *X*[4] *XX*	 *X* *X*	 *X*[4]	 *X*[4]		
Pharmakokinetics[6]			*X*[6] *X*[6]					
Optional Biopsies	*X*			*X*				

1. Studies that are required at screening only (i.e., If done at screening, do not need to be repeated at baseline)
2. Clinical laboratory tests: Week 1: Day 4 or 5 and Week 2: Day 1 and 5.
3. ECG within 1 week baseline, Week 2 Day 1 and Day 5 approximately 45 minutes after ECI301
4. Research labs Week 2 Day 1 EDTA tube only; Week 2 Day 5 EDTA and three CPT tubes; Weeks 4and 6 EDTA only
5. CTC and RTOG until week 4, then RTOG only. Use RTOG acute through 12 weeks, then RTOG late.
6. Pharmacokinetic analysis will be performed on Week 2, Day 1 and Day 4 at time 0, 15 minutes, 30 minutes, 1, 2, 4, 6, 8, and 24 hours after the last dose of ECI301.

3.6 RADIATION THERAPY GUIDELINES:

3.6.1 Simulation.

All patients will undergo CT simulation in the Radiation Oncology Branch. Positioning will be optimized to allow maximal sparing of normal tissues from the treatment field.

3.6.2 Treatment Planning.

The radiated target volume will be defined using the simulation CT with fusion of additional imaging (i.e. diagnostic CT, PET, MRI) as needed for target delineation. The radiated target will be chosen based on a clinical need for treatment. The gross tumor volume (GTV) will be defined as all evident tumor in the radiation target site on clinical examination and radiographic imaging. The clinical target volume (CTV) will be defined as the GTV with a 1 cm margin with corrections in margin allowed for normal barriers to tumor spread (i.e. uninvolved bone or skin). Maximum planning target volume (PTV) margin on CTV will be limited to 1.5 cm with a minimum of 0.5 cm. Three dimensional planning will be used to minimize exposure of surrounding normal tissues. Planning will be optimized such that the planning target volume will be encompassed within the 95% isodose line. Every attempt will be made to ensure that the global maximum dose should not exceed 115% of the prescription dose. Treatment will be delivered with 6MV or greater energy photons or 6MeV or higher electrons. Modifications to the treatment volumes may be made with the approval of the Principal Investigator or Lead Associate Investigator if there is concern that the tumor is inadequately treated or if a normal tissue receives what is considered to be an unsafe dose.

3.6.3 Treatment delivery.

External beam radiation therapy will be administered as 30 Gy delivered over two weeks in 3 Gy daily fractions (Monday through Friday with the exception of federal holidays) in the Radiation Oncology Branch, NCI. Every effort will be made to have the time of day of these treatments be consistent.

3.6.4 Criteria for Removal from Protocol Therapy

- Dose limiting toxicity
- Concurrent illness that in the judgment of the PI would make further protocol therapy unsafe or inappropriate.
- Progression of disease to the extent that in the judgment of the PI continuation of radiotherapy is inappropriate.
- Patient refusal of further protocol therapy.
- Death

3.6.5 Off Study criteria

- Completion of protocol therapy and 6 months of follow up.
- Patient withdrawal of consent for further follow up.
- Death

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off-study. An off-study form from the web site (http://camp.nci.nih.gov/ccr/welcome.htm) main page must be completed and faxed to 301-480-0757.

4 SUPPORTIVE CARE

Any medication that is considered necessary for the patient's safety and well-being, eg, anti-emetics, opioids, may be given at the discretion of the investigator(s) with the exceptions noted below. Concurrent chemotherapy and concurrent targeted agents may not be delivered to patients receiving therapy on this protocol. Patients may continue to use endocrine therapy during protocol therapy (for example androgen suppression or estrogen modulators with the exception of tamoxifen). No other investigational agents, radiotherapy, or chemotherapy may be prescribed during protocol therapy or during the three weeks of follow up after therapy. Growth factors should be avoided during active therapy. The patient must be instructed that no additional medication will be allowed without the prior consent of the investigator. The administration of all medication (including investigational products, prescription, non-prescription or over-the-counter medication) must be recorded in the appropriate sections of the patient's medical record. Concomitant use of anti-inflammatory drugs including NSAIDs and steroids should also be avoided, unless clinically essential. Concurrent use of immunosuppressive medications should be avoided (Appendix III). Concomitant medications should be re-assessed on each weekly on treatment visit and during each post-treatment visit. Vaccinations should be avoided during active therapy and during the first four weeks of follow up.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES:

Plasma (6 cc EDTAtube) will be collected for cytokine analysis pre-treatment, and on Week 1: Day 4 or 5, Week 2: Day 1 and Day 5 and Week 3, week 4 and week6.. Every effort will be made to have the time of day of these collections be consistent. Plasma will be transported immediately on ice to the laboratory of Dr. Deborah Citrin for processing. Aliquots of plasma will be analyzed for cytokines thought to be involved in the dendritic cell natural killer pathway. 1 mL aliquots of plasma will be sent to Huntingdon Life Sciences for evaluation of anti-MIP1α antibodies. Samples will be stored at -80°C in Dr. Citrin's laboratory until complete validation of the assay is available

Pharmacokinetic analysis will be performed on Week 2, Day 1 and Day 4 at time 0, 15 minutes, 30 minutes, 1, 2, 4, 6, 8, and 24 hours after the last dose of ECI301. PK samples will be collected in 3ml EDTA tubes and transported immediately on ice to the laboratory of Dr. Deborah Citrin for processing. 1 mL aliquots of plasma will be sent to Huntingdon Life Sciences for PK analysis. Samples will be stored at -80°C in Dr. Citrin's laboratory until complete validation of the assay is available.

Circulating peripheral mononuclear cells will be collected in 3 CPT tubes (8 ml per tube) pre-treatment, and on Week 1: Day 4 or 5, Week 2: Day 5 and Week 3. . CPT tubes will be transported immediately on ice to the laboratory of Dr. Deborah Citrin for processing.. Lymphocytes will be divided into aliquots for cryopreservation and fixation for additional studies that will include assessment of dendritic cell precursors and assessment of IFNγ secretion. Aliquots of lymphocytes will be provided to Dr. Jane Trepel for flow cytometry based studies.

Optional core tumor biopsies will be obtained prior to treatment and one week after completion of radiation. These biopsies will be obtained from the target lesion (the radiated metastatic site) pretreatment, which will serve as a baseline measurement for all sites. A core biopsy will be

obtained from both the radiated target lesion and a separate non-irradiated lesion at the one week follow up time point. Biopsies will be performed in collaboration with interventional radiology. Standard techniques will be used for percutaneous biopsies which may include CT and / or ultrasound guided biopsy. In some cases, such biopsies may be expedited and facilitated with the use of navigation tools, such as a laser guide or GPS-electromagnetic tracking of the needle or ultrasound, to determine which exact angle the biopsy needle will be inserted.

Following collection, specimens will be snap frozen, transported to the laboratory of Dr. Deborah Citrin, and stored at -80º C until use.

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Core biopsies will be evaluated for inflammatory cell infiltration via immunohistochemistry. Core biopsies will be processed by the laboratory of pathology and paraffin embedded. The panel of markers tested may include CD3, CD4, CD8, and CD20. Depending on the number of lymphocytes observed, additional studies may include markers such as CD56, TIA, and granzyme. Biopsy studies will be coordinated with Dr. Richard Lee in the department of Dermatopathology and Surgical Pathology.

