Protocol Number:	PH&S IRB: 11-062A
Title:	Phase II Randomized Study of High Dose Interleukin-2 Versus Stereotactic Body Radiation (SBRT) and High Dose Interleukin-2 (IL-2) in Patients with Metastatic Melanoma
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PROTOCOL SIGNATURE PAGE

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I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study agent(s) and the conduct of the study.

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1. BACKGROUND AND RATIONALE

1.1 Melanoma and Interleukin-2

The American Cancer Society estimates that there were over 68,000 new diagnoses and 8,100 deaths from melanoma in the United States in 2009(1). Metastatic melanoma has a poor prognosis with less than 5% of patients surviving five years from the manifestation of visceral organ involvement. Disease-specific survival curves in all stages of melanoma have a negative slope, implying that metastatic disease can originate from thin primary lesions and that metastatic melanoma can develop many years after the initial diagnosis. For instance, in survival data compiled by Balch, et al., up to 10% of patients presenting with stage I melanoma (primary site less than 1 mm in depth and no nodal involvement) will die as a consequence of metastatic disease within 10 years(2).(3) Disease recurrence can manifest years or even decades after the initial diagnosis. The potential lethality of early stage melanoma distinguishes it from other solid tumors.

The first publication describing the clinical results of IL-2 appeared in 1985(4).(5) Three of six patients had greater than 50% regression of their metastatic melanoma. A subsequent publication describing the clinical outcomes of 270 patients reported a complete response (CR) rate of 6% and a partial response (PR) rate of 10% (6). The median duration of response was greater than 40 months. An update of this initial report showed that at 15 years over 70% of those achieving a CR were alive and melanoma-free.(6) Approximately 15% of the patients achieving a PR were also alive at 15 years.

The durability of these responses was the main rationale for approval of IL-2 by the FDA, and is also the reason that IL-2 therapy persists in oncological practice. Many regimens that used IL-2 in combination with other agents (e.g. IL-2 plus chemotherapy, IL-2 with interferon, adoptive transfer of various immune cell subsets, and IL-2 with monoclonal antibodies (7-10)) have been tested, but none of these more complex therapies has reported a convincing improvement in response rate or survival compared to IL-2 monotherapy. We and others have investigated the immunological effects of high dose per fraction radiation; which suggested that the combination of radiation and IL-2 could result in better anti-tumor responses. We recently completed a phase I study of SBRT radiation and IL-2 in patients with melanoma and renal cancer showing a 71% objective response rate in metastatic melanoma (reviewed in detail below), which provides the main rationale for this study.(11) Even with the recent FDA approval of ipilimumab showing a 4 month improvement in median survival(12), and targeted agents such as PLX-4032 having a high initial response rate of 70% (with a median duration of response of approximately 7 months)(13), the findings of our pilot study are striking and warrant further investigation.

1.2 Stereotactic Body Radiation Therapy

Stereotactic radiosurgery (SRS) and fractionated stereotactic radiotherapy (SRT) have become routine treatment options for patients with brain metastases.(14-19) When first introduced, the use of a limited number of large dose per fraction treatments in SRS and SRT challenged conventional wisdom in radiation biology and oncology.(20, 21) High fractional dose is more effective for radiation resistant cells. This is suggested by the good local control seen in hypofractionated radiation therapy for nodal metastases and radiosurgery for brain metastases in melanoma.(22-24) However, the risk of long-term normal tissue complication is correlated with the fraction size. In radiosurgery for brain metastases, the high conformality (and thus the avoidance of normal tissue) possible with radiosurgical techniques permits us to safely deliver single doses in the range of 15-20 Gy. Using prototypes for image-guided radiation therapy (IGRT),(25) stereotactic body radiotherapy (SBRT) for small sized lung cancers has been reported where hypofractionated therapy in fractional doses of 12-20 Gy appeared to be relatively safe and effective resulting in local control rates of 80-90%.(26)

The translation of SRS and SRT to extracranial sites was limited by two problems. First, tumors in the body are subject to motion related to natural physiological process like breathing and digestion. Second, because

the treatments are highly focused, image guidance needed to be such that the selected patients are properly treated with limited fields and that the target extent can be determined accurately. By the early 1990s, technological advances in both tumor motion tracking and image guidance allowed the concepts of SRS and SRT to be extended to extracranial sites where it is now known as SBRT. The American College of Radiology and the American Society of Therapeutic Radiology and Oncology have published guidelines that define SBRT and its proper conduct.(27) Patients selected for SBRT should have a limited number of demarcated tumors whose extent can be identified directly on treatment planning image platforms or reliably fused by image registration techniques. Some method of tumor motion control must be used to avoid large margin treatments.

SBRT is an ablative therapy, and it should be understood that targeted tissue is likely to be destroyed. Many beams are brought in from multiple directions so that entrance dose is spread out. Beams are shaped to achieve conformality and rapid dose fall-off. The SBRT dosimetry approach is borrowed from intracranial SRS in which treatments have been well tolerated with long term follow-up.(28)

1.3 High dose local radiation effects on immunity

Curing cancer with immunotherapy has had limited success despite testing many agents and combinations over the last 50 years. Prehn and Main performed one of the earliest experiments showing immunity against tumors in 1957. Mice inoculated with MCA-induced sarcoma cell lines quickly developed tumors and died. If the tumor was resected, then subsequent challenge with the same tumor cells was rejected and the mice survived.(29) This early observation clearly demonstrated that immunity against malignant cells exists. It also suggested that the initial tumor burden somehow suppressed the development of effective initial defensive immune assault, and that by removing the tumor burden, the barrier to effective immunity could be lifted. In clinical practice, surgical tumor debulking is often traumatic to the patient or just too impractical due to the location and/or number of lesions. SBRT can serve as a virtual “scalpel” to reduce tumor burden in patients.

The standard teaching is that radiation is immunosuppressive. However, recent studies have shown that exposure of tumor cells to a single or a few fractions of high dose radiation can augment the release of inflammatory cytokines such as IL-1(30) and TNF-α(31). It can also up-regulate expression of MHC class I(32), B7.1(33), and Fas/CD95(34, 35). Most of these studies *in vivo* and *in vitro* have utilized single radiation doses in the range of 5-10 Gy to induce this response. This fractional dose range is outside the scope of conventional radiation therapy and thus up to now the laboratory findings have not been easily translatable to the clinic. SBRT is a technology by which high fractional doses of radiation can be delivered to sites of metastases, and in conjunction with immunotherapy, may augment immune responses.

The evidence for this is suggested by Chakarborty et al.,(35) who demonstrated that treatment of carcinoembryonic Ag (CEA)-expressing MC38 adenocarcinoma cells with irradiation (20 Gy) *in vitro* enhanced Fas expression at molecular, phenotypic, and functional levels. Furthermore, radiation sensitized these targets to Ag-specific CTL killing via the Fas/Fas ligand pathway. Moreover, localized irradiation of the tumor *in vivo* significantly potentiated tumor rejection by these CEA-specific CTL. The same group later showed that in their model, mice cured of tumors not only demonstrated $CD4^+$ and $CD8^+$ T-cell responses specific for CEA, but also induced T-cell responses to two other antigens (gp70 and p53) overexpressed in the tumors.(36) This indicates epitope spreading induced by local irradiation. This hypothesis is corroborated by the results of Reits et al. who demonstrated that exposure of MC38 tumor cells to single doses of 7-25 Gy increases MHC class I expression, generates radiation injury-induced peptide formation, and makes tumors more susceptible to adoptively transferred cytolytic T cells (CTLs).(32) Lugades et al. have also shown that tumors in murine hosts exposed to three doses of 5-15 Gy per fraction have increased infiltration of tumor-specific CTLs.(37) For tumors that express low levels of antigen, 10 Gy increased antigen release by the tumors; the antigen subsequently was cross-presented by stromal cells making them susceptible to destruction by CTLs, which led to complete tumor rejection.(38)

If high local radiation dose *in vivo* can make tumor cells more immunogenic, then the tumor-specific CTLs that are generated could attack distant metastatic deposits. Chakravarty et al inoculated the hind limb of mice with 3LL/D122 Lewis lung cancer cell line. At week 3, 60Gy was administered. Compared to controls, animals given Flt3/L (dendritic cell maturation factor) developed fewer lung metastases and survived longer. This effect was not seen in nude mice that lack CTLs.(39)

More recently, Demaria et al. inoculated bilateral flanks of mice with 67NR mammary tumor cells and the rump with A20 lymphoma cells as controls. One of the flanks was irradiated with 6Gy and the mice were given Flt3/L. This study demonstrated that local high dose radiation plus Flt3/L induced a tumor-specific CTL response against the tumor located in the opposite flank but not the control tumor.(40)

One of the potential impediments to effective tumor immunotherapy is $CD4^+CD25^+$ foxP3 expressing T cells (T_{reg}) which are found in increased levels in cancer patients, suggesting that the T_{reg} number correlates to the stage of disease and cancer progression.(41-43) Depleting the $CD4^+CD25^+$ population with anti-CD25 mAb at the time of vaccination against a tumor-expressed antigen with the combination of local radiation therapy resulted in elimination of established tumors, whereas the combination of any other two out of three modalities did not eliminate established tumors despite augmenting CD4 and CD8 responses to antigens.(44) Similar augmentation of protective tumor immunity was seen in mice depleted of $CD4^+CD25^+$ population when mice were immunized with dendritic cells pulsed with irradiated tumor cells.(45)

