Masonic Cancer Center
University of Minnesota

Radioembolization of Primary and Secondary Liver Malignancies and the Effect On the Immune System: A Prospective Study of Cytokine Modulation and Immune Cell Infiltration

CRPC #2018LS026

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Key Abbreviations

CTCAE	Common Terminology Criteria for Adverse Events
IRB	Institutional Review Board
PBMC	peripheral blood mononuclear cell
RE	Radioembolization
UMN	University of Minnesota
Y90	Yttrium 90
XRT	External beam radiation

Protocol Synopsis

Radioembolization of Primary and Secondary Liver Malignancies and the Effect On the Immune System
CPRC #2018LS026

Study Design: This is a single institution non-interventional study designed to evaluate the immune reaction to radioembolization (RE) of primary and secondary malignancies of the liver.

RE has been established as a standard of care treatment for both primary and secondary cancers of the liver. The treatment consists of a mapping, or planning angiogram, followed by a delivery angiogram where the dose of yttrium 90 (y90) is delivered. Data has been published on the immune modification powers of external beam radiation (XRT). However, very little data is available on the ways in which RE modifies the immune system. The goal of this study is to determine changes in the peripheral blood monocytes, cytokines and the treated and untreated liver tumors through sample collection prior to and for 12 weeks after standard of care RE.

Prior to the RE delivery procedure, patients will have a blood draw to evaluate for levels of 11 immunologically relevant cytokines (IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12p70, IL-18, TNFα, IFN-Y, Fit ligand 3, and MCP-1). These blood draws will be repeated at 7 days (± 6 days), 4 weeks (± 2 weeks) and 12 weeks (± 2 weeks) after RE.

The patients will also have the infiltration of immune relative cells into treated tumors evaluated. This will be done by the patients undergoing biopsy of the largest tumor to be treated prior to treatment and optionally at 2 weeks (±7 days) following RE. If patients have other areas of tumor, which are not included in the initial treatment site, these areas will also be biopsied.

Finally, the change in immunologically important peripheral lymphocytes will be collected. This will be done with a blood draw on the day of, but prior to RE serving as an internal control. Patients will then also have blood draws performed at 7 days (±6 days), 4 weeks (± 2 weeks) and 12 weeks (± 2 weeks) after RE.

Primary Objective: Determine changes in the peripheral blood lymphocytes after radioembolization (RE) therapy for primary and secondary malignancies of the liver at 12 weeks.

Secondary Objectives:

- Determine changes in the peripheral blood cytokines after radioembolization (RE) therapy for primary and secondary malignancies of the liver.
- Determine the changes in infiltrating immune cells within the treated tumor, which occur after RE therapy for primary and secondary malignancies of the liver.

- Determine the changes in infiltrating immune cells within non-treated liver tumors, which occur after RE therapy for primary and secondary malignancies of the liver.
- Determine changes in the peripheral blood lymphocytes after RE therapy for primary and secondary malignancies of the liver at 7 days,4 weeks, and 12 weeks.

Key Inclusion Criteria:

- Biopsy or image (in the setting of hepatocellular carcinoma (HCC)) diagnosed hepatic malignancy
- Total bilirubin < 2 mg/dL
- ECOG status ≤ 2
- Life expectancy >3 months assessed
- Age >22 years
- Lesion >2.0 cm which is amenable to percutaneously biopsied

Key Exclusion Criteria:

- Unwilling or unable to attend all study related follow ups
- Technetium 99 macro aggregated albumin (MAA) lung shunt fraction >20%
- Arterial anatomy which precludes the ability to safely perform RE
- INR > 3 or platelet count <15,000 which cannot be corrected
- Patients who are unable to hold anticoagulation and/or antiplatelet therapy in the peri-procedural setting

Accrual Objective:

The goal is to accrue 30 patients. It is anticipated that approximately 10 patients per year can be enrolled; meaning enrollment is expected to take 60 months.

1 Objectives

1.1 Primary Objective

The primary objective is to determine changes in the peripheral blood lymphocytes after RE therapy for primary and secondary malignancies of the liver.