5.2 SAMPLE STORAGE, TRACKING, AND DISPOSITION

- Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA.
- In the lab, all samples will be barcoded, with data entered and stored in the Labmatrix data base. This is a secure program, with access to the Labmatrix data base limited to defined personnel, who are issued individual user accounts. The program creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix data base access.
- The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).
- Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the ROB lab area of NIH Building 10, B2.
- Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in the Labmatrix data base and are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and any unused samples must be returned to the ROB. Plasma, serum, urine and blister fluid samples collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study. However, this

research may only be done if the risks of the new questions were covered in the consent document.

- No germline mutation testing will be performed on any of the samples collected.
- At the completion of the protocol, the investigator will dispose of all specimens 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.
- Any new use of the samples will require prospective IRB review and approval.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

- Clinical data and acquired images will be recorded in a NCI CCR database (C3D)**.**
- Clinical data collection for participants receiving radiation therapy will include: demographic information, pathologic diagnosis, clinical stage, history including prior and concurrent therapies, CT reports, lab reports, vital signs, dose of radiation delivered, and toxicity assessment.
- The results of any procedures and/or tests will be included in the patients' hospital chart and/or research records as appropriate. Data from these records may be used in research and scientific publications.
- Data collected may be used anonymously, for publications not originally specified, concerning the natural history of disease processes and long term effects of treatment. The data may also be used as the basis for entirely new protocols.

6.2 MEASUREMENT OF EFFECT AND RESPONSE CRITERIA

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated at 6 and 12 weeks and 6 months. Lesions treated with radiotherapy will be considered radiated target lesions. As many as two sites of metastatic disease may receive radiotherapy simultaneously. Additional metastatic sites (non-irradiated target lesions), up to 3 additional lesions, will be followed for evidence of response.

Patients who have received complete therapy with ECI301 and radiation will be considered evaluable for response. Patients with objective disease progression prior to completion of therapy will also be considered evaluable for response rate. Target (irradiated and non-irradiated) lesion measurements will be recorded at each assessment time point for each patient and response will be determined. An analysis of response rate based on a subset of patients must explain which patients were excluded and for which reasons.

6.2.1 Antitumor Effect – Solid Tumors

Response and progression will be evaluated in this study using the Revised Response Evaluation Criteria in Solid Tumors (RECIST) Committee [European Journal Cancer. 45(2):228-247, 2009]. Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

6.2.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with ECI301.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received a complete treatment, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of treatment will also be considered evaluable.)

6.2.1.2 Disease Parameters

Measurable disease.
Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
• 10mm by CT scan (CT scan slice thickness no greater than 5 mm.
• 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
• 20mm by chest X-ray.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed

Non-measurable disease. All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with P10 to <15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Special considerations regarding lesion measurability Bone lesions, cystic lesions, and lesions previously treated with local therapy:

Bone lesions:
- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CTor MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic lesions:
- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are not considered measurable unless there has been demonstrated progression in the lesion.

Target lesions.

For the purposes of this protocol, lesions will be considered a radiated target lesion if they are encompassed in the radiation treatment field. Up to two sites will be radiated and considered evaluable. When more than one measurable lesion is present at baseline all non-irradiated lesions up to a maximum of three lesions total (and a maximum of two lesions per organ/site) should be identified as unirradiated target lesions and will be recorded and measured at baseline.

Radiated target lesions are chosen based on clinical need for treatment. Non-irradiated target lesions for measurement should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of $\geq$15mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, saggital or coronal). The smaller of these measures is the short axis. All other pathological nodes (those with short axis $\geq$10mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10mm are considered non-pathological and should not be recorded or followed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes 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’, ‘absent’, or in rare cases ‘unequivocal progression’ (more details to follow). In addition, it is possible to record multiple nontarget lesions involving the same organ as a single item on the case record form (e.g. ‘multiple enlarged pelvic lymph nodes’ or ‘multiple liver metastases’).

Non-target lesions. All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Non-target lesions should be outside the radiated field. Measurements are not required and these lesions should be followed as ‘present’, ‘absent’, or in rare cases ‘unequivocal progression’. In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. ‘multiple enlarged pelvic lymph nodes’ or ‘multiple liver metastases’).

6.2.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a caliper if clinically assessed. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial and ≥10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest x-ray Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

Conventional CT and MRI CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Ultrasound (US) Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor markers Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response. Because tumor markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis. Tumor markers will not be used to assess response on this protocol.

Cytology, Histology These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment, the cytological confirmation of the neoplastic origin of any

effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

6.2.1.4 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

6.2.1.5 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).

Incomplete Response/

Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

6.2.1.6 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the end of measurements (up to 6 months after therapy) taking into account any requirement for confirmation. 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
CR	Not-evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD
CR=complete response, PR= partial response, SD=stable disease, PD=progressive disease, and NE=not evaluable			

6.2.1.7 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or 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 CR 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.

6.2.1.8 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression.

6.3 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. Adverse events occurring during the study will be graded according to the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) found at: http://ctep.cancer.gov/reporting/ctc_v30.html. In addition, the study will use the RTOG/EORTC Morbidity Scoring Scheme to score acute and late effects of radiation treatment. These criteria are available from the RTOG website http://rtog.org/members/toxicity/main.html

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

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 must be recorded on the AE case report form.

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.

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

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

7.1.2 Attribution:

An assessment of the relationship between the AE and the medical intervention**.** CTCAE does not define an AE as necessarily "*caused by a therapeutic intervention"*. After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

RELATIONSHIP	**ATTRIBUTION**	**DESCRIPTION**
Unrelated to investigational agent/intervention[1]	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE *is **doubtfully related*** to the intervention
Related to investigational agent/intervention[1]	Possible	The AE ***may be related*** to the intervention
	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

[1]**NOTE**: AEs listed as "possibly, probably, or definitely" related to the investigational agent/intervention are considered to have a suspected "reasonable causal relationship" to the investigational agent/intervention (ICH E2A).

7.1.3 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.

7.1.4 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.

7.1.5 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.

7.1.6 Disability

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

7.1.7 Life-threatening adverse drug experience

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

7.1.8 Protocol Deviation (NIH Definition)

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

7.1.9 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.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

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

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

- Is related or possibly related to participation in the research; **AND**

- 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 NCI-IRB REPORTING

7.2.1 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 working days of PI awareness via iRIS.

7.2.2 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.

7.2.3 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.

7.3 FDA REPORTING CRITERIA

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

7.3.1.1 Expedited reporting to the FDA

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

The study Sponsor will notify FDA in writing of any suspected adverse reaction that is both serious and unexpected as soon as possible but no later than 15 calendar days after initial receipt of the information using the MedWatch Form 3500a. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

The study Sponsor will also report expeditiously as above:

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

7.3.1.2 Exclusions to expedited reporting to the FDA

The following events will not be reported in an expedited manner but will be included in the annual report:

- Pain due to disease
- Events associated with vascular access devices not implanted or used on this protocol

7.3.2 FDA Annual Reports (Refer to 21 CFR 312.33)

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

7.3.3 Expedited Adverse Event Reporting Criteria to the IND Manufacturer (ECI, Inc)

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

The study Sponsor will notify ECI, Inc in writing of any suspected adverse reaction that is both serious and unexpected as soon as possible but no later than 15 calendar days after initial receipt of the information.

