**VI. PROTOCOL**

# Imaging CCR2 Receptors with $^{64}$Cu-DOTA-ECL1i in Head and Neck Cancer


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**Imaging CCR2 Receptors with $^{64}$Cu-DOTA-ECL1i in Head and Neck Cancer**
**Protocol Revision History**

**Initial submission version: 10/14/2019**

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|---|---|---|---|
| 11/13/2019 | 6.10.0 Regulatory and Reporting Requirements | Updated to match SOP for Siteman Cancer Center Received 11/2019 | Pending RDRC review<br>Pre HRPO Review<br>Pre FDA submission<br>FDA submitted Jan 2020 |
| 04/14/2020 | Title Page | Update HRPO number, CT.Gov number, protocol version number and date | PRMC approved 07/22/2020<br>FDA<br>Dec 2020 |
| 04/14/2020 | Footer all pagers | Update date and add page numbers | PRMC approved 07/22/2020 FDA Dec 2020 |
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| 04/14/2020 | 6.5.2 Patient Population | Add information on analysis of HPV+ and HPV- tissue samples | PRMC approved 07/22/2020<br>FDA Dec 2020 |
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| 03/31/21 | Throughout protocol | Correct syntax/punctuation | |
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| 03/31/21 | Schema | Revise to include patients who will receive other definitive therapy | |
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| 03/31/21 | 6.5.2 Patient Population | Revise to include patients who will receive other definitive therapy | |
| 03/31/21 | 6.5.8 Safety & Clinical Follow-Up | Revise to include patients who will receive other definitive therapy | |
| 03/31/21 | 6.12.0 Data Submission Schedule | Add Tissue Collection Form and submission schedule | |
| 03/31/21 | 6.13 Study Calendar | Add tissue collection | |

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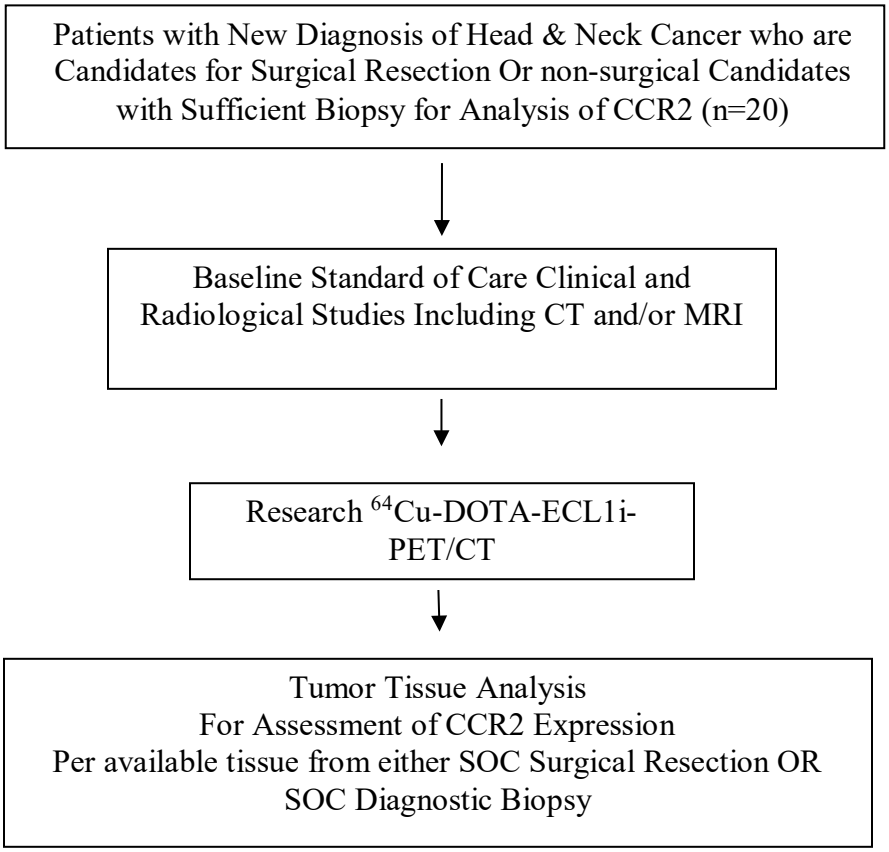
| 08/28/2023 | Title Page | Update protocol version number and date for renewal submission<br>Remove study coordinators | Pending PRMC<br>Pending FDA |
| | Footer all pagers | Update version number and date Fix pagination error(s) | |
| | Formatting all pages | Document consistency fix spacing etc | |
| | Contents | Update table of Contents | |
| | 6.5.3 PET Imaging / 6.13 Study Calendar | Clarify metabolites are collected whenever possible | |
| | 6.8.0 Risks and Benefits | Fix typographical error referencing PDAC &Clarify metabolites are collected whenever possible | |