1.2 Secondary Objectives

- Determine changes in the peripheral blood cytokine after REtherapy for primary and secondary malignancies of the liver.
- Determine the changes in infiltrating immune cell within the treated tumor which occur after RE therapy for primary and secondary malignancies of the liver.
- Determine the changes in infiltrating immune cell within non-treated liver tumors which occur after RE therapy for primary and secondary malignancies of the liver.

2 Background and Rationale

The importance of the immune system in the treatment of cancer has been well established over the last several decades, leading to the development of immunotherapies (1). It has also been noted that certain loco-regional therapies such as external beam radiation (XRT) and thermal ablation induce immunologic responses (2-6). However, to date basic research into the immune response induced by RE, a recently established standard of care treatment for primary and secondary malignancies of the liver, is lacking (7,8).

Rarely, loco-regional therapy can induce a positive immune response systemically, termed an abscopal effect. Pre-clinical studies with XRT have suggested that single high dose radiation, not clinically obtainable with XRT, but regularly performed in RE, make abscopal events more frequent (9-11). This suggest RE may be an excellent method of inducing abscopal effects as the deliverable dose to the tumor can be as high as 200-300 Gy, much higher than clinically obtainable by XRT (11). However, despite the apparent potential of RE to positively modulate the immune system and the dramatic benefits this may provide, little research has been performed in this area.

Five prior papers have investigated cytokine changes following RE (12-16). Both Carpizo et al and Lewandowski et al focused on the release of angiogenic factors which have been shown to be tumor protecting, however, are not focused on immunomodulation. Wickremeskera et al did study changes in several immunomodulating cytokines (IL-1β, IL-6, IL-8, IL-10, IL12, TNFα, and IFN-Y) however, only did so over the first 48 hours. The half-life of yttrium 90 is 64.1 hours, therefore much of the radiation dose and potentially the immunomodulation, will not have occurred at 48 hours. Fernandez-Ros et al. focused on the induction of liver regeneration following RE and through that studied among other factors the change in IL-6, IL-8, and TNF-α before and at 4 time points following treatment out to 2 months (15). Finally, Seidensticker et al. investigated the prognostic effects of IL-1, IL-2, IL-4, IL-6, IL-8, and TNF-α on overall survival and liver dysfunction with serum blood draws prior to and at 3 days and 6 weeks after radioembolization (16). They found that pre-

treatment IL-6 and IL-8 values portended a worse prognosis (16). These studies have also found that RE leads to modifications in several cytokines (IL-1 and IL-6) (12, 15, 16) however, they do not focus on the immunomodulatory capabilities of these cytokines, include a comprehensive cytokine profile, nor correlate cytokine changes with immune cell infiltration or peripheral lymphocyte changes.

Similarly, while the ability of XRT to induce immune cell infiltration has been investigated (17,18), this has not been documented in RE. Multiple cells including cytotoxic T cells, natural killer cells, helper T cells, dendritic cells, and macrophages have been shown to play roles in immune mediated tumor cell death and tumoral cell protection pathways (17). The infiltration of these cells into a treated tumor following RE is significant in the understanding of how RE induces immune modulation. Recently Chew et al published the first study to investigate immune cell infiltration following RE (19). The demonstrated higher expression of granzyme B and infiltration of $CD8^+$ T cells, $CD56^+$ NK cells and $CD8^+$ $CD56^+$ NK cells into treated tumors. They also demonstrate an increase in the TNF-α on both $CD8^+$ and $CD4^+$ Tcells as well as an increase in the percentage of antigen presenting cells after RE.

3 Patient Selection

Study entry is open to patients 22 years of age and older regardless of gender, race, or ethnic background. While there will be every effort to seek out and include minority patients, the patient population is expected to be no different than that of other studies enrolling a similar population at UMN.