7.3.3.1 Exclusions to expedited reporting to the Manufacturer

The following events will not be reported in an expedited manner but will be included in the annual report:

- Pain due to disease
- Events associated with vascular access devices not implanted or used on this protocol

7.4 DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

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

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

7.4.2 Sponsor Monitoring Plan

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

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

8 STATISTICAL SECTION

8.1 STUDY DESIGN/ENDPOINTS

Dose escalation of ECI301 will proceed in cohorts of 3-6 patients are started at each dose level. DLT will be defined as CTCAE v4.0 any grade 3 or greater non-hematologic toxicity, any grade 3 neutropenia or thrombocytopenia, any grade 4 anemia and nausea, vomiting, diarrhea, tumor pain, or pre-existing hyponatremia, dyselectrolytemia, or orthostatic hypotension has been optimally treated with anti-emetics, anti-diarrheal, analgesics, or hydration and which persists for over 48 hours despite maximal medical therapy. Toxicity resulting in cumulative radiation treatment interruptions of 4 or more days will be considered a DLT. In addition, grade 3 or higher RTOG acute toxicity attributed as being possibly, probably, or definitely related to ECI301 is observed, this will be defined as a DLT. If 0/3 experience first course DLT, the next cohort starts one dose level higher. If 1/3 experience first course DLT, up to 3 more patients are started at that same dose level. If 2 or more experience first course DLT, no further patients are started at that dose and the next lower dose level is expanded to a total of 6 patients if only three were treated at that dose. The MTD or recommended Phase II dose is the dose level at which no more than 1 of 6 patients experience DLT during treatment and the three weeks after completion.

8.2 SAMPLE SIZE/ACCRUAL RATE

The number of patients required to complete this trial involves 5 cohorts of 3 to 6 patients. The total number of patients that will accrue to this study is therefore between 3 to 30, at a rate of 2 patients per month.

No group (except patients with cognitive impairment unable to give informed consent and those less than 18 years of age) is being excluded from participation. Subjects of both genders, from all racial and ethnic groups are eligible for this trial. Efforts will be made to extend accrual to a representative population.

8.3 ANALYSIS OF SECONDARY ENDPOINTS

Analysis of inflammatory cell infiltration in tumors, immune cell profiles, and immune cell IFN production will be performed. The statistical analysis will involve only descriptive statistics and exploratory graphical representations of the data. That is, no inferential analysis will be conducted. Exploratory modeling of dose-response relationship in terms of anti-tumor activity

and immunologic changes will be performed. These results of these studies will be for descriptive purposes.

The final PK analyses and evaluation for anti-MIPα antibodies will be the responsibility of ECI, Inc. The ECI301 concentration-time data, along with the derived PK parameters, will be listed and summarized appropriately. Additional parameters may be determined if deemed appropriate:

ECI301: C_{max}, t_{max}, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $C_{min,}$ CL/F

The PK parameters will be derived using non-compartmental analysis. The maximum plasma concentrations (C_{max}) and the time to reach the maximum plasma concentrations (t_{max}) will be determined by visual inspection of the plasma concentration-time profiles. The area under the plasma concentration-time curve from zero to 8 hours post dose $AUC_{(0-12)}$ and zero to 24 hours post-dose $AUC_{(0-24)}$ will be calculated by the linear trapezoidal rule. The total oral clearance from plasma (CL/F) after an oral dose will be calculated from the dose divided by the AUC during the appropriate dosing interval.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION:

This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met. For safety reasons, pregnant women and children are excluded from this study. This study will be recruited through internal referral, our physician referral base, and through various cancer information hotlines (i.e., Clinical Studies Support Center, 1-800-4Cancer.) Participants should realize that there is no guarantee of benefit to them from participation in this trial. The results of this trial may benefit future cancer patients. To date, there is no information that suggests that differences in drug metabolism or effect on tumor would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but a balance must be struck between participant safety considerations and limitations on the number of individuals exposed to potentially ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, a follow-up study may be written to investigate those differences more fully. Inclusion of Women and Minorities: This study will be open to all individuals regardless of gender, ethnicity, or race provided that the aforementioned inclusion and exclusion criteria are met.

Pregnant women are excluded from this study because the effects of ECI301 on the developing fetus is unknown and because radiation is a known teratogen. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ECI301, breastfeeding should be discontinued if the mother is treated with ECI301. Participants with unstable or serious medical conditions such as uncontrolled diabetes, uncontrolled hypertension, symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within the past 6 months, uncontrolled cardiac arrhythmia; or psychiatric illness/social situations that would limit compliance with study requirements are excluded due to the possibility that the underlying condition may obscure the attribution of effect and adverse events and may limit study compliance. HIV-positive patients are ineligible because they harbor an incompletely effective immune system which could impair response to ECI301. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when

indicated. Patients with autoimmune diseases or hepatitis are excluded due to the possibility that ECI301 could worsen inflammatory processes.

9.2 PARTICIPATION OF CHILDREN:

This study includes patients 18 years of age and older. Because insufficient dosing or adverse event data are currently available on the use of ECI301 in patients <18 years of age, children are excluded from this study, but may be eligible for future pediatric trials. Studies will be performed in patients <18 years of age when it is appropriate to do so.

9.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS:

Participants will receive treatment with radiotherapy to sites of metastatic disease and the risks of this therapy may be increased with the use of ECI301 delivered concurrently as a radiation sensitizer. The risks of radiotherapy to a metastatic site are highly dependent on the area receiving treatment with the exception of fatigue and skin reaction. Patients will be provided with a separate consent specific to the area receiving radiotherapy before treatment that will detail the specific risks of radiotherapy to that site as is standard practice. Participants may also experience risks from delivery of the therapeutic agent ECI301 relating to the infusion or drug reactions. Infusional risks include line infection and bleeding. The protocol therapy could enhance the effects of radiotherapy and result in more durable palliation.

In addition to the risks of protocol therapy, participants may undergo optional biopsies. The risks of biopsy are also dependent on the region biopsied and may include bleeding, pain, and infection.

9.4 RISKS/BENEFITS ANALYSIS:

This is a first in man study of ECI301. The side effects of ECI301 infusions in man are unknown. Animal toxicology studies have suggested that ECI301 is considered to have an acceptable safety margin at the likely doses to be studied in this Phase I protocol with evidence of reduction in circulating monocytes transiently. Human studies of a similar agent, BB-10010 showed acute changes in monocyte counts which rapidly resolved and no other observable toxicity. The expected discomforts of infusions of ECI301 are those to be expected from everyday infusions: pain and discomfort from venipuncture. In this study using ECI301, a conservative approach to starting dose has been chosen to minimize patient risk.

It is possible that ECI301 will enhance tumor sensitivity to radiation or could enhance the immune system's recognition of tumor at unirradiated sites. This could result in more durable palliation and reduction in metastatic burden. It is also possible that ECI301 could increase the effects of radiation on normal tissues that are irradiated by resulting in inflammation. For this reason, we have chosen small margins of additional tissue to include in our treatment volume. It remains a possibility that patients may have a greater degree of acute toxicity from radiotherapy than would be expected which could include dermatitis, fatigue, or pain.

In summary the potential risk: benefit ratio for Phase I studies is considered to be acceptable in light of potential benefits, the initial patient eligibility criteria, and proposed monitoring of patients.

Optional biopsies may also result in a risk to the patient. The risks of biopsy include bleeding, infection, pain, and site specific side effects. Biopsies will only be performed if they are felt to be of minimal risk and if the patient consents.

9.5 CONSENT AND ASSENT PROCESS AND DOCUMENTATION:

- The procedures and treatments involved in this protocol, with their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the treatment subject. A signed, informed consent document will be obtained from the subjects by one of the physician investigators.
- Consent forms: The original signed informed consent documents will be kept with patient medical records. Central Registration will also retain a copy of the informed consent document. A copy of their own signed informed consent documents will also be given to study participants.
- Central Registration will ascertain the date of IRB approval before registering the first subject.
- The treatment subject informed consent forms contain all elements required for consent. In addition, the Principal Investigator, or their designee will obtain oral consent and will be available to answer all patient questions.

10 PHARMACEUTICAL INFORMATION:

10.1 ECI301

10.1.1 Source:

Effector Cell Institute.