## Contents

## SCHEMA

### Imaging CCR2 Receptors with $^{64}$Cu-DOTA-ECL1i in Head and Neck Cancer



### 6.1.0 Background

Chemokines, also known as chemoattractant cytokines, are a super family of small secreted proteins initially characterized by their ability to induce leukocyte migration. They are small proteins known as cytokines that have chemoattractant properties and, thus, are called chemokines. One of their most critical functions is governed by the specific amino acid sequence (or motif) of Glu-Leu-Arg (or ELR) immediately preceding the first cysteine of their CXC motif. ELR+ CXC chemokines are considered angiogenic, whereas ELRCXC chemokines are considered angiostatic[1]. To date, more than 50 chemokines and 20 chemokine receptors have been identified. Chemokines and their receptors have a highly conserved sequence throughout the genetic tree [2]. They are grouped into 4 distinct groups (C, CC, CXC, and CX3C), which are based on the number and position of conserved cysteine residues near the NH2 terminus of the molecule [3]. The specific effects of the chemokines on their target cells are mediated by members of a family of 7-transmembrane-spanning, G-protein–coupled chemokine receptors. Most chemokines are able to bind with high affinity to multiple receptors while their receptors usually are restricted to a single subclass and induce an intensive signaling cascade of activated second messengers that lead to cell motility and multiple other functional effects in the target cells. Chemokines insert their cellular trafficking and processes are influenced significantly by chemokines via their receptors.

Chemokine Receptors: Most chemokine receptors bind specifically, but indiscriminately to their chemokine ligands. Chemokine receptors exist in many epithelial and hematopoietic cells. These receptors are members of the 7-TM GPCR super family. Like chemokines, chemokine receptors also share structurally conserved/homologous regions, which allow their subgrouping classification. In general, upon binding to their ligands, chemokine receptors undergo conformational changes that allow the binding of G proteins to intracellular loop epitopes and the carboxy terminal tail of the receptors [3]. The second intracellular loop of the receptors contains an Asp-Arg-Tyr-Leu-Ala-Ile-Val (DRYLAIV) motif, which is missing in the nonsignaling receptors and does not permit G-protein coupling [2] .The chemokine receptors that do not bind to G proteins may act in synchrony with other proteins to support their effects. Examples of these alternative cohorts include b-arrestin, integrins, and growth factor receptors [4-9].

Chemokines are vital for leukocyte migration and activation, and physiologically they have been implicated in various immune, inflammatory and activation responses such as allergic disease, atherosclerosis, and wound healing. Furthermore, chemokines play an important role in the neoplastic processes of many organs and in determining the location of the metastatic spread of cancer cells. For example in squamous cell carcinoma of head and neck, chemokine receptors orchestrate critical functions in oral tumorigenesis by favoring angiogenesis, tumor growth and metastasis [10,11]. In prostate cancer, multiple chemokines and their receptors (including CCR5, CCR7, CCR9, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR7) are involved in the multistep process of metastasis [5,8,12-15]. In cervical cancer, CXCR4 receptor has been shown to play an important role during cervical cancer invasion [16]. In addition, CXCR7 expression has been shown to predict poor disease-free and disease-specific survival in cervical cancer patients, and might be a promising new therapeutic marker. In a large majority of cases, CXCR7 is co-expressed with CXCR4 and/or EGFR, supporting the hypothesis that these receptors assist in CXCR7 signal transduction [17]. CXCR4 has been found to be upregulated in several other cancers such as breast, prostate, lung, bladder, ovarian, renal, oesophageal, colorectal, and pancreatic cancer, and lymphoma, melanoma, osteosarcoma, neuroblastoma, etc. [18]. In addition, high level of CXCR4 expression correlated with tumor progression and metastasis [19,20], as well as poor prognosis and resistance to chemotherapy [21,22]. Thus, these receptors provide an important target for imaging and treatment of various types of cancers.