One of the immunological effects of SBRT may be the elimination of $CD4^+CD25^+$ cells within the tumor and a decrease in their number or activity in the periphery. Because SBRT involves multiple beams traversing through the body and intersecting at the tumor of interest, the body receives low dose exposure. The effect of low dose exposure is unclear, but it is possible that this may preferentially augment immunity by suppressing the T_{reg} population. Animal data exist suggesting that this phenomenon exists: 1) 0.1 Gy TBI in mice given 6-15 hours prior to tumor transplantation increases TD50 of tumor cells(46); 2) 0.2 Gy TBI in rats transplanted with KDH-8 hepatoma cell lines decreases metastases to lung(47); 3) subset of Lyt-1,2^+ CTL can be killed *in vitro* with 0.1-0.25Gy(48); and 4) tumor bearing rats given 0.2Gy TBI (but not to tumor) increased IFN-γ and TNF-α expression in splenocytes, but did not increase IL4, IL6 or IL10.(49) Safwat et al have also shown that the combination of low dose TBI and IL-2 has a synergistic effect in a murine metastatic melanoma model.(50)

1.4 Rationale for Combining Radiation and IL-2

As stated before, IL-2 is the only medical therapy that has the potential to cure metastatic melanoma, albeit only in a small minority of patients. The reasons for the low probability of response are not completely understood despite greater than 25 years of investigation, but recent data show a strong link between Treg activity and response in patients receiving IL-2. For instance, Cesana and colleagues reported that melanoma patients have high baseline Treg and those responding to high-dose IL-2 have decreased Treg number while patients who do not respond have increasing numbers.(50) Similar data exist in RCC showing that Treg and myeloid-derived suppressor cells are elevated in many patients with metastatic disease and can decrease with successful treatment(51). Since radiation modulates Treg, it may counterbalance some of the inhibitory properties of IL-2 as IL-2 can also induce proliferation of Treg.

IL-2 also causes proliferation of T lymphocytes. If a population of melanoma antigen-specific cytotoxic T-cells is induced by radiation or radiation decreases the inhibitory influence of Treg, then IL-2 could induce proliferation of a T-cell subset with enhanced melanoma activity resulting in enhanced tumor regression.

1.4.1: **Clinical Experience with Radiation and IL-2**

Radiation has been used prior to IL-2 with no enhancement of IL-2 response(52). This study treated 14 melanoma and 12 renal cell cancer patients with 500 cGy BID to a total of 10 Gy- 20 Gy over 1-2 days, 2-24 hours prior to the initiation of IL-2 with no improvement in patient outcomes. There are several possible

reasons why this radiation and IL-2 combination was ineffective. The patients were treated between 1989-1990, likely via 2-D treatment planning (the details of the treatment plan were not defined by the authors). 2-D treatment plans generally overestimate the tumor volume, and often field sizes from this era were generous, possibly negating any synergistic effect from excess by-stander irradiation. Tumor draining lymph nodes are important in generating radiation-induced CTL tumor regression.(53) The wide margins from 2-D treatment planning techniques would likely include tumor draining lymph nodes as well, thus eradicating T cells and antigen-presenting cells that are instrumental to establishing an anti-tumor immune response. In addition, the fractional radiation dose was not adequate to eradicate the melanoma or renal cancer metastatic deposits treated. Most of the pre-clinical studies demonstrating the immune-enhancing effects of radiation utilized fraction sizes > 5 Gy.(31-33, 35, 37, 38) It is probable that higher fractional radiation doses are more immunogenic than lower doses as evidenced by abscopal tumor regressions that have been observed in patients with renal cell cancer treated with SBRT using fractions sizes of at least 8 Gy.(54)

We have maintained a database on patients receiving high-dose IL-2 since 1997 that encompasses over 1000 immunotherapy admissions to our Biotherapy Program at PPMC. We compiled an anecdotal experience in 8 melanoma patients and 2 renal cancer patients who had high-dose per fraction radiation for clinical palliation the week before starting IL-2. Three patients had gamma knife or stereotactic radiosurgery for brain metastases, three had treatment of painful bone metastases and four had pulmonary lesions treated to relieve bronchial obstruction. Five of the 8 patients with melanoma responded (PR = 2 and CR = 3) by RECIST criteria of the non-irradiated lesions for an overall response rate of 62.5%. The two patients with renal cancer achieved PR by RECIST with minor radiographic abnormalities. None of the responding patients has required additional therapy for their malignancy. Based on these anecdotal experiences and the scientific rationale detailed above, we conducted and recently completed a phase I study of SBRT and IL-2.

Our phase I study enrolled patients with metastatic melanoma or renal cell carcinoma who had received no prior medical therapy for their advanced disease and were not candidates for surgical resection. The main eligibility requirements were sufficient cardiopulmonary reserve to tolerate high-dose IL-2 and at least one pulmonary or hepatic metastatic deposit to which SBRT could be safely administered. Patients were enrolled in consecutive cohorts to 1, 2 or 3 doses of SBRT (20 Gy per fraction) with the last radiation dose 3 days before starting the first IL-2 cycle. IL-2 was administered at 600,000 international units per kg via intravenous bolus infusion every 8 hours for a maximum of 14 doses per cycle. A second cycle of IL-2 was repeated after a 2-week rest period. Four weeks after the second IL-2 cycle imaging studies were repeated and patients with regressing disease received up to 6 cycles of IL-2 therapy. Blood samples for immune monitoring were obtained at baseline, 3 days after SBRT (just before the first IL-2 dose) and 3 days after completing the first IL-2 cycle.

Twelve patients were included in the intent-to-treat analysis and 11 completed treatment per the study design. RECIST criteria were used to assess overall response in non-irradiated measurable disease. Eight out of 12 patients (66.6%) achieved a complete or partial response (1 CR and 7 PR). Six of the patients with PR on CT had a CR by PET imaging. Five out of 7 patients with melanoma had disease regression (71.4%, all PET CRs), 3 of 5 patients with renal cancer had a partial response (60%) and one had stable disease.(11) Tumor regression occurred at all SBRT dose levels and all lesions treated with SBRT regressed. Immune monitoring showed significantly greater proliferation of $CD4^{+}$T cells with a memory phenotype ($CD3^{+}CD4^{+}Ki67^{+}CD25^{+}FoxP3^{-}CCR7^{-}CD45RA^{-}CD27^{+}CD28^{+}$) in responding patients, a finding not described previously. The expected toxicities of high-dose IL-2 were observed, but there was no increase in the severity or frequency of IL-2 toxicity associated with the addition of SBRT. A maximum tolerated dose of SBRT was not reached.

The response rate SBRT and IL-2 in patients with melanoma was significantly higher than expected and among the highest observed in any early phase trial in metastatic melanoma. All of the visceral responses have been durable and no patient with a response has required subsequent medical therapy thus far

(although one required surgery for a brain metastasis, which in retrospect was present at the start of the SBRT + IL-2 treatment).

1.5 Rationale for Study Design

This randomized phase II study is designed to confirm the response rate of the SBRT + IL-2 compared to high-dose IL-2 with 80% power to detect a difference between the historical IL-2 response rate of 16% versus an assumed 60% response rate for SBRT + IL-2 (this is a more conservative assumption than the 71% response rate we observed in the completed phase I trial). Twenty-two patients will be enrolled to each treatment arm. All patients will receive IL-2 at 600,000 international units per kg IVB q8h x 14 planned doses with an additional cycle 14 days after the first (this is the current standard IL-2 regimen in our Biotherapy Program). Responding patients with regressing disease are eligible for up to 6 IL-2 cycles. Among the first 20 patients enrolled, SBRT will be administered as a single 20Gy dose of radiation on the Friday before IL-2 begins. Patients 21 through the completion of the study will receive two SBRT dose fractions based on an interim analysis of data obtained from the first 14 patients who completed treatment on the study. The salient findings from this interim analysis are as follows:

1) Serum uric acid on day 8 was significantly higher in patients receiving SBRT ($p < 0.005$), which indicates tumor break down as expected from radiation.

2) Although as a group the patients who received SBRT had higher uric acid values on day 8, two of the individuals who received SBRT has no change in uric acid and did not respond. This indicates that the goal of tumor destruction by radiation was not achieved in these individuals,

3) In contrast to the pilot study where all SBRT-treated lesions regressed, at least one of the patients in the interim analysis had regrowth of a radiated lesion,

4) The sum of the largest diameters of the tumor deposits treated with one fraction of radiation in the pilot study was significantly smaller than the sum of largest diameter of lesions treated with radiation in the interim analysis.

5) There were also significant differences in serum IL-12 and RANTES on day 8 comparing the SBRT and IL-2 groups in the interim analysis.

The findings of the interim analysis are consistent with our hypothesis that radiation causes tumor breakdown and re-directs inflammation to the site of the tumor. A single dose of radiation is not sufficient in all patients to induce the biological changes that our hypothesis suggests are necessary to facilitate a systemic anti-tumor effect from IL-2, therefore two SBRT fractions will be used for patients enrolled after patient 20 in the study accrual. This modification will also permit a more extensive comparison of the clinical and immunological effects of one versus two SBRT doses.

This SBRT dosing regimen was also based on our completed phase I trial that tested up to three SBRT doses of 20 Gy. We found no increase in IL-2 toxicity of IL-2 after SBRT and also found no difference in response by SBRT dose (data submitted for publication).