10.1.2 Toxicity

ECI301 has not been delivered to humans. Single dosing studies of IV (bolus) administration of ECI301 in 5-week-old male ICR mice at a dose of 0 (PBS; non-treated [NT], n=2), 1, 10, or 300 mg/kg ECI301 (n=3/dose) were completed. All mice were fasted prior to dosing and were observed for 4 hours post dose for signs of general condition and mortality. Body weight measurements were taken on Days -1, 0, 1, and 2. Animals were sacrificed 2 days after dosing. Parameters evaluated included: hematology for albumin/globulin ratios, blood urea nitrogen, creatinine, lactic dehydrogenase, aspartate aminotransaminase, alanine aminotransaminase, and inorganic phosphorus of the isolated plasma. Urine was analyzed for glucose, urobilinogen, and pH. Histological assessment of brain, lung, liver, kidney, thymus, intestine, and colon was conducted. No animals died following administration of single doses of ECI301 up to 300 mg/kg. There were no significant treatment-related differences in clinical signs, body weights, plasma examination, histology, or organ weights in any dose group. Protein levels in urine showed minor elevations in the 10-mg/kg and 300-mg/kg groups. There were no other observed changes. These results indicate that a single dose of ECI301 of up to 300 mg/kg did not cause any overt toxicity during observations for 2 days post-treatment. It was concluded that a single dose of ECI301, up to 300 mg/kg, was well tolerated, and therefore the NOAEL was 300 mg/kg for this study. [28]

A single dose study was conducted in accordance with GLP to evaluate the acute toxicity of IV administration of ECI301 in rats. A single dose of 0 (0.01% polysorbate 80 in PBS), 0.05, 0.5, or 5 mg/kg ECI301 was administered IV (bolus) to Crl:CD® (SD) IGS BR rats (5/sex/dose). All rats were observed daily for 14 days after dosing for general clinical condition, signs of toxicity, and mortality. All animals were subjected to necropsy and macroscopic investigation. An additional group of animals was administered a single dose of 0.05, 0.5, or 5 mg/kg ECI301 IV for toxicokinetic analysis (6/sex/dose). Animals were bled at 5, 15, and 30 minutes, and 1, 2, and

4 hours postdose for toxicokinetic analysis. There were no treatment-related deaths for this study. There were no significant treatment-related clinical signs or effects on body weight. Organ weights were not affected, and there were no treatment-related macroscopic findings. The NOAEL was determined to be greater than the 5-mg/kg ECI301 used in this study. The toxicokinetic results indicated that the rate and extent of systemic exposure of rats to ECI301 appeared to be characterized by nonlinear (dose-dependent) kinetics over the dose range. Increasing the dose of ECI301 above 0.05 mg/kg was considered likely to result in a higher systemic exposure than would be predicted from a linear relationship, which is consistent with the possibility of a capacity-limited process for ECI301 elimination. There were no differences between the sexes for systemic exposure. It was concluded that a single dose of ECI301 at 5 mg/kg did not cause any toxicological effects. [28]

Repeated dosing studies of ECI301 in 5- to 7-week-old ICR mice were conducted as a non-GLP investigation to evaluate the potential toxicity of repeat IV administration. ECI301 was administered IV at 0 (PBS; 2/dose), 1, 10, or 100 mg/kg/day (3/dose) for two 5-day dosing cycles, split by a 3-day off-dose period. An additional group receiving bovine serum albumin (BSA; 100 mg/kg) was added to serve as an additional control group for using a different protein. Animals were observed for 4 hours post-dose on Day 3 and Day 13 for signs of general condition and mortality. Animals were autopsied 24 hours following the last dose. Parameters evaluated included daily body weights, urinalysis, and hematology. Organ weights and histology of brain, lung, liver, kidney, thymus gland, and colon tissue were conducted. There was no treatment-related mortality. There were no treatment-related effects on body weights or any of the hematological parameters measured. The protein in urine increased slightly in 1 of 3 animals in the 10-mg/kg group and 1 of 3 animals in the 100-mg/kg group. At 100 mg/kg ECI301, there was an increase in lung and spleen weights. Histology of the kidney showed an increase in the adhesion of glomerular basement membrane and renal glomeruli, and disruption of the glomerulus structure in all animals in the group receiving ECI301 at 100 mg/kg, and 1 of 3 animals in the 10-mg/kg group. It is unclear if these results are due to administration of ECI301 or to immune complex deposition. In spleen samples from both BSA and ECI301 (100 mg/kg), the boundary between the red and white pulp was unclear. There was structural damage to the white pulp, and the central artery was unclear. There were no treatment-related changes in any of the other organs or tissues. This experiment demonstrated that repeated doses of ECI301, to a maximum total dose of 1000 mg/kg (1000 times the effective dosage), had no critical effect on the kidney, but abnormal tissue images and urine protein elevations were observed that indicated some renal damage. Further study is required to determine if this damage is attributable to ECI301 or another protein. Data from this non-GLP study supported further study of repeat doses over 2 to 4 weeks.[28]

A separate study was conducted in accordance with GLP to evaluate the systemic toxicity and toxicokinetics of IV administration of ECI301 alone or in combination with docetaxel in rats for three 5-day dosing cycles. A single dose of 0 (0.01% polysorbate 80 in PBS), 0.05, 0.15, or 1.5 mg/kg/day ECI301, 12.5 mg/kg docetaxel, or a 0.05-/12.5- mg/kg/day combination of ECI301 and docetaxel, was administered IV (bolus) to Crl: CD® (SD) IGS BR rats daily for 5 days. After 51 days, 10 animals/sex/dose were sacrificed and an additional 5 animals/sex/dose were sacrificed following a 10-day recovery period. All rats were observed daily after dosing for general clinical condition, signs of toxicity, and mortality. Additionally, food consumption, ophthalmology, hematology, blood chemistry, urinalysis, anti-ECI301 antibody assessments, peripheral blood immunophenotyping, and organ weights were analyzed. All animals were

subject to necropsy with macroscopic and microscopic pathology. An additional group of animals for each group (6/sex/dose) except control (3/sex/dose) was bled for toxicokineticanalysis of ECI301 and docetaxel plasma levels. Animals were bled at 5, 15, and 30 minutes, and 1, 2, and 4 hours post dose for toxicokinetic analysis. There were no deaths in this study attributable to ECI301 treatment alone. Six animals died following docetaxel treatment. There were no treatment-related clinical signs or any effect upon bodyweight and food consumption. Similarly, there were no treatment-related ophthalmoscopic findings or any effect upon the composition of the urine. The hematological examination indicated a slight increase of lymphocyte count after the first treatment cycle, resulting in an increased total leukocyte count, in males given 0.15 or 1.5 mg/kg, with similar trends also occurring at all doses in females after Cycle 3. This was not evident in the males after Cycle 3 or in the females at the end of the recovery period. Biochemical changes in the blood plasma were confined to a reduction of glucose concentrations after Cycles 1 and 3 in males given 1.5 mg/kg and after Cycle 3 in males given 0.15 mg/kg and a small increase of plasma urea after Cycle 3 in males given 1.5 mg/kg. An analysis of organ weights after the completion of the third dosing cycle indicated a small reduction of spleen and thymus weight in males given 0.05 mg/kg, which was attributed to treatment since this dose was at the maximum biological effect level for ECI301. There were no treatment-related macroscopic or histopathological findings. The plasma samples taken from the control animals were below the limit of quantification in all cases, demonstrating that there was no quantifiable contamination with ECI301 in these animals.[28]