**CCR2 in Head & Neck Cancer:** The importance of chemokines and their cognate receptors in head and neck cancers is being revealed by increasing amount of studies. Recently,

the expression of CXCR2 was reported substantially higher than that in paraneoplastic tissue in laryngeal squamous cell carcinoma [23]. Elevated expression was significantly related with lymph node metastasis, histological grade, and 5-year survival [23]. Thus, CXCR2 expression could be considered as a potent prognostic marker for laryngeal squamous cell carcinoma patients [23]. Several studies have also documented the significance of CXCR4 in HNSCC tumor progression and organ-specific metastasis[24,25]. Wang et al investigated the expression of CXCR4 in nasopharyngeal carcinoma tissues, and they found that CXCR4 expression was elevated in tumor tissues and the increased expression was correlated with metastatic rates in patients as well as poor overall survival [26]. This finding was consistent with another study which reported that CXCR4 mRNA was significantly higher in HNSCC tissues than in paraneoplastic tissues and its expression were associated with lymph node metastasis and distant metastasis [27]. These findings clearly demonstrate that CXCR4 could also be used to predict prognosis and metastasis in HNSCC patients.

Chen et al. reported that a 64I mutation of the CC chemokine receptor-2 (CCR2) is significantly associated with oral cancer susceptibility [28]. Also, another group demonstrated that mutation of MCP-1 gene, which encodes CCR2 ligands, increased the expression of MCP-1 resulting in elevated CCR2 activity which could increase the risk for developing oral squamous carcinoma[29]. The authors predicted that MCP-1 and CCR2 could be used as genetic markers for diagnosis of oral carcinoma squamous[29]. In addition, Fujita et al. found that The CCL2-CCR2 axis is associated with lymphatic metastasis in oral squamous cell carcinoma[30]. Yang et al. have shown that CCL2-CCR2 axis could promote nasopharyngeal carcinoma (metastasis by activating ERK1/2-MMP2/9 pathway. This study helps to develop novel therapeutic targets for distant metastasis in nasopharyngeal carcinoma[31]. Another CC chemokine receptor, i.e., CCR7, which plays a critical role in the migration of activated dendritic cells to regional lymph nodes, was also found elevated in HNSCC tumor tissues compared with paraneoplastic tissues and the elevated expression of CCR7 was correlated with lymph metastasis and tumor tissue differentiation status[27]. Another study evaluated the expression of CCR7 in primary and metastatic tumor cell lines and biopsies from both primary and metastatic lesions, and it was found that CCR7 expression was elevated in metastatic cells and tissues[27]. These findings reveal an important role of CCR7 in mediating the metastasis and tumor malignancy in head and neck squamous cell cancer patients. Taken together, accumulating evidence indicates that the altered expressions of various chemokine receptors (and their cognate ligands as well) in head and neck squamous cell cancer are associated with different cellular events including cell survival, tumor progression, and metastasis. The expression of chemokine receptors is associated with tumor cell differentiation and prognosis of head and neck squamous cell cancer patients. Moreover, their expression is also, to some degree, histologically specific, i.e., the expression of these chemokine receptors indicates it is the squamous cell carcinoma rather than other types (such as adenoid cystic carcinoma)[32]. These features, along with other tumor biomarkers, may help oncologists to assess their patients more thoroughly

In the last few decades, human papillomavirus (HPV) has emerged as the key etiologic factor driving the rising incidence of oropharyngeal squamous cell cancer [33], [34] HPV-related oropharyngeal squamous cell cancer has emerged as a distinct clinical entity of head and neck cancer with expected high survival. This recognition has led to the investigation of whether a population of patients can be identified who can safely undergo treatment de-escalation, in an effort to minimize long-term treatment toxicity while maintaining excellent survival. The relationship of HPV and CCR2 has not been established in head and neck cancer. As the number of patients in this study is limited and we often do not know the HPV status of the patients, it will be extremely important to assess the CCR2 expression in the cancer tissues of HPV+ and HPV- patients to understand better the relationship of CCR2 expression and HPV. In future studies, we will be able to assess whether presence of CCR2 in each group is predictive of worse prognosis

and more aggressive therapy is needed. This will be very important as treatment deintensification for HPV+ squamous cell cancer of head and neck cancer is being evaluated.

**Positron Emission Tomography (PET) Using CCR-Based Tracers**: PET imaging of tumor metabolism has been extensively used for staging, treatment planning and monitoring of response in various types of malignancies. Due to the importance of chemokine receptors, attention has been made to develop radiolabeled compounds that targets these receptors.