Patients assigned to the IL-2 arm who have melanoma progression after the first two IL-2 cycles (using RECIST criteria) are eligible for SBRT followed by 2 additional cycles of IL-2. Melanoma responses that occur in this crossover group will not be counted in the initial response tabulation, which is the primary objective of this study. Responses will be determined using modified response evaluation of solid tumors (RECIST) criteria 1.1 of lesions not treated by SBRT (detailed below). Extensive immunological monitoring will be performed in an attempt to determine the mechanism of antitumor activity of SBRT + IL-2.

2. OBJECTIVES

2.1 Primary objective:

2.1.1 Perform a randomized phase II study to compare the response rate of high dose IL-2 to SBRT + IL-2 in patients with metastatic melanoma**.**

2.2 Secondary objectives:

2.2.1 Measure the response of SBRT + IL-2 in patients with melanoma who have disease progression after high-dose IL-2.

2.2.2 Perform immunological monitoring to assess:

2.2.3 Proliferation of $CD4^+$T cells with a memory phenotype ($CD3^+CD4^+Ki67^+CD25^+FoxP3^-CCR7^-CD45RA^-CD27^+CD28^+$) after high-dose IL-2 and SBRT + IL-2.

2.2.4 Pro-calcitonin, DAMPs and other inflammatory mediators that may influence the interaction between SBRT and IL-2.

2.2.5 Ab response and correlate with gene expression by autologous tumor

2.2.6 T cell response to melanoma cell lines and antigens identified by protein array.

2.2.7 Biopsies of melanoma lesions before and after SBRT to study histological changes and immune cell infiltrates.

2.2.8 Compare the immunological and clinical effects of one versus two SBRT doses.

3. PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Histological confirmation of melanoma will be required by previous biopsy or cytology.

3.1.2 Patients must be ≥ 18 years of age.

3.1.3 Tumors amenable to SBRT in lungs (central locations preferred), mediastinum, chest wall, bones with a soft tissue component (other than long bones), or liver, liver hilum and associated lymph nodes (inclusive of immediately adjacent masses), **1 – 3 foci; no minimum size, but none greater than 7 cm**. Patients may have other metastases but only a maximum of 3 will be treated.

3.1.4 ECOG performance status of 0-1.

3.1.5 Women of childbearing potential must have a serum or urine pregnancy test performed within 72 hours prior to the start of protocol treatment. The results of this test must be negative in order for the patient to be eligible. In addition, women of childbearing potential as well as male patients must agree to take appropriate precautions to avoid pregnancy.

3.1.6 Patients must sign a study-specific consent form.

3.2 Exclusion Criteria

3.2.1 No metastatic site amenable to SBRT.

3.2.2 Patients with brain metastases not candidates for radiosurgery alone.

3.2.3 Previous radiation to sites proposed for SBRT.

3.2.4 Patients with active systemic, pulmonary, or pericardial infection.

3.2.5 Pregnant or lactating women, as treatment involves unforeseeable risks to the embryo or fetus.

3.2.6 Evidence of ischemia on exercise tolerance test, stress thallium study, or baseline EKG.

3.2.7 DLCO, FEV1 or FEV1/FVC less than 70% of predicted due to clinically significant underlying pulmonary disease. For any pulmonary function test values less than predicted values, the PI will review, and document the patient's suitability for high dose IL-2 therapy.

3.2.8 WBC < 3.0 x $10^{9/}$L, Hgb < 9.0 g/dL, AST/ALT > 3 times ULN, total bilirubin > 1.9 g/dL, creatinine > 1.9 g/dL.

3.2.9 Need for chronic steroids.

3.3 Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. Given the racial demographics of Oregon, 86.6% white, and the reduced incidence of melanoma in the non-white population, we expect that few of the patients enrolled will be non-white. The expected distribution of men and women enrolled is based on our experience with other clinical trials at our Cancer Center. The anticipated study population is illustrated in the table below.

Table 1.

	Race/Ethnicity					
Gender	White, not of Hispanic Origin	Black, not of Hispanic Origin	Hispanic	Asian or Pacific Islander	Unknown	Total
Male	24	0	0	0	0	24
Female	20	0	0	0	0	20
Total	44	0	0	0	0	44

4. REGISTRATION PROCEDURES

4.1 Registration and Cancellation Guidelines

To register a patient, the investigator will call the Data Management Office of the Robert W. Franz Cancer Research Center at (503) 215-2613 and speak to one of the Nurse Coordinators for the trial. The following information will be requested:

4.1.1 Investigator's name
4.1.2 Patient's Identification
4.1.3 Patient's name or initials and chart number
4.1.4 Patient's Social Security number
4.1.5 Eligibility Verification

Patients must meet all of the eligibility requirements and undergo all pre-study procedures.

If a patient enrolls in the study, but does not receive study therapy, the patient's enrollment may be canceled. Reasons for cancellation will be documented in writing. Any patient whose enrollment was canceled before receiving study therapy will be replaced. Any patient who enrolled in the study but did not receive SBRT or IL-2 will be replaced.

4.2 Assignment of Study Numbers

Study Numbers will be assigned at enrollment based on order of enrollment and study ID, as follows: SI-01, SI-02, SI-03, etc.

All case report forms, study reports, and laboratory samples for research tests, including immune parameters or pharmacokinetics, will be labeled with the full patient Study Number.

5. STUDY DESIGN

Eligible patients will be registered (see Section 4) and randomized to treatment arm using closed envelope method. The patients will receive SBRT radiation as specified in Section 7.

IL-2 treatment will begin on the Monday following the radiation treatment. The dose level of interleukin-2 will be the same for all enrolled patients (600,000 IU per kilogram IVB q8h x 14 planned doses with an additional cycle 14 days after the first cycle).

6. STUDY CALENDAR

		Study Treatment*					Follow Up	
Study Days	**Screening. -30 days**	**SBRT Tx [2]**	**Days 1-5**	**Day 8**	**Week 4 Days 22-26**	**Week 7 (+/- 1 wk)**	**Follow-up/restaging[7]**	**Survival F/U[7]**
Medical history[9]	X	X	X		X	X		
Physical exam	X	X	X		X	X	X	
Vital Signs weight	X		X		X	X	X	
CBC, Diff, plt	X	X	X	X	X	X	X	
Chemistry panel[1]	X		X	X	X	X	X	
TSH/T4	X						X	
PT/PTT	X							

Tumor Biopsy[8]	X			X				
Immunologic monitoring[6]	X	X	X	X	X	X		
ECG	X							
CT (chest/abd/pelvis)	X					X	X	
PET scan[3]	X						X	
PFTs[4]	X							
Brain MRI	X							
Pregnancy test	X							
Cardiac stress test[5]	X							

* The last SBRT dose will be on a Friday, with IL-2 administered the following Monday. Biotherapy Program guidelines will be used to manage IL-2-related toxicity.

1 Electrolytes, BUN/creatinine, LFTs, Magnesium Phosphorus. Procalcitonin, LDH and uric acid on Days 1, 2, 5, and 8

2 SBRT will precede cycle 1 IL-2 in patients assigned to the SBRT + IL-2 arm. For patients who are assigned to IL-2 monotherapy and have progressive disease after 2 IL-2 cycles, they can then receive SBRT before cycle 3 IL-2 commences.

3 PET scans are required after the completion of IL-2 and if clinically indicated at other time points.

4 Includes routine spirometry, lung volumes and diffusion capacity (within 8 weeks prior to study treatment).

5 For patients over 50 years of age or with significant cardiac risk factors.

6 Immunologic monitoring samples: collect baseline samples as follows: for patients randomized to receive radiation collect on the day radiation is to start before radiation is administered. For patient randomized to Il-2 alone collection of sample during screening is acceptable ensuring that patient is eligible for study treatment. For both arms collect samples on day 1 the day IL-2 starts (before IL-2 is administered), day 2 (at 9AM after planned dose 3 of IL-2), day 5, day 8 and at the investigators' discretion, day 22-26 sample collect any day during week 4.For patients who receive SBRT prior to cycle 3 of IL-2 (crossover patients), samples should be obtained on the same schedule. Refer to Appendix D for description of tubes to be collected at each time point

7 Patients will be followed every 3 months for 2 years, then every 6 months during year 3, and yearly thereafter for years 4 and 5 with the indicated tests (starting three months after the last Course of IL-2). For patients who progress, patient status will be followed for survival. Survival follow-up will consists of a follow-up phone call every 6 months to assess survival status of progressing patients from end of treatment to 5 years.

8 CT- or ultrasound-guided core biopsy of lesion to be treated with SBRT if technically feasible

9 Collect medical history at screening and adverse events grade 3 or higher throughout the study up to week 7 or 8 (IL-2 restaging), report only the highest grade for any reportable event.

10 To be collected at PI discretion as additional immunologic monitoring

7. ADMINISTRATION OF STUDY TREATMENTS

7.1 Radiation Therapy

7.1.1 Dose Specifications

Stereotactic Targeting and Treatment: The term "stereotactic" for the purposes of this protocol implies the targeting, planning, and directing of therapy using beams of radiation along any trajectory in 3-D space toward a target of known 3-D coordinates. This protocol will require treatments to be conducted with the use of a fixed 3-D coordinate system defined by fiducials. The coordinate system defined by the fiducials should be directly related to the radiation-producing device (e.g., couch and gantry) in a reproducible and secure fashion. Capability should exist to define the position of targets within the patient according to this same 3-D coordinate system. As such, the patient is set up for each treatment with the intention of directing the radiation toward an isocenter or target according to the known 3-D coordinates as determined in the process of treatment planning. The nature of the fiducials themselves may include radio-opaque markers or rods placed at known locations in a frame or fixed structure adjacent to the patient as well as use of the tumor itself as a fiducial (e.g. acquiring tomographic views of the tumor simultaneously with the treatment).