The toxicokinetic investigations indicated that the extent of systemic exposure of rats to ECI301 appeared to be characterized by non-linear (dose-dependent) kinetics over the dose range 0.05 to 1.5 mg/kg/day on Days 1 and 47. Increasing the dose of ECI301 above 0.05 mg/kg/day resulted in a higher systemic exposure than would be predicted from a linear relationship, indicative of limited elimination capacity. In general, there were no differences in the systemic exposure of males and females to ECI301 and there was no accumulation after repeated doses. One female given 0.05 mg/kg ECI301 showed a positive anti-ECI301 antibody response. All other animals showed negative responses. Immunophenotyping after completion of Cycle 3 indicated, in animals given 1.5 mg/kg, an increased percentage of CD4+ T cells and the males showed a corresponding decrease in the percentage of CD8+ T cells, although the total numbers for all lymphocyte subsets studied were not affected. The numbers of NK lymphocytes were reduced in females given 1.5 mg/kg. Following recovery, males given 1.5 mg/kg still showed a reduced percentage of CD4+ lymphocytes and an increased percentage of CD8+ lymphocytes. It was concluded that IV (bolus) administration of ECI301 to CD rats at doses up to 1.5 mg/kg during three 5-day cycles of treatment, each separated by 16 days, was well tolerated, was not immunogenic, and caused no toxicologically significant findings. Docetaxel treatment 2 days after each cycle in which animals were given ECI301 at 0.05 mg/kg (a pharmacologically active dose) at 12.5 mg/kg in the first cycle in both sexes and the second cycle in males, subsequently reduced to 6.25 mg/kg for the remaining cycles, caused a range of findings that were attributable to its anti-mitotic activity. Compared with animals given docetaxel on the same study days, but with no ECI301 treatment, all differences in the response to docetaxel were attributed to normal biological variation in response to treatment and, consequently, there was no evidence that ECI301 modulated the effects of docetaxel treatment.[28]

A separate study was conducted to evaluate the potential for systemic toxicity of 3 different doses of ECI301, when administered IV to common marmosets for a 5-day dosing period with an 18-day observation period, to confirm doses to be used in the main toxicity study. Common

marmosets (*Callithrix jacchus*) received 0.05, 0.15, or 1.5 mg/kg ECI301 (1/sex/group) IV bolus, once a day, for 5 days. Animals were observed for 18 days for clinical condition, injection-site reactions, and body weight. Hematology, blood chemistries, organ weight, and macroscopic pathology investigations were undertaken. There were no treatment-related deaths during this study. There were no treatment-related clinical signs of toxicity, and body weights were unaffected. There was no effect of treatment on organ weights or macropathology. All animals had reduced hematocrit, hemoglobin concentration, and/or erythrocyte count on Day 9 and/or Day 22, often accompanied by increased reticulocyte count. Mean cell volume was increased in the male receiving 0.05 mg/kg, the female receiving 0.5 mg/kg, and both animals receiving 1.5 mg/kg. Mean cell hemoglobin concentration was decreased in the male receiving 0.05 mg/kg and the female receiving 1.5 mg/kg. Anisocytosis was reported on the blood film for all females. There was no strong trend with dosage, and it is possible that these changes may have been caused by repeated blood sampling. However, with the absence of control animals on this study, the possibility that these changes were due to treatment with ECI301 cannot be excluded. An increase in monocyte count occurred on Day 9 in the female marmosets receiving 0.05 mg/kg or 0.15 mg/kg. These values normalized by Day 22. Minor blood chemistry changes occurred on Day 9 and Day 23, but these changes were small and were not dose related in degree. All animals showed reduced alkaline phosphatase activity on Day 23. Creatinine concentrations were reduced on Day 23 in the males given 0.05 mg/kg and in animals given 0.15 mg/kg or 1.5 mg/kg. Total triglyceride concentrations were high on Day 9 and Day 23 for all animals except males given 1.5 mg/kg. In the animals given 0.05 mg/kg and the females given 0.15 mg/kg, there was a reduction of total cholesterol level. All animals showed an increase of sodium concentration on Day 9 and, at 0.15 and 15 mg/kg, this was associated with increased chloride concentration. Potassium concentrations were low on Day 9 in all animals, and in the males, this persisted to Day 23. Total protein concentrations were low on Day 23 in animals given 0.05 mg/kg or 0.15 mg/kg, but not in those given 1.5 mg/kg, and most showed an increase in the albumin to globulin ratio. It was concluded that the IV administration of ECI301 to common marmosets for a single 5-day dosing period was well tolerated up to doses of 1.5 mg/kg. There were no toxicologically significant findings, and therefore it was concluded that 1.5 mg/kg was a suitable dose for future studies in marmosets. [28]

Study EFC0010 was conducted to evaluate the systemic toxicity and toxicokinetics of IV administration of ECI301 to *Callithrix jacchus*, the common marmoset for three 5-day cycles. A single dose of 0 (0.01% polysorbate 80 in PBS), 0.05, 0.15, or 1.5 mg/kg ECI301 was administered IV (bolus) to marmosets (3/sex/dose). Radiation with chemotherapy is standard of care regimen for treatment of cancer. Docetaxel is one of the common chemotherapy drugs; however, significant AEs such as suppression of bone marrow function are associated with the administration of docetaxel. In phase 1 clinical study, ECI301 is expected to be administered to cancer patients in combination with radiation treatment. However, it may be necessary for docetaxel to be administered to patients in addition to ECI301. Therefore, toxicity data of ECI301 in combination with docetaxel were found to be necessary. The design of the study was as indicated below and also included 2 groups that were dosed with docetaxel, one in combination with ECI301. All animals were observed daily throughout the study period for general clinical condition, injection-site observations, body weight, ophthalmology, electrocardiography, blood pressure, hematology, blood chemistry, urinalysis, peripheral blood immunophenotyping, organ weights, macroscopic and microscopic pathology, and mortality. An additional group of animals for each group except control (2/sex/dose) were bled for

toxicokinetic analysis. Animals were bled at 5, 15, and 30 minutes, and 1, 2, and 4 hours postdose for toxicokinetic analysis of plasma concentrations of ECI301 or docetaxel, and anti-ECI301antibody assessment. Two animals were sacrificed prematurely during this study. Neither death was attributed to ECI301 treatment, but 1 of them was determined to be possibly due to docetaxel treatment. There were no clinical signs that were attributable to treatment, and there was no effect upon bodyweight. One male receiving 0.15 mg/kg was killed on Day 59 due to gradual decline in its general clinical condition. The macroscopic and histopathological examinations did not identify any finding that would account for the ill health of this animal and, consequently, this death was not attributed to treatment. There were no treatment-related ophthalmoscopic findings and no effect upon the electrophysiology of the heart or blood pressure. There were no hematological findings or changes in blood plasma or urinary composition that were attributable to treatment. There were no toxicologically significant organ weight differences after 3 cycles of dosing with ECI301 and no macroscopic or histopathological findings that were due to treatment.[28]

The plasma samples taken from the control animals were below the limit of quantification in all cases, demonstrating that there was no quantifiable contamination with ECI301 in these animals. Mean plasma concentrations (Cmax) and ECI301 concentration-time curves estimated up to 4 hours post dose (AUC4) on Day 1 and 47 are also shown. The toxicokinetic investigations indicated that the extent of systemic exposure of marmosets to ECI301 appeared to be characterized by non-linear (dose-dependent) kinetics over the dose range 0.05 to 1.5 mg/kg on Days 1 and 47. Increasing the dose of ECI301 above 0.15 mg/kg was considered likely to result in a higher systemic exposure than would be predicted from a linear relationship, which is consistent with the possibility of a capacity limited process for the elimination of ECI301. In general, there were no differences in the systemic exposure of male and female marmosets to ECI301, and there was no accumulation after repeated doses. All animals showed negative anti-ECI301 antibody responses. Immunophenotyping after completion of Cycle 3 indicated no significant change at any dose.[28]