Non-English speakers will not be actively sought out for the purposes of this study, however, they would not be excluded given the availability of in house interpretive services.
Translation services will be provided for all participants who self-identify either as requiring translators or if it is felt by the investigator at any time to be necessary to utilize a translator to ensure full understanding of a concept or term. If a potential participant is non-English speaking, a written short form consent will be provided and reviewed with an interpreter. In addition, an interpreter will go through the main consent with the participant orally. If interested in the study, the participant will sign the short form, but not the full English consent form. A witness, who is not study staff or family, will sign both the short form and the main consent form. The study staff who is responsible for obtaining consent will sign only the main consent form. If the participant is expected to be participating for more than 30 days following the consenting process, the main consent will translated into their language and provided to the participant. Once a long form consent is translated for a given language, only the long form and not the short form will be used with subsequent participants who speak that language.
A potential participant must meet all of the inclusion and exclusion criteria to be considered eligible for study participation.

3.1 Inclusion Criteria

3.1.1 Diagnosis of primary hepatocellular carcinoma or biopsy proven hepatic metastases from another solid tumor primary for which RE is planned

3.1.2 Hepatic lesion >2.0 cm which is amenable to percutaneously biopsy

3.1.3 Age 22 years or older
3.1.4 EOCG PS ≤ 2 – refer to Appendix II
3.1.5 Total bilirubin ≤ 2.0 mg/dL
3.1.6 Expected life expectancy of >3 months assessed
3.1.7 Participant provides voluntary written consent prior to the performance of any research related tests or procedures

3.2 Exclusion Criteria

3.2.1 Pregnant or breastfeeding - Females of childbearing potential must have a negative pregnancy test (serum or urine) within 7 days of study registration
3.2.2 Technetium 99 macro aggregated albumin (MAA) lung shunt fraction >20%
3.2.3 Arterial anatomy which precludes the ability to safely perform RE
3.2.4 INR > 3 or platelet count <15,000/mcL
3.2.5 Patients who are unable to hold anticoagulation and/or antiplatelet therapy in the periprocedural setting
3.2.6 In the opinion of the enrolling investigator, the patient may be unwilling or unable to attend all study related follow up due to physical, psychiatric, or logistical circumstances

3.3 Anticipated enrollment time line:

It is anticipated that approximately 10 patients per year can be enrolled in this study. Therefore, it is believed that it will take approximately 60 months to enroll the goal of 30 patients.

4 Patient Registration and Study Enrollment

Written consent must be obtained prior to the performance of any research related tests or procedures. Consent is usually obtained before final eligibility is determined.

4.1 Registration with the Masonic Cancer Center Clinical Trials Office

Any patient who has been consented is to be registered in OnCore by the Primary Clinical Research Coordinator (PCRC) or designee. If a patient is consented, but not enrolled, the patient's record is updated in OnCore as a screen failure and reason for exclusion recorded.

4.2 Study Enrollment with the Masonic Cancer Center Clinical Trials Office

To be eligible for study enrollment, the patient must sign the research consent and meet each of the inclusion criteria and none of the exclusion on the eligibility checklist (Appendix I) based on the eligibility assessment documented in the patient's medical record.

The Primary Clinical Research Coordinator (PCRC) or designee will add the "on-treatment" date to complete enrollment.

5 Study Plan

Patients enrolled in this study receive standard of care (RE therapy but agree to serial sample collections for research purposes as detailed in Section 7.

Direct study participation ends after the final blood sample is collected 12 weeks after the RE therapy unless any of the following occurs:

- The patient is non-compliant or withdraws consent
- The patient is discharged to hospice for terminal cancer care

As part of the consent process, the participant will be asked to opt in to the storage of any leftover blood or tumor samples for future unspecified research once testing related to this study is completed and verified.

6 Expected Adverse Events and Potential Risks

6.1 Risks of Blood Sample Collection

The risk of blood sample collection is very minimal and include infection and hematoma.

6.2 Risks of Hepatic Tumor Biopsy

Risks of hepatic tumor biopsy include significant hemorrhage, requiring transfusion or secondary procedure, infection, track seeding, or accidental damage to surrounding structures. However, the risks for each of these complications is ≤ 1%.