7.1.2 Dose Fractionation

Patients 1 – 20 will receive a single fraction of radiation. Patients 21 through the completion of the study will receive two fractions. The dose for all patients will be 20 Gy per fraction to the prescription line at the edge of the planning treatment volume (PTV) with

the last dose delivered on a Friday before IL-2 administration. For patients receiving two radiation doses, the doses can be administered on the Wednesday and Friday before IL-2 starts. Patients who are assigned to IL-2 monotherapy and have progressive disease after two IL-2 cycles are then eligible to receive SBRT before cycle 3 of IL-2 commences, single fraction for patients 1-20 and two fractions for patients 21- end of study.

The target dose is determined based on the study dose level and the normal tissue requirements specified in 7.1.11. Treatment at the allocated dose level is only permitted if the normal tissue criteria are met. If the normal tissue criteria are not met at that dose, treatment at a lower dose level is permitted, as long as the normal tissue constraints are met at the lower dose level. No dose lower then 15Gy is allowed. If normal tissue constraints are not met at this lowest dose permitted, then the patient will not proceed with radiation on study.

7.1.3 Premedications

Analgesic premedication to avoid general discomfort during long treatment durations also is recommended when appropriate. If a portion of the stomach or small intestine is treated, H2 blocker or proton pump inhibitors is recommended to attempt to decrease the chance of late GI bleeding

7.1.4 Physical Factors

Only photon (x-ray) beams of energies 4-10 MV will be allowed. Photon beam energies greater than 10 MV but not more than 15 MV will only be allowed for a limited number (≤ 2) beams that must travel more than a cumulative distance of 10 cm through soft tissue (not lung) to reach the isocenter.

7.1.5 Minimum Field Aperture (Field Size) Dimension

Due to uncertainties in beam commissioning resulting from electronic disequilibrium within small beam apertures, a minimum field dimension of 3.5 cm is required for any field used for treatment delivery for 3-D conformal techniques. It is understood that this may exceed the technical requirements for small lesions (< 2.5 cm axial GTV dimension or < 1.5 cm cranio-caudal GTV dimension). In such cases, the prescription dose is still prescribed to the edge of the defined PTV. This minimum field dimension does not apply to plans using IMRT, VMAT, or tomotherapy.

7.1.6 Patient Positioning

Patients will be positioned in a stable position capable of allowing accurate reproducibility of the target position from treatment to treatment. Positions uncomfortable for the patient should be avoided so as to prevent uncontrolled movement during treatments. A variety of immobilization systems may be utilized including stereotactic frames that surround the patient on three sides and large rigid pillows (conforming to patients external contours) with reference to the stereotactic coordinate system (see Section 7.1.1). All positioning systems must be validated and accredited by the treating physician prior to enrolling or treating patients on this trial. Patient immobilization must be reliable enough to insure that the Gross Tumor Volume (GTV) does not deviate beyond the confines of the PTV with any significant probability (i.e., < 5%).

7.1.7 Inhibition of Effects of Internal Organ Motion

Special considerations may be made to account for the effect of internal organ motion (i.e., breathing, etc.) on target positioning and reproducibility. Acceptable maneuvers include reliable abdominal compression, accelerator beam gating with the respiratory cycle, and active breath-holding techniques. All systems used to account for internal organ motion must be validated and accredited by the treating physician prior to enrolling or treating

patients on this trial. Internal organ inhibition maneuvers must be reliable enough to insure that the Gross Tumor Volume (GTV) does not deviate beyond the confines of the Planning Treatment Volume (PTV) with any significant probability (i.e., < 5%).

7.1.8 Localization

Isocenter port localization films (anterior/posterior and lateral) should be obtained at each treatment on the treatment unit (or patients should undergo a tomographic imaging study, e.g., cone-beam CT (CBCT), if available) immediately before treatment to ensure proper alignment of the geometric center (i.e.: isocenter) of the simulated fields. Verification CT scans and portal films may be taken at the discretion of the treating physician, but are not required for protocol participation.

7.1.9 Image Acquisition

Computed Tomography (CT) will be the primary image platform for targeting and treatment planning. The planning CT scans must allow simultaneous view of the patient anatomy and fiducial system for stereotactic targeting. Axial acquisitions with gantry 0 degrees will be required with spacing ≤ 3.0 mm between scans.

The target lesion will be outlined by an appropriately trained physician and designated as the gross tumor volume (GTV). All contours will be reviewed prior to treatment planning by the treating radiation oncologist and another radiation oncologist. Dr. Seung or Dr. Crittenden will review all treatment plans at all participating study sites. The treatment plan reviews by Drs. Seung or Crittenden can occur after SBRT is administered. **This primary target will not be enlarged for prophylactic treatment (including no "margin" for presumed microscopic extension); rather, only include abnormal CT signal consistent with gross tumor (i.e., the GTV = Clinical Target Volume, CTV)**. An additional 0.5-1.0 cm in the axial plane and in the longitudinal plane (cranio-caudal) may be added to the GTV to constitute the planning treatment volume (PTV). If 4D-CT is performed to generate the internal tumor volume (ITV), the ITV will may be expanded up to 3-5 mm (i.e.: ITV + 3 mm= PTV) uniformly. As multiple tumors may be treated, the respective volumes will be labeled as follows: GTV1 or ITV1, PTV1 for the first tumor; GTV2 or ITV2, PTV2 for the second etc. for all tumors treated up to a maximum of three tumors.

7.1.10 Dosimetry

Three-dimensional coplanar or non-coplanar beam arrangements will be custom designed for each case to deliver highly conformal prescription dose distributions. Non-opposing, non-coplanar beams are preferable. Typically, 7-10 beams of radiation will be used with roughly equal weighting. Generally, more beams are used for larger lesion sizes. When static beams are used, a minimum of 7 non-opposing beams should be used. For arc rotation techniques, a minimum of 340 degrees (cumulative for all beams) should be utilized. For this protocol, the isocenter is defined as the common point of gantry and couch rotation for the treatment unit. Field aperture size and shape should correspond nearly identically to the projection of the PTV along a beam's eye view (i.e. no additional "margin" for dose build up at the edges of the blocks or MLC jaws beyond the PTV). The only exception will be when observing the minimum field dimension of 3.5 cm when treating small lesions (see above). As such, prescription lines covering the PTV will typically be the 60-90% line (rather than 95-100%); however, higher isodoses (hotspots) must be manipulated to occur within the target and not in adjacent normal tissue. The isocenter in stereotactic coordinates will be determined from system fiducials (or directly from the tumor) and translated to the treatment record.

The treatment dose plan will be made up of multiple static beams or arcs as described above. The plan should be normalized to a defined point corresponding closely to the center of mass of the PTV (COM_{PTV}). Typically, this point will be the isocenter of the beam rotation; however, it is not a protocol requirement for this point to be the isocenter. Regardless, the point identified as COM_{PTV} must have defined stereotactic coordinates and receive 100% of the normalized dose. Because the beam apertures coincide nearly directly with the edge of the PTV (little or no added margin), the external border of the PTV will be covered by a lower isodose surface than usually used in conventional radiotherapy planning, typically around 80% but ranging from 60-90%. The prescription dose will be delivered to the margin of the PTV and fulfill the requirements below. As such, a "hot spot" will exist within the PTV centrally at the COM_{PTV} with a magnitude of total dose (Gy) times the reciprocal of the chosen prescription isodose line (i.e., 60-90%).

For purposes of dose planning and calculation of monitor units for actual treatment, correction for tissue heterogeneity is strongly encouraged.

Successful treatment planning will require accomplishment of all of the following criteria:

1) Normalization

The treatment plan should be normalized such that 100% corresponds to the center of mass of the PTV (COM_{PTV}). This point will typically also correspond (but is not required to correspond) to the isocenter of the treatment beams.

2) Prescription Isodose Surface Coverage

The prescription isodose surface will be chosen such that 95% of the target volume (PTV) is conformally covered by the prescription isodose surface, and 99% of the target volume (PTV) receives a minimum of 90% of the prescription dose.

3) High Dose Spillage

a) *Location*

Any dose greater than 105% of the prescription dose should occur primarily within the PTV itself and not within the normal tissues outside of the PTV. Therefore, the cumulative volume of all tissue outside of the PTV receiving a dose greater than 105% of prescription dose should be no more than 15% of the PTV volume.

7.1.11 Critical Organ Dose-Volume Limits

The following table lists maximum dose limits to a point or volume within several critical organs. **These are absolute limits, and treatment delivery that exceeds these limits will constitute a major protocol violation.** The dose in table 1 is listed as a single fraction for the one fraction treatment. Total dose and dose per fraction are listed in table 2 for 2-fraction treatment. These limits were extrapolated from RTOG using known tolerance data, radiobiological conversion models, norms used in current practice at academic centers, (55-61) and the experience of several years of irradiation using these large fractions at Indiana University (62-64) and centers in Sweden, Germany, and Japan (65-67), and the AAPM Task Group 101 report on SBRT.(68)

In order to verify each of these limits, the organs must be contoured such that appropriate dose volume histograms can be generated. Instructions for the contouring of these organs will follow below.