The toxicokinetic investigations indicated that the co-administration of ECI301 with docetaxel generally appeared to have little effect on the systemic exposure of male and female marmosets to docetaxel. The co-administration of ECI301 with docetaxel did not elicit an anti-ECI301 antibody response in any animal. Peripheral blood immunophenotyping indicated a reduction in the number of CD3+ T lymphocytes in males and females given docetaxel alone and a reduction in CD20+ lymphocytes in the males. These males also showed an increase in the percentage of CD3+ lymphocytes and a corresponding decrease in the percentage of CD20+ B lymphocytes. This T/B cell ratio change was not seen in the females. A reduction in the number and percentage of CD14+ monocytes was also seen in the males, but not in the females. It was concluded that IV (bolus) administration of ECI301 to common marmosets at doses up to 1.5 mg/kg during three 5-day cycles of treatment, each separated by 16 days, was well tolerated, was not immunogenic, and caused no toxicologically significant findings. Docetaxel treatment at 3 mg/kg, 2 days after each cycle in which animals were given ECI301 at 0.05 mg/kg (a pharmacologically active dose), caused several findings that were attributable to its anti-mitotic activity. There was no evidence in this study that ECI301 modulated adversely the effect of docetaxel treatment, but ECI301 coadministration did appear protective with regard to the effect upon lymphocyte populations in the peripheral blood.[28]

10.1.3 Formulation and preparation

The ECI301 drug product is presented as a sterile solution for injection, 1.3 mL in a 6 mL glass vial, with a closure consisting of a rubber stopper and crimp cap. It was made according to current Good Manufacturing Practice (cGMP) for ECI by Toyobo, Japan. The excipients (inactive ingredients) in the ECI301 drug product are of compendial grade and are commonly used in parenteral dosage forms. The quantitative composition of the ECI301 drug product is shown in Table 4, including the pharmacopoeial reference for each component, where applicable.

TABLE 4

Quantitative Composition of the ECI301 Drug Product

Component	Amount (per mL)	Pharmacopeial/Quality Standard
Drug substance: ECI301	1.00 mg	In-house
Inactive Ingredients:		
Sodium hydrogen phosphate hydrate	2.90 mg	JP, USP
Potassium dihydrogen phosphate	0.20 mg	NF
Sodium chloride	8.00 mg	JP, USP
Potassium chloride	0.20 mg	JP, USP
Polysorbate 80	0.10 mg	JP, USP
Water for injection	q.s.	JP, USP

10.1.4 Stability and storage

The ECI301 drug product is stored frozen at -20°C until use, and may not be re-frozen. It is stable at -20°C for up to 36 months. ECI301 is stable at room temperature for at least two hours.

10.1.5 Administration procedures

The concentration of ECI301 in the drug product is 1000 µg/mL. Dose escalation will be achieved by extracting the correspondingly larger volumes from multiple vials as required, according to the following table (Table 5). ECI301 will be administered without dilution (Table 5). The study drug will be filtered with 0.20 or 0.22-micron pore size in the pharmacy and then administered as a bolus injection via an in-dwelling peripheral intravenous line (PICC line preferred) at a rate of 1 mL/second. The catheter will immediately be flushed with 20 mL 0.9% NaCl at 1 mL/sec The dose will be delivered from the pharmacy in a single syringe; accordingly, doses requiring volumes greater than 10 mL will be delivered in larger syringes.

TABLE 5

Volume of Drug Product Required for the ECI301 Dose Levels

ECI301 Dose (µg/kg)	ECI301 Dose (µL/kg)	Volume Required in µL	Final Volume to be Injected
25	25	W x 25	W x 25 µL (no dilution)

ECI301 Dose (µg/kg)	**ECI301 Dose (µL/kg)**	**Volume Required in µL**	**Final Volume to be Injected**
50	50	W x 50	W x 50 µL (no dilution)
82.5	82.5	W x 82.5	W x 82.5 µL (no dilution)
125	125	W x 125	W x 125 µL (no dilution)
175	175	W x 175	W x 175 µL (no dilution)

W = weight of patient in Kgs

10.1.6 Incompatibilities

No incompatibilities or drug interactions have been described.

11 REFERENCES

1. Friedman, E.J., *Immune modulation by ionizing radiation and its implications for cancer immunotherapy.* Curr Pharm Des, 2002. **8**(19): p. 1765-80.
2. Sharp, H.J., et al., *Synergistic antitumor activity of immune strategies combined with radiation.* Front Biosci, 2007. **12**: p. 4900-10.
3. Maurer, M. and E. von Stebut, *Macrophage inflammatory protein-1.* Int J Biochem Cell Biol, 2004. **36**(10): p. 1882-6.
4. Rollins, B.J., *Chemokines.* Blood, 1997. **90**(3): p. 909-28.
5. Wolpe, S.D. and A. Cerami, *Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines.* Faseb J, 1989. **3**(14): p. 2565-73.
6. Wazer, D.E. and B.L. Willett, *Clinical considerations in palliative treatment of metastatic prostate carcinoma.* Int J Radiat Oncol Biol Phys, 1987. **13**(1): p. 145-6.
7. Wu, J.S., et al., *Meta-analysis of dose-fractionation radiotherapy trials for the palliation of painful bone metastases.* Int J Radiat Oncol Biol Phys, 2003. **55**(3): p. 594-605.
8. Muranski, P., et al., *Increased intensity lymphodepletion and adoptive immunotherapy--how far can we go?* Nat Clin Pract Oncol, 2006. **3**(12): p. 668-81.
9. Paulos, C.M., et al., *Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling.* J Clin Invest, 2007. **117**(8): p. 2197-204.
10. Klebanoff, C.A., et al., *Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy.* Trends Immunol, 2005. **26**(2): p. 111-7.
11. Roses, R.E., et al., *Radiation therapy and Toll-like receptor signaling: implications for the treatment of cancer.* Oncogene, 2008. **27**(2): p. 200-7.
12. Hauser, S.H., et al., *Radiation-enhanced expression of major histocompatibility complex class I antigen H-2Db in B16 melanoma cells.* Cancer Res, 1993. **53**(8): p. 1952-5.
13. Klein, B., et al., *The effect of irradiation on expression of HLA class I antigens in human brain tumors in culture.* J Neurosurg, 1994. **80**(6): p. 1074-7.
14. Hallahan, D.E., et al., *X-ray-induced P-selectin localization to the lumen of tumor blood vessels.* Cancer Res, 1998. **58**(22): p. 5216-20.
15. Gulley, J.L., et al., *Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer.* Clin Cancer Res, 2005. **11**(9): p. 3353-62.
16. Dewan, M.Z., et al., *Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody.* Clin Cancer Res, 2009. **15**(17): p. 5379-88.
17. Demaria, S., et al., *Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer.* Clin Cancer Res, 2005. **11**(2 Pt 1): p. 728-34.
18. Brody, J.D., et al., *In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study.* J Clin Oncol. **28**(28): p. 4324-32.
19. Yoneyama, H., et al., *Regulation by chemokines of circulating dendritic cell precursors, and the formation of portal tract-associated lymphoid tissue, in a granulomatous liver disease.* J Exp Med, 2001. **193**(1): p. 35-49.
20. Zhang, Y., et al., *Mobilization of dendritic cell precursors into the circulation by administration of MIP-1alpha in mice.* J Natl Cancer Inst, 2004. **96**(3): p. 201-9.