7 Study Calendar/Schedule of Tests and Procedures

7.1 Required Clinical Care Evaluations

	Screening	
	within 28 days of enrollment	within 14 days of enrollment
Written Consent	X	
Medical history	X	
Confirmation of hepatic metastases meeting study eligibility requirements	X	
Provider assessment for eligibility	X	
ECOG performance status	X	
Complete Blood Count with diff (CBC), INR		X
Total bilirubin		X
Pregnancy test (urine or serum) WOCBP		X[1]

[1]For women of childbearing potential (WOCBP): urine pregnancy test within 14 days prior to study registration. If a urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

Medical History: This will be obtained during the standard of care pre-RE clinical visit with the treating interventional radiology. The Interventional radiologist will collect the information directly from the patient. At this time ECOG status will also be determined by the interventional radiologist

Laboratory values: The laboratory values (CBC, INR, total bilirubin, and Pregnancy test) will be gathered from Epic, as this testing is standard of care prior to RE.

7.2 Research Related Sample Collection

	Prior to RE	Time post RE			
		1 week (± 6 days)	2 weeks (±7 days) *optional*	4 weeks (± 2 weeks)	12 weeks (± 2 weeks)
Assess for adverse events		X	X	X	X
Peripheral blood for cytokines	X	X		X	X
Peripheral blood for lymphocytes	X	X		X	X
Tumor biopsy	X		X a treated and an untreated tumor		

Research sample collection will be tied to the clinical care time points whenever possible. It is recognized that with novel therapies as used in this study, the timing of protocol directed research samples may miss important patient specific events. For this reason, up to 3 extra samples for a total of 180 ml of blood may be drawn at additional time points that are not specified above. This blood would be drawn in the case that blood draws from specified time points were damaged, lost, or unusable.

7.3 Endpoints, detailed:

The primary endpoint for this study is to determine changes in the peripheral blood monocytes after RE therapy for primary and secondary malignancies of the liver at 12 weeks.

Secondary endpoints include the changes in peripheral blood monocytes at 7 days and 4 weeks. The changes in immunologically relevant cytokines *(IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12p70, IL-18, TNFα, IFN-Y, Fit ligand 3, and MCP-1)* at 7 days, 4 weeks, and 12 weeks will also be determined. Biopsies of the treated tumor prior to treatment and 2 weeks after treatment (if done) will also be performed to assess immune cell infiltration following treatment. The 2-week after treatment biopsy will be performed strictly for research purposes and will be optional. The second biopsy was made optional as it was found to be a significant barrier to enrollment. Furthermore, the change in PBMCs is the most scientifically important aspect of the study. Additionally, when available, a non-treated tumor will be biopsied two weeks after treatment to assess for immune cell infiltration.

7.4 Research data collection and lab analyses detailed:

Basic characteristics of the malignancy (type of malignancy, size of largest tumor, and number of tumors) will be recorded. RE will be completed in a manner which has been previously described (20). All of the preprocedural work up and procedure costs will be billed to insurance as this is standard of care.

Clinical follow up with magnetic resonance imaging (MRI) or CT will occur at 1 month and 3 months. Radiologic response will be evaluated at 3 months using Response Evaluation Criteria in Solid Tumors (RECIST) and modified RECIST guidelines. Overall survival will be defined as time from RE to death, and patients will be censored for transplantation. Progression free survival will be defined as time from RE to progression as defined by RECIST and mRECIST.

Peripheral blood monocytes will be collected and processed for flow cytometric analyses and cryostorage for later batch studies. The effect RE on lymphocyte repertoire, numbers and phenotype will be assessed at baseline and at defined time points during the trial (7 days (+/- 6 days), 4 weeks (+/- 2 weeks), and 12 weeks (+/- 2 weeks). The frequency and percentage of peripheral blood monocytes and T-cell subsets (CD4 and CD8 effector memory, central memory and Treg subsets), NK cells and myeloid-derived suppressor cells will be determined. Peripheral blood tumor-specific T cell subsets will be evaluated by flow cytometry in cooperation with the Translational Therapy Laboratory, Masonic Cancer Center. Cytotoxic T lymphocytes (CD8+ T cells) and CD4+ helper T cells will be isolated using fluorescence-activated cell sorting (FACS) from cryopreserved cells followed by mRNA expression analysis to assess gene expression changes resulting from therapy. Testing will be performed in the UMN Flow Cytometry and Genomics Core Laboratories. Peripheral blood monocytes may also be tested in functional assays (i.e. IFN-γ ELISpot) for responses to known antigens as well as to predicted neoantigens from whole-exome/transcriptome sequencing of melanoma tumors. All patients will also undergo HLA testing to enable epitope discovery using available MHC binding prediction algorithms.