Table 1: Critical Organ Dose Volume Limits for One SBRT Dose

Organ	Volume	Dose
Spinal Cord	Any point	12 Gy
Esophagus*	Any point	15.4 Gy
Ipsilateral Brachial Plexus	Any point	17.5 Gy
Heart/Pericardium	Any point	20 Gy
Great Vessels	Any point	37 Gy
Trachea and Large Bronchus*	Any point	20 Gy
Skin	Any point	20 Gy
Rib**	Any point	20 Gy
Liver	D30% D50%	<12 Gy <7 Gy
Kidney	35% of total kidney (L+R)	<15 Gy
Small bowel	Any point	12.4 Gy
Stomach	Any point	12.4 Gy
Parallel Tissue	**Critical Volume (cc)**	**Critical Volume Dose Max (Gy)**
Lung (Right and Left)	1500 cc	7 Gy
Lung (Right and Left)	1000 cc	7.4 Gy
*Avoid circumferential radiation **If rib structure lies within PTV the limit may be exceeded		

Table 2: Critical Organ Dose Volume Limits for Two SBRT Doses

Organ	Volume	Dose (Gy)
Spinal Cord	Any point	14.6 (7.3 Gy/fx)
Esophagus*	Any point	16.8 (8.4 Gy/fx)
Ipsilateral Brachial Plexus	Any point	16 (8 Gy/fx)
Heart/Pericardium	Any point	20 (10 Gy/fx)
Great Vessels	Any point	30 (15 Gy/fx)
Trachea and Large Bronchus*	Any point	20 (10 Gy/fx)
Skin	Any point	22 (11 Gy/fx)
Rib**	Any point	24.6 (12.3 Gy/fx)
Liver	700 cc	9.6 (4.8 Gy/fx)
Kidney	35% of total kidney (L+R)	12.4 (6.2 Gy/fx)
Small bowel	Any point	16.8 (8.4 Gy/fx)
Stomach	Any point	14.8 (7.4 Gy/fx)
Parallel Tissue	**Critical Volume (cc)**	**Critical Volume Dose Max (Gy)**
Lung (Right and Left)	1500 cc	5.8 (2.9 Gy/fx)
Lung (Right and Left)	1000 cc	6.2 (3.1 Gy/fx)
*Avoid circumferential radiation **If rib structure lies within PTV the limit may be exceeded		

a. Contouring of Normal Tissue Structures

Spinal Cord: The spinal cord will be contoured based on the bony limits of the spinal canal. It should be contoured at least 10 cm above and below the superior and inferior extent of the PTV respectively.

Esophagus: The esophagus will be contoured starting at least 10 cm above and below the superior and inferior extent of the PTV respectively.

Brachial Plexus: The ipsilateral brachial plexus will for the purpose of this normal tissue constraint will include on the major trunks of the plexus and will be contoured using the subclavian and axial vessels as markers to identify the location of the plexus. It will originate at the bifurcation of the brachiocephalic trunk into the jugular subclavian veins and extend along the sublclavian vein to the axillary vein ending where the vein crosses the second rib.

Heart: The heart and pericardial sack will be contoured. The superior border will begin at the inferior aspect of the aortic arch and extend to the apex of the heart.

Trachea and Proximal Bronchial Tree: The trachea will be divided into two sections proximal and distal. The distal trachea will be included as a structure with the proximal bronchial tree.

Proximal Trachea: The proximal trachea should extend 10cm above the superior extent of the PTV or 5 cm superior to the carina (whichever is most superior) and continue inferiorly to 2 cm above the carina.

Proximal Bronchial Tree: This will include the inferior 2 cm of the trachea and the proximal airways on both sides. The following airways will be included: the distal 2 cm of the trachea, the carina, the right and left mainstem bronchi, the right and left upper bronchi, the bronchus intermedius, the right middle lobe bronchus, the lingular bronchus and the right and left lower lobe bronchi.

Whole lung: Both the right and left lungs should be contoured as one structure and as separate individual lungs. GTV should not be included in this structure. In most cases the dose limits will be met for the individual lung.

Proximal Bronchial Tree Plus 2 cm: Central lesion will be defined as lesions falling within the structure that is defined as 2 cm larger in all directions from the proximal bronchial tree. If the GTV (ITV) falls inside this structure, patients will be considered ineligible for this study.

Skin: The skin will be defined as the outer 0.5 cm of the body surface.

Rib: Ribs within 5 cm of the PTV will be contoured by outlining the bone and the marrow. The intervening soft tissue should not be contoured.

Liver: The liver is defined as the portion of liver not involved by gross tumor. There must be at least 1000 cc of normal liver. This may mean subtracting non-treated liver metastasis from the liver volume to get an accurate calculation of the functional liver that the patient has.

Stomach: The stomach will be contoured in its entirety

Kidney: Both the right and left kidney will be contoured in their entirety. They will be defined as 2 structures.

Small Bowel: The entire small bowel will be contoured 5 cm above and below the GTV.

7.1.12 Documentation Requirements

In general, treatment interruptions should be avoided by preventative medical measures and nutritional, psychological, and emotional counseling. Treatment breaks, including indications, must be clearly documented on the treatment record.

7.1.13 Dosimetry Compliance

Section 7.1.10 describes appropriate conduct for treatment planning dosimetry. Criteria for both major and minor deviations are provided in the table in Section 7.1.10. See section 7.1.11 for dose volume limits for specific organs and structures. Exceeding these limits by more than 2.5% constitutes a minor protocol violation. Exceeding these limits by more than 5% constitutes a major protocol violation.

7.1.14 Treatment Delivery Compliance

Set-up films will be compared to digitally reconstructed radiographs from the same beam's eye view. Deviations of less than 0.5 cm in the transverse plane and 1.0 cm in the cranio-caudal plane will be considered compliant. Deviations from 0.5-1.0 cm in the transverse plane and 1.0-1.25 cm in the craniocaudal plane will be considered minor protocol deviations. Deviations greater than those listed as minor will be considered major protocol deviations.

7.1.15 RT Quality Assurance Reviews

The Principal Investigator will perform an RT Quality Assurance Review as appropriate during the study.

7.1.16 Radiation Toxicity

<u>Radiation pneumonitis</u>

Radiation pneumonitis is a subacute (weeks to months from treatment) inflammation of the end bronchioles and alveoli. Any patient suspected of having radiation pneumonitis will be assessed by a medical oncologist, and radiation oncologist, as the clinical picture may be very similar to acute bacterial pneumonia with fatigue, fever, shortness of breath, non-productive cough, and a pulmonary infiltrate on chest x-ray. The infiltrate on chest x-ray should include the area treated to high dose, but may extend outside of these regions. The infiltrates may be characteristically "geometric" corresponding to the radiation portal, but may also be ill-defined.

Patients reporting symptoms as above will be promptly evaluated and treated. Mild radiation pneumonitis may be treated with non-steroidal anti-inflammatory agents. More significant pneumonitis will be treated with systemic steroids, bronchodilators, and pulmonary toilet after discussion with the biotherapy physician. Infections should be treated with antibiotics. Consideration of prophylaxis of opportunistic infections should be considered in immuno-compromised patients.

It is unlikely that symptomatic pneumonitis will occur during the weeks radiation is actually delivered to the patients. However, if a patient experiences pneumonitis prior to completing therapy, therapy should be put on hold until symptoms resolve. At that point, a clinical decision whether to finish therapy will be made.

<u>Bronchial Injury</u>

In the Indiana University, the majority of patients treated at doses of 20 Gy times 3 fractions = 60 Gy or higher ultimately experienced atelectasis (collapse) of lung

downstream from the area of treatment(69). This was felt to be related to injury of bronchi or bronchioles within or near the treated tumor. By unknown mechanisms over a period of 3-6 months, damage to pulmonary parenchyma distal to the site of bronchial injury results in this focal lung collapse. In the majority of patients, this effect noted on imaging studies was asymptomatic. In others, the injury appeared to correlate with a drop in diffusing capacity and arterial oxygen tension on pulmonary function tests. This process of collapse was not reversible in the Indiana University experience. This injury is the justification for the fractional dose for central and hilar tumors from this protocol so as to avoid substantial (or total) lung collapse.

Bronchial injury with subsequent focal collapse of lung may impair overall pulmonary status. It also makes further assessment of tumor response more difficult as the collapsed lung approximates the treated tumor. Since atelectatic lung and tumor have similar imaging characteristics, radiology reports will often describe the overall process as progressive disease while the actual tumor may be stable or shrinking. Investigators are referred to the strict criteria for local failure in Section 8.1.1 of this protocol to avoid such mischaracterization.

The consequences of bronchial toxicity, e.g., cough, dyspnea, hypoxia, impairment of pulmonary function test parameters, pleural effusion or pleuritic pain (associated with collapse), should all be graded according to the NCI Common Terminology Criteria For Adverse Events (CTCAE) version 4.0.

Liver Injury

Radiation therapy should be held at any point in the protocol for CTCAE v4.0 hepatic adverse event Grade 4. It is expected that a proportion of patients treated for right lower lobe lung or liver lesions will have transient elevation of liver enzymes following treatment. If elevation of liver enzymes is observed up to Grade 3 levels, more frequent measurements (at least twice weekly) of the liver enzymes are recommended until the enzymes stabilize or return to baseline levels. Repeat of all Grade 4 blood work is required at least 5 days following the first abnormal lab value to determine if the Grade 4 levels are transient (defined here as < 5 days) or persistent.