21. Teitz-Tennenbaum, S., et al., *Radiotherapy potentiates the therapeutic efficacy of intratumoral dendritic cell administration.* Cancer Res, 2003. **63**(23): p. 8466-75.
22. Shiraishi, K., et al., *Enhancement of antitumor radiation efficacy and consistent induction of the abscopal effect in mice by ECI301, an active variant of macrophage inflammatory protein-1alpha.* Clin Cancer Res, 2008. **14**(4): p. 1159-66.
23. Broxmeyer, H.E., et al., *Myeloid progenitor cell proliferation and mobilization effects of BB10010, a genetically engineered variant of human macrophage inflammatory protein-1alpha, in a phase I clinical trial in patients with relapsed/refractory breast cancer.* Blood Cells Mol Dis, 1998. **24**(1): p. 14-30.
24. Clemons, M.J., et al., *A randomized phase-II study of BB-10010 (macrophage inflammatory protein- 1alpha) in patients with advanced breast cancer receiving 5-fluorouracil, adriamycin, and cyclophosphamide chemotherapy.* Blood, 1998. **92**(5): p. 1532-40.
25. Hunter, M.G., et al., *BB-10010: an active variant of human macrophage inflammatory protein-1 alpha with improved pharmaceutical properties.* Blood, 1995. **86**(12): p. 4400-8.
26. Marshall, E., et al., *Clinical effects of human macrophage inflammatory protein-1 alpha MIP-1 alpha (LD78) administration to humans: a phase I study in cancer patients and normal healthy volunteers with the genetically engineered variant, BB-10010.* Eur J Cancer, 1998. **34**(7): p. 1023-9.
27. Bernstein, S.H., et al., *A randomized phase II study of BB-10010: a variant of human macrophage inflammatory protein-1alpha for patients receiving high-dose etoposide and cyclophosphamide for malignant lymphoma and breast cancer.* Br J Haematol, 1997. **99**(4): p. 888-95.
28. ECI, I., *ECI301 Investigator Brochure.* 2010.
29. Kaech, S.M., E.J. Wherry, and R. Ahmed, *Effector and memory T-cell differentiation: implications for vaccine development.* Nat Rev Immunol, 2002. **2**(4): p. 251-62.
30. Thompson, E.D., et al., *Tumor masses support naive T cell infiltration, activation, and differentiation into effectors.* J Exp Med. **207**(8): p. 1791-804.

12 APPENDICES

12.1 APPENDIX I : ACUTE RTOG TOXICITY SCORING

	[0]	[1]	[2]	[3]	[4]
SKIN	No change over baseline	Follicular, faint or dull erythema/ epilation/dry desquamation/ decreased sweating	Tender or bright erythema, patchy moist desquamation/ moderate edema	Confluent, moist desquamatiom other than skin folds, pitting edema	Ulceration, hemorrhage, necrosis
MUCOUS MEMBRANE	No change over baseline	Injection/ may experience mild pain not requiring analgesic	Patchy mucositis which may produce an inflammatory serosanguinitis discharge/ may experience moderate pain requiring analgesia	Confluent fibrinous mucositis/ may include severe pain requiring narcotic	Ulceration, hemorrhage or necrosis
EYE	No change	Mild conjunctivitis with or without scleral injection/ increased tearing	Moderate conjunctivitis with or without keratitis requiring steroids &/or antibiotics/ dry eye requiring artificial tears/ iritis with photophobia	Severe keratitis with corneal ulceration/ objective decrease in visual acuity or in visual fields/ acute glaucoma/ panopthalmitis	Loss of vision (unilateral or bilateral)

EAR	No change over baseline	Mild external otitis with erythema, pruritis, secondary to dry desquamation not requiring medication. Audiogram unchanged from baseline	Moderate external otitis requiring topical medication/ serious otitis medius/ hypoacusis on testing only	Severe external otitis with dischange or moist desquamation/ symptomatic hypoacusis/tinnitus, not drug related	Deafness
SALIVARY GLAND	No change over baseline	Mild mouth dryness/ slightly thickened saliva/ may have slightly altered taste such as metallic taste/ these changes not reflected in alteration in baseline feeding behavior, such as increased use of liquids with meals	Moderate to complete dryness/ thick, sticky saliva/ markedly altered taste	-------	Acute salivary gland necrosis
PHARYNX & ESOPHAGUS	No change over baseline	Mild dysphagia or odynophagia/ may require topical anesthetic or non-narcotic analgesics/ may require soft diet	Moderate dysphagia or odynophagia/ may require narcotic analgesics/ may require puree or liquid diet	Severe dysphagia or odynophagia with dehydration or weight loss(>15% from pre-treatment baseline) requiring N-G feeding tube, I.V. fluids or hyperalimentation	Complete obstruction, ulceration, perforation, fistula

LARYNX	No change over baseline	Mild or intermittent hoarseness/cough not requiring antitussive/ erythema of mucosa	Persistent hoarseness but able to vocalize/ referred ear pain, sore throat, patchy fibrinous exudate or mild arytenoid edema not requiring narcotic/ cough requiring antitussive	Whispered speech, throat pain or referred ear pain requiring narcotic/ confluent fibrinous exudate, marked arytenoid edema	Marked dyspnea, stridor or hemoptysis with tracheostomy or intubation necessary
UPPER G.I.	No change	Anorexia with <=5% weight loss from pretreatment baseline/ nausea not requiring antiemetics/ abdominal discomfort not requiring parasympatholytic drugs or analgesics	Anorexia with <=15% weight loss from pretreatment baseline/nausea &/ or vomiting requiring antiemetics/ abdominal pain requiring analgesics	Anorexia with >15% weight loss from pretreatment baseline or requiring N-G tube or parenteral support. Nausea &/or vomiting requiring tube or parenteral support/abdominal pain, severe despite medication/hematemesis or melena/ abdominal distention (flat plate radiograph demonstrates distended bowel loops	Ileus, subacute or acute obstruction, performation, GI bleeding requiring transfusion/abdominal pain requiring tube decompression or bowel diversion

LOWER G.I. INCLUDING PELVIS	No change	Increased frequency or change in quality of bowel habits not requiring medication/ rectal discomfort not requiring analgesics	Diarrhea requiring parasympatholytic drugs (e.g., Lomotil)/ mucous discharge not necessitating sanitary pads/ rectal or abdominal pain requiring analgesics	Diarrhea requiring parenteral support/ severe mucous or blood discharge necessitating sanitary pags/abdominal distention (flat plate radiograph demonstrates distended bowel loops)	Acute or subacute obstruction, fistula or perforation; GI bleeding requiring transfusion; abdominal pain or tenesmus requiring tube decompression or bowel diversion
LUNG	No change	Mild symptoms of dry cough or dyspnea on exertion	Persistent cough requiring narcotic, antitussive agents/ dyspnea with minimal effort but not at rest	Severe cough unresponsive to narcotic antitussive agent or dyspnea at rest/ clinical or radiologic evidence of acute pneumonitis/ intermittent oxygen or steroids may be required	Severe respiratory insufficiency/ continuous oxygen or assisted ventilation
GENITOURINARY	No change	Frequency of urination or nocturia twice pretreatment habit/ dysuria, urgency not requiring medication	Frequency of urination or nocturia which is less frequent than every hour. Dysuria, urgency, bladder spasm requiring local anesthetic (e.g., Pyridium)	Frequency with urgency and nocturia hourly or more frequently/ dysuria, pelvis pain or bladder spasm requiring regular, frequent narcotic/gross hematuria with/ without clot passage	Hematuria requiring transfusion/ acute bladder obstruction not secondary to clot passage, ulceration or necrosis

HEART	No change over baseline	Asymptomatic but objective evidence of EKG changes or pericardial abnormalities without evidence of other heart disease	Symptomatic with EKG changes and radiologic findings of congestive heart failure or pericardial disease/ no specific treatment required	Congestive heart failure, angina pectoris, pericardial disease responding to therapy	Congestive heart failure, angina pectoris, pericardial disease, arrhythmias not responsive to non-surgical measures
CNS	No change	Fully functional status (i.e., able to work) with minor neurologic findings, no medication needed	Neurologic findings present sufficient to require home case/ nursing assistance may be required/ medications including steroids/anti-seizure agents may be required	Neurologic findings requiring hospitalization for initial management	Serious neurologic impairment which includes paralysis, coma or seizures>3 per week despite medication/hospitalization required
HEMATOLOGIC WBC (X 1000)	>=4.0	3.0 - <4.0	2.0 - <3.0	1.0 - <2.0	<1.0
PLATELETS (X 1000)	>=100	75 - <100	50 - <75	25 - <50	<25 or spontaneous bleeding
NEUTROPHILS	>=1.9	1.5 - <1.9	1.0 - <1.5	0.5 - <1.0	<0.5 or sepsis
HEMOGLOBIN (GM %)	>11	11-9.5	<9.5 - 7.5	<7.5 - 5.0	-------
HEMATOCRIT (%)	>=32	28 - <32	<28	Packed cell transfusion required	-------

GUIDELINES: The acute morbidity criteria are used to score/grade toxicity from radiation therapy. The critera are relevant from day 1, the commencement of therapy, through day 90. Therafter, the EORTC/RTOG Criteria of Late Effects are to be utilized.