Patients will undergo biopsy of the largest percutaneous available tumor to be treated within a 12-week period prior to treatment and optionally at 2 weeks (± 7 days) following RE. If the pre-treatment biopsy is not standard of care, it will be done for research. The 2-week after treatment biopsy will be performed strictly for research purposes. The optional post RE biopsy will include a treated (defined as a lesion which had y90 delivered to it as determined on standard of care Bremsstrahlung scan after RE) and non-treated lesion (defined as a lesion which did not receive y90 as determined on standard of care Bremsstrahlung scan after RE). Four 18-gauge core needle biopsies will then be obtained. One of the four cores will immediately be placed on ice and sent to our CLIA approved research laboratory. This sample will then be flash frozen and

stored at -80 degrees. Three of the cores will immediately be placed into formalin and submitted to the research laboratory where they will undergo paraffin embedding. These samples will also be stored until analysis can be undertaken. A paraffin embedded sample will then undergo immunohistochemistry analysis. The samples will be analyzed using a standard streptavidin biotinylated alkaline phosphatase method or a streptavidin biotinylated horseradish peroxidase method as described previously (17,18). The slides will then be analyzed using a standard light microscope and CCD-camera at 20x objective. The slides will be scanned into digital format using an Aperio XT scanner (Leica Biosystems Inc. Buffalo Grove, IL). A pathologist will then demarcate the tumor area on the slide. This area of interest will be analyzed using the counting software "FiJI-ImageJ" (FIJI) will be used to quantify the number of infiltrating cells (cell/mm^2) in the tumor. They will also be analyzed by the number of infiltrating cells per 100 tumor cells. These analytic standards have been previously described (17,18). The infiltration of immune cells into the tumor before and after treatment will then be compared with each patient serving as their own control.

On the day of RE patients will have labs drawn, as is consistent with current practice. In addition to standard labs (total bilirubin, international normalized ratio (INR), complete blood count (CBC)) the patients will have an additional tube drawn to be analyzed for cytokine level. To determine the effects on cytokines *(IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12p70, IL-18, TNFα, IFN-Y, Fit ligand 3, and MCP-1)* over a period of time the cytokines will be analyzed following RE at 7 days (+/- 6 days), 4 weeks (+/- 2 weeks) and 12 weeks (+/- 2 weeks). All cytokines will be measured in a similar way through the Cytokine Reference Laboratory at the UMN. This Clinical Laboratory Improvement Amendments (CLIA) approved lab specializes in the analysis of cytokines, chemokines, and growth factors, and as such is very qualified to perform these tests which they have been performing for several years on a regular basis. The tests will be performed in a similar fashion to prior authors (15-17).

The blood cells, blood serum and the biopsy samples will then be stored at the clinical research lab at the University of Minnesota

8 Event Monitoring, Documentation, and Reporting

Toxicity and adverse events will be classified and graded according to NCI's Common Terminology Criteria for Adverse Events version 5.0 (CTCAE V5) and reported on the schedule below. A copy of the CTCAE can be downloaded from the CTEP home page. (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50).

The following definitions of adverse events (AEs) and serious adverse events (SAEs) will determine whether the event requires expedited reporting via the SAE Report Form in addition to routine documentation in the OnCore AE case report form (CRF).

Note: throughout this section the generic term "study drug" refers to the study related procedures.

8.1 Event Terminology

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Serious Adverse Event: An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious 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 Event: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in protocol-related documents (e.g. protocol, consent documents), or is not listed at the specificity or severity that has been observed or given the characteristics of the subject population being studied.