Radiation Induced Liver Disease (RILD) is a clinical syndrome of anicteric ascites, hepatomegaly and elevation of alkaline phosphatase (ALP) relative to other transaminases that may occur 2 weeks to 3 months following radiation to the liver. ALP must be at least 2-fold increased above the baseline ALP. In this setting, due to the difficulty in distinguishing RILD from disease progression, if ascites develops at any time within 3 months following treatment, an abdominal CT and paracentesis with pathological evaluation of the ascitic fluid is required. If the ascitic fluid does not reveal malignancy and there is no evidence of disease progression in the liver or abdomen, it will be assumed that RILD has occurred. If disease progression in the liver or abdomen has occurred, no diagnosis of RILD can be made. Treatment for RILD with repeat paracenteses, diuretics (Spironolactone), and close follow-up is recommended. For ease of reporting, any patient with a Grade 3 or higher ALP (5-fold increase above upper limit of normal) in the presence of ascites and absence of disease progression will be labeled as having RILD. In patients who have elevation of liver enzymes near Grade 4 levels and/or in patients with early non-specific signs or symptoms of liver injury, close follow-up is recommended with repeat blood work. If no tumor progression is documented in these patients, liver injury will be presumed to be treatment related.

7.2 Immunotherapy

7.2.1 Schedule

IL-2 shall commence on the Monday following completion of SBRT, administered at a dose of 600,000 IU per kilogram IVB every 8 hours for a maximum of 14 doses each cycle. The second cycle is planned 16 days after cycle 1 but may be delayed up to one week to allow toxicity to resolve. The maximum number of IL-2 doses that can be administered during two cycles (one course) will be 28 doses.

7.2.2 Administration

The IL-2 (dose = 600,000 IU /kg) will be diluted in 50 ml of D5W and administered over 15 minutes.

7.2.3 Venous Access Catheters

Patients will have a triple lumen central venous catheter or PICC placed prior to initiating each course of therapy. Central venous catheters will be placed preferentially in the subclavian vein. Catheters should be removed at the end of each treatment cycle. Patients will receive antibiotic prophylaxis with cephalexin 250 mg PO every 8 hours, or cefazolin 1 gm IV every 12 hours (or appropriate alternative for patients with allergies to cephalosporins) while the central catheter is in place. If catheter related bacteremia develops, the catheter should be removed and parenteral antibiotic treatment (e.g. Vancomycin 1 gm IV q 12-24 hours depending on renal function) may be required.

7.2.4 Concurrent Therapy

Steroids will not be permitted unless cleared by the biotherapy physician.

Patients should discontinue any antihypertensive therapy at least 24 hours prior to initiating each cycle of IL-2.

Supportive medications will be given according to institutional standard of care.

7.2.5 Additional Immunotherapy

- Patients assigned to the SBRT + IL-2 treatment who achieve a complete or partial response or stable disease by RECIST criteria may receive up to 2 more courses of IL-2 under institutional standard of care (6 IL-2 cycles total).
- Patients assigned to the IL-2 arm who have disease progression after cycle 2 and then receive SBRT before cycle 3 of IL-2 will receive a maximum of 6 IL-2 cycles if disease regression or stability is documented thereafter.
- Treating physicians will follow the institutional standard of care for patients who continue on IL-2 treatment for additional courses (after Course 1) and for follow up after treatment is completed.
- IL-2 patient treatment information and follow up will be tracked as appropriate in the institution's IL-2 database. This database will be used to compile patient treatment response, complications and follow up.

7.3 Study Treatment Discontinuation

All reasons for discontinuation of treatment must be documented. All patients will be followed for survival or until death post-treatment.

7.3.1 Criteria for Removal from Study Treatment

- Disease progression after SBRT + IL-2 or the follow up period; the patient should be re-staged and sites of recurrence and/or progression documented.
- Unacceptable toxicity
- The patient may elect to withdraw from study treatment at any time for any reason.
- Development of intercurrent, non-cancer related illnesses that prevent either continuation of therapy or regular follow up.

8. MEASUREMENT OF EFFECT

Patients should be reevaluated for response at the completion of Course 1 of IL-2 treatment (Week 7), and after each subsequent IL-2 course. After completion of IL-2, imaging shall be done every 12 weeks until best response and as clinically indicated thereafter. Baseline scans shall be done within 28 days of starting IL-2.

8.1 Evaluation of SBRT Target Lesions

SBRT target lesions, defined as the lesions treated with SBRT, will be evaluated with a modified version of the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1(70). Additional definitions beyond the RECIST 1.1 guidelines specific to this protocol are incorporated to define local control as described in Section 8.1.1.

The longest diameter (LD) for *the SBRT target lesion* will be calculated **from the treatment planning CT scan** using appropriate tissue-specific windowing and reported as the baseline LD. The baseline LD will be used as reference by which to characterize the objective tumor. For follow-up assessment, diagnostic CT scans performed using a 5 mm contiguous reconstruction algorithm using pulmonary windowing taken as part of scheduled protocol follow-up are preferred as the method of evaluation for response. When CT scans are not available, chest x-ray determination will be allowed as long as the target lesion is clearly visible.

Local treatment effects in the vicinity of the tumor target may make determination of tumor dimensions difficult. For example, bronchial or bronchiolar damage may cause patchy consolidation around the tumor that over time may coalesce with the residual tumor. In cases where it is indeterminate whether consolidation represents residual tumor or treatment effect, it should be assumed that abnormalities are residual tumor.

8.1.1 Response Criteria

Local Enlargement **(LE)**	At least a 20% increase in the LD of target lesion, taking as reference the smallest LD recorded since the treatment started; Ideally, this determination will be made based on CT image evaluation.
Local Failure **(LF)**	Refers to the primary treated tumor after protocol therapy and corresponds to meeting both of the following two criteria: 1) Increase in tumor dimension of 20% as defined above for local enlargement (LE); 2) The measurable tumor with criteria meeting LE should be avid on Positron Emission Tomography (PET) imaging with uptake of a similar intensity as the pretreatment staging PET, OR the measurable tumor should be biopsied confirming viable carcinoma. The EORTC criteria for post-treatment PET evaluation will be used as a basis for evaluation in cases more difficult to assign as to whether the uptake is pathological for cancer recurrence vs. inflammation.
Local Control **(LC)**	The absence of Local Failure.

8.2 Evaluation of Systemic Disease Response

Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, Version 1.1(70). Changes in only the LD (unidimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below.

8.2.1 Definitions

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (LD to be recorded) as ≥20 mm with conventional techniques (CT, MRI, x-ray) or as ≥10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target lesions: All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the LD for all target lesions will be calculated and reported as the baseline, which will be used as reference by which to characterize the objective tumor response.

Non-target lesions: All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

8.2.2 Guidelines for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. 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. Tumor lesions that are situated in a previously irradiated area will not be considered measurable, unless there is clear evidence of progression on physical exam or imaging studies.

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 is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US): When the primary endpoint of the study is objective response evaluation, US should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy, Laparoscopy: Can be useful to confirm complete pathological response when biopsies are obtained, but will not be used for tumor measurements.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific additional criteria for standardized usage of prostate-specific antigen (PSA) and CA-125 response in support of clinical trials are being developed.

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

8.2.3 Response Criteria

Evaluation of Target Lesions	
Complete Response **(CR**):	Disappearance of all target lesions or no abnormal FDG uptake on PET.
Partial Response **(PR):**	At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD
Progressive Disease **(PD)**	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
Stable Disease **(SD)**	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of Non-Target Lesions	
Complete Response **(CR**):	Disappearance of all non-target lesions and normalization of tumor marker level
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)**	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the PIs.

8.2.4 Evaluation of Best Overall Response

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

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

Note:
Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic

deterioration." Every effort should be made to document the objective progression, even after discontinuation of treatment.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

8.2.5 Confirmatory Measurement/Duration of Response

Confirmation:

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed between 4 and 8 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of eight weeks.

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.

9. REGULATORY AND REPORTING REQUIREMENTS

9.1 Adverse Event Reporting

9.1.1 Definitions

Serious adverse event:

A serious adverse drug experience is defined as any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or 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.

Unexpected adverse event:
Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or

severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

Associated with the use of the drug / intervention:
There is a reasonable possibility that the experience may have been caused by the drug.

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

Life-threatening adverse event:
Any adverse drug experience that places the patient or subject, in the view of the investigator, 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.

Unanticipated Problem

An unanticipated problem is an adverse event that is (i) unexpected; (ii) serious; and (iii) felt by the investigator to be possibly, probably, or definitely related to the research intervention. Only adverse events that meet this definition need be reported to the IRB.

For more information on the definition of an unanticipated problem and reporting requirement, consult the current PH&S AE Guidelines published on the IRB website (http://in.providence.org/or/departments/reviewboard/Pages/default.aspx).

9.1.2 Reporting

PH&S IRB:
An unanticipated event that is serious and definitely or probably caused by the study treatment (drugs or device) will be reported to the IRB in accordance with their guidelines and within their timelines. Collaborating sites must comply with all local IRB requirements and copies of all IRB approvals from these sites must be provided by the site's clinical research staff to the PH&S Regional Research Office.