The evaluator must attempt to discriminate between disease- and treatment-related signs and symptoms.

An accurate baseline evaluation prior to commencement of therapy is necessary.

All toxicities Grade 3, 4 or 5* must be verified by the Principal Investigator.

*ANY TOXICITY WHICH CAUSED DEATH IS GRADED 5.

12.2 APPENDIX II: LATE RTOG TOXICITY SCORING

ORGAN TISSUE	0	Grade 1	Grade 2	Grade 3	Grade 4
SKIN	None	Slight atrophy Pigmentation change Some hair loss	Patch atrophy; Moderate telangiectasia; Total hair loss	Marked atrophy; Gross telangiectasia	Ulceration
SUBCUTANEOUS TISSUE	None	Slight induration (fibrosia) and loss of subcutaneous fat	Moderate fibrosis but asymptomatic Slight field contracture <10% linear reduction	Severe induration and loss of subcutaneous tissue Field contracture >10% linear measurement	Necrosis
MUCOUS MEMBRANE	None	Slight atrophy and dryness	Moderate atrophy and telangiectasia Little mucous	Marked atrophy with complete dryness Severe telangiectasia	Ulceration
SALIVARY GLANDS	None	Slight dryness of mouth Good response on stimulation	Moderate dryness of mouth Poor response on stimulation	Complete dryness of mouth No response on stimulation	Fibrosis
SPINAL CORD	None	Mild L'Hermitte's syndrome	Severe L'Hermitte's syndrome	Objective neurological findings at or below cord level treated	Mono, para quadraplegia
BRAIN	None	Mild headache Slight lethargy	Moderate headache Great lethargy	Severe headaches Severe CNS dysfunction (partial loss of power or dyskinesia)	Seizures or paralysis Coma

ORGAN TISSUE	0	Grade 1	Grade 2	Grade 3	Grade 4
EYE	None	Asymptomatic cataract Minor corneal ulceration or keratitis	Symptomatic cataract Moderate corneal ulceration Minor retinopathy or glaucoma	Severe keratitis Severe retinopathy or detachment Severe glaucoma	Panopthalmitis/ Blindness
LARYNX	None	Hoarseness Slight arytenoid edema	Moderate arytenoid edema Chondritis	Severe edema Severe chondritis	Necrosis
LUNG	None	Asymptomatic or mild symptoms (dry cough) Slight radiographic appearances	Moderate symptomatic fibrosis or pneumonitis (severe cough) Low grade fever Patchy radiographic appearances	Severe symptomatic fibrosis or pneumonitis Dense radiographic changes	Severe respiratory insufficiency/ Continuous O_2/ Assisted ventilation
HEART	None	Asymptomatic or mild symptoms Transient T wave inversion & ST changes Sinus tachycardia >110 (at rest)	Moderate angina on effort Mild pericarditis Normal heart size Persistent abnormal T wave and ST changes Low ORS	Severe angina Pericardial effusion Constrictive pericarditis Moderate heart failure Cardiac enlargement EKG abnormalities	Tamponade/ Severe heart failure/ Severe constrictive pericarditis
ESOPHAGUS	None	Mild fibrosis Slight difficulty in swallowing solids No pain on swallowing	Unable to take solid food normally Swallowing semi-solid food Dilatation may be indicated	Severe fibrosis Able to swallow only liquids May have pain on swallowing Dilation required	Necrosis/ Perforation Fistula

ORGAN TISSUE	0	Grade 1	Grade 2	Grade 3	Grade 4
SMALL/LARGE INTESTINE	None	Mild diarrhea Mild cramping Bowel movement 5 times daily Slight rectal discharge or bleeding	Moderate diarrhea and colic Bowel movement >5 times daily Excessive rectal mucus or intermittent bleeding	Obstruction or bleeding requiring surgery	Necrosis/ Perforation Fistula
LIVER	None	Mild lassitude Nausea, dyspepsia Slightly abnormal liver function	Moderate symptoms Some abnormal liver function tests Serum albumin normal	Disabling hepatitic insufficiency Liver function tests grossly abnormal Low albumin Edema or ascites	Necrosis/ Hepatic coma or encephalopathy
KIDNEY	None	Transient albuminuria No hypertension Mild impairment of renal function Urea 25-35 mg% Creatinine 1.5-2.0 mg% Creatinine clearance >75%	Persistent moderate albuminuria (2+) Mild hypertension No related anemia Moderate impairment of renal function Urea>36-60 mg% Creatinine clearance (50-74%)	Severe albuminuria Severe hypertension Persistent anemia (<10g%) Severe renal failure Urea >60 mg% Creatinine >4.0 mg% Creatinine clearance <50%	Malignant hypertension Uremic coma/Urea >100%

ORGAN TISSUE	0	Grade 1	Grade 2	Grade 3	Grade 4
BLADDER	None	Slight epithelial atrophy Minor telangiectasia (microscopic hematuria)	Moderate frequency Generalized telangiectasia Intermittent macroscopic hematuria	Severe frequency and dysuria Severe generalized telangiectasia (often with petechiae) Frequent hematuria Reduction in bladder capacity (<150 cc)	Necrosis/ Contracted bladder (capacity <100 cc) Severe hemorrhagic cystitis
BONE	None	Asymptomatic No growth retardation Reduced bone density	Moderate pain or tenderness Growth retardation Irregular bone sclerosis	Severe pain or tenderness Complete arrest of bone growth Dense bone sclerosis	Necrosis/ Spontaneous fracture
JOINT	None	Mild joint stiffness Slight limitation of movement	Moderate stiffness Intermittent or moderate joint pain Moderate limitation of movement	Severe joint stiffness Pain with severe limitation of movement	Necrosis/ Complete fixation

GUIDELINES: The late morbidity criteria are used to score/grade toxicity from radiation therapy. The critera are relevant from day 90 after completion of therapy.

The evaluator must attempt to discriminate between disease- and treatment-related signs and symptoms.

All toxicities Grade 3, 4 or 5* must be verified by the Principal Investigator.

*ANY TOXICITY WHICH CAUSED DEATH IS GRADED 5.

12.3 APPENDIX III. EXCLUDED IMMUNOSUPRESSIVE MEDICATIONS

Adalimumab (HUMIRA)
Azathioprine
Basiliximab (Simulect)
Certolizumab pegol (Cimzia)
Cyclophosphamide
Cyclosporin
Daclizumab (Zenapax)
Dactinomycin
Etanercept (Enbrel)
Golimumab (Simponi)
Ifliximab (Remicade)
Methotrexate
Mercaptopurine
Muromonab-CD3
Mycophenolate
Rituxan
Sirolimus
Tacrolimus
Oral or intravenously delivered glucocorticoids