The following definitions are from the Masonic Cancer Center's Data and Safety Monitoring Plan (http://z.umn.edu/dmsp)

Major Deviation: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject's willingness to participate in the research. Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

8.2 Event Monitoring and Documentation

This study involves collecting samples for research related purposes in patient undergoing standard of care RE therapy. Event monitoring will focus on risks associated with study procedures, including sample collection (blood and tumor) and breach of confidentiality. However, all AEs will be documented and reported as required.

8.3 Expedited Reporting Requirements

The following situations must be reported to the UMN IRB in an expedited manner:

Agency reporting to	Criteria for reporting	Timeframe	Form to Use	Submission address/email address
U of MN IRB	Unanticipated death of a locally enrolled subject(s); New or increased risk; Any adverse event that require a change to the protocol or consent form – refer to the IRB website for complete details	5 Business Days	IRB Report Form	irb@umn.edu
	Major Deviations that occur at MCC, as defined in Section 10.1.	5 Business Days	OnCore Deviation Form and IRB Report Form	

9 Study Data Collection and Monitoring

9.1 Data Management

Participants will be registered in OnCore as detailed in Section 4.1. Study data will be collected in REDCap.

9.2 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dsmp

For the purposes of data and safety monitoring, this study is classified as high risk (investigator initiated). Therefore, the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the study's progress at least quarterly.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in Section 10.3 to the Masonic Cancer Center's SAE Coordinator and the University of Minnesota IRB.

9.3 Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the Masonic Cancer Center or their designee, IRB, government regulatory bodies, and University of Minnesota compliance groups. All study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.) will be made available. The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

9.4 Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at least 6 years after the study file is closed with the IRB.

10 Statistical Considerations

Descriptive statistics will be calculated for outcomes and variables of interest. Continuous variables will be summarized using mean and standard deviation, or median and interquartile range, as appropriate. Categorical variables will be summarized using frequencies and percentages. Plots of each peripheral lymphocyte at baseline and each subsequent time of measurement (as stated in the protocol synopsis section) will be generated. This will help determine if individuals have different baseline peripheral lymphocyte values or different peripheral lymphocyte value trends over time. Similar plots will be generated for each cytokine *(IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12p70, IL-18, TNFα, IFN-Ɣ, Fit ligand 3, and MCP-1).*

Primary analysis: To determine if there is a change in peripheral lymphocytes following RE, a mixed model with random intercept and random slope for time, and adjustment for baseline peripheral lymphocyte value will be carried out for each lymphocyte. The fixed effect for time, representing the slope over time, will be tested for significance, and a 95% confidence interval will be reported. Depending on if the outcome is skewed, appropriate transformations will be used. Similar models may be carried out with adjustment for certain variables such as age, sex, and size of tumor. Additionally, to investigate specific changes in peripheral lymphocytes from baseline, pairwise comparisons between baseline and each measurement following RE, using paired t-tests or Wilcoxon signed-rank tests, will be performed with a Bonferroni adjustment for multiple comparisons.

Secondary analysis: To determine if there is a change in active cell infiltration into tumors following RE, paired t-tests or nonparametric Wilcoxon signed-rank tests will be used, depending on the distribution of the outcome. These analyses will be performed for the different tumor types (treated and non-treated tumors). In the case that certain variables such as age, sex and size of tumor are of interest, a mixed model will be obtained using an appropriate distribution assumption of the outcome (e.g. Gaussian, Poisson or negative binomial).

To determine if there is a change in cytokine production following RE, an analysis similar to the primary analysis will be performed for each cytokine.

Statistical analyses will be performed using SAS 9.4 (SAS Institute Inc., Cary, NC) or R 3.4.2. All p-values reported will be two-sided and a significance level of 0.05 will be used.

11 Ethical and Regulatory Considerations

11.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

11.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

11.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved Consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

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Appendix I – Patient Eligibility Checklist

Appendix II – ECOG Performance Status Criteria

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

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