9.2 Data Reporting

Clinical data will be recorded on study-specific case report forms (CRFs), which will be provided by PH&S clinical research staff. All forms must be legible and complete. Black ink must be used in completing these forms. All corrections must noted with a single line strike though inaccurate entries and will be initialed and dated. All data entries to CRFs must be supported by a clinical source document. No direct entry of patient data to the CRF is permitted.

9.3 Continuing Review and Final Reports

An annual progress report (continuing review) will be submitted to the IRB of record for the duration of the study. A final report to the IRB of record will be submitted at the summation of the study.

9.4 Protocol Modifications and Amendments

All modifications or amendments to the protocol or informed consent document must be approved by the Principal Investigator and submitted to the Providence Health & Services Regional Institutional Review Board (IRB) for review and approval. All modifications and amendments will be

documented with a new version number and date. All changes to the informed consent document will include the date of the revision on the form.

No changes will be implemented until IRB approval is obtained except when a potential threat to patient safety exists.

The IRB will be notified of any significant deviations from the approved protocol. Documentation of all IRB correspondence will be maintained in the central regulatory file according to section 10.5.

Collaborating sites must comply with all requirements of the IRB of record, and copies of all IRB approvals from these sites must be provided by the site's clinical research staff to the PH&S Regional Research Office.

9.5 Record Retention

According to 21 CFR 312.62(c), the investigator shall retain required records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated. If no application is to be filed or if the application is not approved for such indication, the investigator shall retain these records until 2 years after the investigation is discontinued or the IND is withdrawn and the FDA is notified.

The investigator must retain protocols, amendments, IRB/IBC approvals, completed, signed, dated consent forms, patient source documents, case report forms, quality monitoring reports, drug accountability records and all documents of any nature regarding the study or patients enrolled. All records will be maintained under restricted access by the Clinical Trials Department at Providence Portland Medical Center while the study remains active. Records may be placed in long-term storage after the study is completed. The location of long-term storage will be secure and easily accessed for regulatory purposes.

9.6 Quality Assurance Plan

The Providence Health System Quality Assurance (QA) plan for cancer clinical trials comprises Standard Operating Procedures (SOPs) that require ongoing review of activities associated with all investigator-initiated trials including protocol compliance, accuracy of data and safety of participants.

9.6.1 Study Monitoring

Study monitoring activities (Quality Control Reviews) are performed by a Contract Research Organization (CRO) or clinical research staff members who have completed specialized training in study monitoring procedures and human subjects' protections. Individuals who perform study monitoring activities do not report to Principal Investigators or research scientists and may not monitor studies for which they have direct responsibility.

Study monitoring activities are conducted regularly and include (but are not limited to) review and verification of the following:

- Eligibility
- Informed Consent process
- Adherence to protocol treatment plan
- Case Report Forms (CRFs)
- Source Documentation
- Adverse Events
- Regulatory Reporting

Results of study monitoring activities will be reported to applicable study personnel, the Clinical Trials Manager and Quality Assurance.

9.6.2 Quality Assurance

Quality Assurance (QA) personnel review study monitoring reports and if necessary, determine follow-up actions to resolve significant findings. QA has the authority to request immediate corrective action if significant patient safety issues are identified.

QA will track and trend results from study monitoring reports as well as associated corrective and preventive actions. A QA summary report will be provided to the IRB at the time of continuing review.

QA personnel do not have a direct reporting relationship to the Principal Investigator and are not responsible for enrollment or coordination of care for study participants.

9.6.3 Plans For Assuring Accuracy Of Data

All case report forms will undergo quality assurance review. All quality assurance reviews will include verification of the accuracy and integrity of data entered to case report forms. Incorrect data will be identified and corrected. The existence of adequate source documents for all data will be verified. All annual reports (continuing reviews) or publications will be reviewed by a staff person not associated with patient care coordination, data completion and submission, or writing such reports.

10. STATISTICAL CONSIDERATIONS

10.1 Study Endpoints

The primary study endpoint is to determine the best overall tumor response of high dose IL-2 versus SBRT + high dose IL-2 using RECIST criteria applied to all target and non-target lesions with the exclusion of sites treated with SBRT. For patients who have SBRT after progression on IL-2 monotherapy, the response rate will be recorded, but not counted as a response for the primary objective. There will be a comparison of the overall tumor response of patients receiving one versus two SBRT doses.

The secondary objectives are hypothesis generating and have not been included in determining the sample size. These include tests of the proliferation of $CD4^+$T cells with a memory phenotype ($CD3^+CD4^+Ki67^+CD25^+FoxP3^-CCR7^-CD45RA^-CD27^+CD28^+$) by flow cytometry, exploratory studies of pro-calcitonin, DAMPs and other inflammatory mediators that may influence the interaction between SBRT and IL-2 analysis of antibody response and correlations with gene expression by autologous tumor, characterization of T cell response to melanoma cell lines and antigens identified by protein array and study of biopsies of melanoma lesions before and after SBRT. Descriptive statistics will be used in the analysis of these hypothesis-generating studies.

10.2 Sample Size

Our sample size determination is based on our initial phase I study in which we observed a 71% response rate. The 95% confidence interval (Clopper-Pearson) for a response rate of 5 CRs out of

7 patients is (29%, 96%). If the high-dose IL-2 group has a response proportion of 0.16 based on the published literature versus a response proportion of 0.70 in the SBRT + IL-2 group, then15 patients **in each group** (for a total of 30 patients) would have 80% power with a significance level of 0.05 to detect the stated difference with the Pearson Chi-Square test with continuity correction. The table gives the sample size per group (Two Group Test) to detect difference in response 80% power, α=0.05, Response Proportion of 0.16 vs. Column Heading and using Pearson Chi-Square test with continuity correction.

Proportion Responders in SBRT + IL-2 Group				
.50	**.60**	**.70**	**.80**	**.90**
34	22	15	11	8

For this study we will assume a more conservative response rate of 60% in the SBRT + IL-2 group. This will require 22 patients to be treated in each group (44 total) to achieve an 80% power.

10.3 Patient Accrual

The IL-2 service currently treats approximately 3 melanoma patients per week. In addition to the IL-2 service, Providence Cancer Center enrolls an average of two melanoma patients per month to clinical trials. It is estimated that we can enroll one patient per month to this trial and can complete accrual within one year.

10.4 Immunological Monitoring

As detailed in the study schema, samples for immunological monitoring will include blood and melanoma tumor biopsies. The main objectives of the monitoring will be to characterize circulating T-cell subsets.

The immunological monitoring lab will perform flow cytometry with a panel of markers including CD3, CD4, CD8, CD27, CD28, CD95, Ki-67, CD25, CD127, CCR-7, FoxP3, and CD45RA. T cell sub-populations of interest include T_{reg}, central memory and effector cells. This polychromatic flow assay will be used to identify proliferating T cells.

T cell response to melanoma cell lines and antigens identified by protein array will be analyzed using a bank of 245 melanoma specimens that were triple enzyme digested and cryopreserved at the EACRI in Dr. Fox's lab. We will start by evaluating whether post treatment aphereses samples recognize HLA-matched melanoma cells. If we identify patients with detectable responses against matched melanoma cells we will then evaluate whether the same response exists in the limited number of pretreatment cryopreserved PBMC.

Markers of tumor lysis, inflammation and immune activation will be explored by measuring serum lactate dehydrogenase, uric acid and phosphate and test each of these as surrogate markers of cytotoxicity-mediated antigen release. We will also measure serum levels of danger associated molecular patterns (DAMPs) and pro-calcitonin. Radiation therapy has been shown to result in release of DAMPs and these DAMPs may be important immune adjuvants that influence the response to chemotherapy and radiation. Additional evaluations of the immune response will include a phenotypic analysis of changes in myeloid, T, B and NK cell populations, over time. We will measure Treg (natural versus induced) and Treg activation status via a flow cytometry panel that includes CD3, CD4, Ki-67, CD25, FoxP3, PD-1, CTLA-4,

ICOS, CD134. HELIOS and ICOS. The NK/ B cell panel includes CD3, CD8, CD25, CD56, NKG2A, NKG2D, KLR1, CD19. The MDSC panel includes, CD14, CD15, CD45 and HLA-DR.

We will also examine the frequency of tumor-specific T cells in collaboration with Dr. Ton Schumacher at The Netherlands Cancer Institute. HLA typing will be performed using DNA from the first immune monitoring sample. Using these results, de-identified samples from HLA-A2+ patients will be shipped to the Netherlands Cancer Institute for analysis. Using a novel high throughput technology, researchers will screen patient material for reactivity against very large panels of melanoma-associated epitopes using peptide MHC multimers. Clinical data (without PHI) may also be shared (e.g., treatments, outcomes, lab/scan results, etc.)

It is also possible that additional collaborations with other outside researchers will be established. De-identified biospecimen samples and clinical data (without PHI) may be shared in those efforts as well."

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APPENDIX A
ECOG PERFORMANCE SCALE

0 Fully active, able to carry on all predisease activities without restriction (Karnofsky 90-100).

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. For example, light housework, office work (Karnofsky 70-80).

2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours (Karnofsky 50-60).

3 Capable of only limited self-care, confined to bed or chair 50% or more of waking hours (Karnofsky 30-40).

4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair (Karnofsky 10-20).

5 Death (Karnofsky 0)

APPENDIX B
AJCC Staging for Melanoma and Renal Cell Carcinoma

Melanoma:

Primary Tumor (T)

TX Primary tumor cannot be assessed.
T0 No evidence of primary tumor.
Tis Melanoma *in situ*
T1 Melanoma ≤ 1.0 mm in thickness with or without ulceration
T1a Melanoma ≤ 1.0 mm in thickness and level II or III, no ulceration
T1b Melanoma ≤ 1.0 mm in thickness and level IV or V or with ulceration
T2 Melanoma 1.01-2mm in thickness with or without ulceration
T2a Melanoma 1.01-2mm in thickness, no ulceration
T2b Melanoma 1.01-2mm in thickness, with ulceration
T3 Melanoma 2.01-4mm in thickness, with our without ulceration
T3a Melanoma 2.01-4mm in thickness, no ulceration
T3b Melanoma 2.01-4mm in thickness, with ulceration
T4 Melanoma > 4mm in thickness with or without ulceration
T4a Melanoma > 4mm in thickness, no ulceration
T4b Melanoma > 4mm in thickness, with ulceration

Regional Lymph Nodes (N)

NX Regional lymph nodes cannot be assessed.
N0 No regional lymph nodes metastasis
N1 Metastasis in one lymph node
N1a Clinically occult (microscopic) metastases
N1b Clinically apparent (macroscopic) metastases
N2

Distant Metastasis (M)

MX Distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis present
Note: M1 includes separate tumor nodule(s) in a different lobe (ipsilateral or contralateral)
STAGE GROUPING
Occult Carcinoma TX N0 M0
Stage 0 Tis N0 M0
Stage IA T1 N0 M0
Stage IB T2 N0 M0
Stage IIA T1 N1 M0
Stage IIB T2 N1 M0
T3 N0 M0
Stage IIIA T1 N2 M0
T2 N2 M0
T3 N1 M0
T3 N2 M0
Stage IIIB Any T N3 M0
T4 Any N M0
Stage IV Any T Any N M1

APPENDIX C
Management of Interleukin-2 Toxicity

A. Dose Modification for Toxicity

Modification of the treatment protocol will occur by withholding doses of IL-2 rather than continuing therapy at a reduced dose.

Dose of IL-2 may be withheld for:

- hypotension refractory to fluids and pressors or requiring unacceptably high pressor doses (≥ 100 mcg neosynephrine);
- anuria for>24 hours and unresponsive to fluid replacement and low-dose dopamine;
- respiratory distress requiring oxygen>4 liters to maintain O_2 saturation>95%;
- confusion (mental status changes can progress to paranoia despite discontinuation of IL-2; it is imperative that the IL-2 be stopped at any sign of persistent confusion or disorientation);
- sustained ventricular tachycardia or any sign or symptom of myocardial ischemia or myocarditis. Patients experiencing sustained ventricular tachycardia or myocardial ischemia should not receive further treatment with IL-2;
- metabolic acidosis with HCO_3<18, despite attempts to correct with IV HCO_3;
- atrial fibrillation or myocarditis;
- documented systemic infection;
- any other serious toxicity that is not controlled at time of next dose.

B. Specific Toxicity Management

Several treatment-related toxicities have been uniquely associated with the administration of high-dose IL-2. Recommendations for management of the more significant toxicities typically seen with high-dose bolus IL-2 are as follows (the following are only suggested guidelines for toxicity management):

B1 Fluid Replacement

Excessive fluid replacement will increase the patient's likelihood of developing pulmonary edema. Fluid replacement with normal saline at 75 ml/hr IV may be required for patients who do not have adequate oral intake or who experience significant diarrhea. Once patients have gained greater than 5% of baseline weight, additional fluid boluses should not be given to maintain blood pressure. Lasix should not be given unless symptomatic fluid retention develops and blood pressure is adequate.

B2 Hypotension

Administration of high dose IL-2 leads to decreased peripheral vascular resistance and consequent hypotension. In order to manage this toxicity, the physician may utilize the following guidelines:

1. Monitor patients in a setting capable of providing vasopressor support.

2. Prior to starting IL-2 therapy determine a minimum tolerated blood pressure (MTBP). For patients under age 40 and with no prior history of ischemic or valvular heart disease, the MTBP may be a systolic blood pressure (SBP) of 80 mmHg, while the MTBP for all other patients should be a SBP of 85-90 mmHg based on perceived risk of cardiac toxicity.

3. When a patients systolic BP falls below the MTBP, suggested therapy may involve (in the following order):
   a) Begin fluid boluses (250cc normal saline IV over 15 min. may repeat x 2) until SBP is $\geq$ MTBP.
   b) Should fluid boluses fail make the SBP$\geq$MTBP, treatment with neosynepherine should also be instituted (1-4 ug/kg/min) to maintain SBP ≥ MTBP.

B3 Management of Arrhythmias/Myocarditis

If significant arrhythmias occur at any stage in the patient's treatment (whether on pressor agents or not), the possibility of myocardial ischemia/infarction must be excluded by both EKG and cardiac enzyme assessment. Patients who develop atrial fibrillation should have IL-2 doses held. Therapy may resume when the patient converts to normal sinus rhythm and is hemodynamically stable. If a significant supraventricular arrhythmia occurs while a patient is on dopamine therapy; neosynepherine should also be substituted for dopamine as initial blood pressure support. Patients experiencing sustained ventricular tachycardia or documented myocardial ischemic episodes during therapy should not receive further treatment with IL-2. Patients with myocarditis may resume treatment in subsequent cycles if CPK returns to normal. A cardiac ECHO documenting normal cardiac function should be performed prior to restarting therapy.

B4 Management of Neurotoxicity

Doses are held rather than reduced for neurotoxicity. If Grade 4 neuro-cortical toxicity is encountered and is not reversible within 48 hours, no further treatment should be given and the patient should be removed from the study. If Grade 4 toxicity is reversible to Grade 1 within 48 hours, future treatment may be considered (in subsequent cycles) if the patients shows and evidence of tumor regression.

B5 Metabolic Acidosis

During IL-2 therapy, when a patient's HCO_3 falls to below 20, $NaHCO_3$ should be added to the maintenance IV infusion. Should the HCO_3 level fall below 18, IL-2 therapy should be held, and bolus infusions of $NaHCO_3$ should be instituted. IL-2 therapy may resume if repeat HCO_3 $\geq$ 18.

B6 Pulmonary Toxicity

Pulse oximetry and clinical exam are routinely used at the time of each IL-2 dose to ascertain if the patient has sufficient pulmonary reserve to proceed with the planned dose. Due to the concern that pulmonary toxicity may be exacerbated by the combination of SBRT and IL-2, routine chest radiography and DLCO will also be used in IL-2 dose decisions. IL-2 doses will be held if the CXR shows an effusion greater than 1/3 the vertical dimension of the lung, if pneumonitis is present in areas outside those treated by SBRT or if DLCO is less than 75% on the first day of each IL-2 cycle.

B7 Other Toxicities

Increases in the serum creatinine to 2.0-3.5 mg/dl and total bilirubin to 3.0-10.0 mg/dl are common and reversible upon cessation of treatment. Doses of IL-2 have not generally been withheld for renal and hepatic dysfunction alone. Although toxicity may become severe, recovery usually occurs following cessation of IL-2 and vigorous supportive care is warranted. Additional IL-2 toxicity management guidelines are detailed in the Biotherapy Program Standard Operating Procedures.

C. Management of Grade 4 Toxicity

Patients with Grade 4 (Life Threatening) toxicity may be treated with dexamethasone 4 mg qid until side effects improve to an acceptable level. In the clinical trials with IL-2, dexamethasone has been used to treat patients with pulmonary edema requiring assisted ventilation, although many patients may be managed successfully without steroids.

D. Toxicity Criteria for Discontinuing Treatment

Patients will not be considered for further therapy if the following toxicities are encountered:

1. Pulmonary toxicity requiring endotracheal intubation not resolved within 72 hrs (to Grade1)
2. Renal dysfunction requiring dialysis not reversible within 72 hrs
3. Grade 4 cardiac dysrhythmia or Grade 2 or 3 dysrhythmia not easily controlled with medical management.
4. Myocardial ischemia (Grade 3 or 4) or infarction or symptomatic myocarditis (note: asymptomatic CPK or CPK-MB band elevations without EKG changes are not a contraindication to further treatment).
5. Coma
6. Life-threatening sepsis
7. Pericardial tamponade
8. Bowel ischemia or perforation
9. Any other severe or life-threatening toxicity which, in the opinion of the investigator, would preclude further treatment with these agents, or has not resolved to baseline 8 weeks after treatment.

APPENDIX D
Immunologic Monitoring Schedule

Baseline: One 3cc lavender top tube
Ten 10 cc green top tubes
One 10 cc tiger top tube

Day 1: One 3cc lavender top tube
Three10 cc green top tubes
One 10 cc tiger top tube

Day 2: One 3cc lavender top tube
Three10 cc green top tubes
One 10 cc tiger top tube

Day 5 One 3cc lavender top tube
Three10 cc green top tubes
One 10 cc tiger top tube

Day 8: One 3cc lavender top tube
Three10 cc green top tubes
One 10 cc tiger top tube

Week 4: One 3cc lavender top tube
Three10 cc green top tubes
One 10 cc tiger top tube

Week 7 (+/- 1 week) if pheresis product cannot be collected:
One 3cc lavender top tube
Ten 10 cc green top tubes
One 10 cc tiger top tube