**$^{64}$Cu-DOTA-ECL1i-PET/CT:**

Our group has developed a CCR2 specific PET radiotracer based on the peptide, ECL1i (d(LGTFLKC)) and radiolabeled with $^{64}$Cu ($^{64}$Cu-DOTA-ECL1i) [35]. The CCR2 detection sensitivity and specificity has been demonstrated in multiple preclinical studies and ex vivo human tissues [36-38]. We have tested this tracer in preclinical inflammation models, human pancreatic cancer and human head and neck cancer and demonstrated a positive correlation between the PET signals and CCR2 expression.

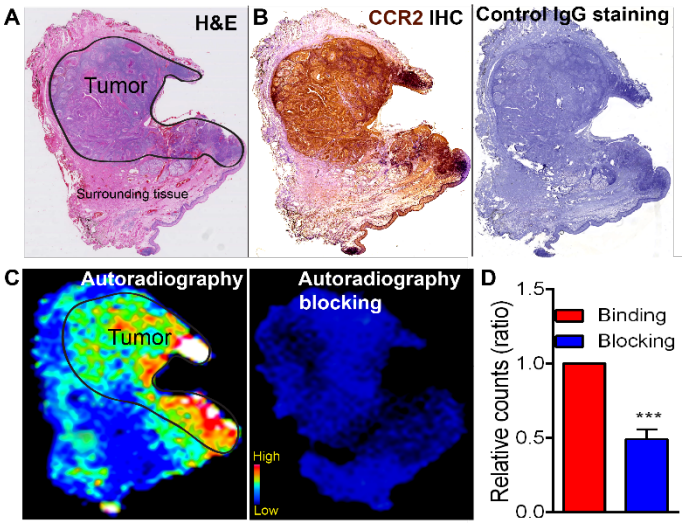



**Figure 1.** *Ex vivo* characterization of $^{64}$Cu-DOTA-ECL1i binding to CCR2 in human head and neck cancer tissue. (A) H&E. (B) CCR2 DAB staining showed high expression of the receptor in the area full of tumor cells. Control IgG staining confirmed the receptor staining specificity. (C) Autoradiography showed tracer binding to the cancerous area of the tissue and the specificity was verified by the blocking. (D) Quantitative comparison of counts in binding and blocking tissues.

Ex vivo characterization of $^{64}$Cu-DOTA-ECL1i binding to CCR2 expressed in human head and neck cancer tissue: CCR2 immunohistochemistry was performed on the de-identified human cancerous tonsil tissue collected from the SCCTPC (**Figure 1A**). As shown in **Figure 1B**, strong CCR2 (brown color) signals were detected in the area of tumor cells. Low CCR2 signals were also measured in the muscle and soft tissue, likely due to the expression of the receptors on the infiltrated macrophages or inflammatory cells. The control IgG staining showed negative signal of CCR2, confirming the specific expression of CCR2 on these tumor cells. The *ex vivo* binding assay of $^{64}$Cu-DOTA-ECL1i using autoradiography showed strong binding to the cancerous area with signals in similar profile to the CCR2 IHC, indicating the specific binding of the tracer to the CCR2 receptors on tumor cells (**Figure 1C**). The competitive receptor blocking using non-radiolabeled DOTA-ECL1i conjugate in excess amount (DOTA-ECL1i: $^{64}$Cu-DOTA-ECL1i molar ratio = 100:1) significantly blocked the binding to the tissue. Quantitative comparison of counts in binding and blocking tissues demonstrated 50.8 ± 0.6% (n=4, $p<0.005$) blocking efficiency (Binding was set as 1 for each specimen), confirming the binding specificity (**Figure 1D**).

**Summary:** CCR2 is a significant prognostic biomarker in head and neck cancer. Currently there is no clinical biomarker to study CCR2, its prognostic significance or to select patients for CCR2-targeted therapy and to monitor response to such therapy. Our group has developed a CCR2 specific PET radiotracer based on the peptide, ECL1i (d(LGTFLKC)) and radiolabeled with $^{64}$Cu ($^{64}$Cu-DOTA-ECL1i). We also have found that $^{64}$Cu-DOTA-ECL1i specific binding has been demonstrated in human head and neck cancer tissue. Currently $^{64}$Cu-DOTA-ECL1i is being studied in several clinical settings under IND (#137620) at Washington University. Our group is studying $^{64}$Cu-DOTA-ECL1i in patients with head and neck cancer. The use of $^{64}$Cu-DOTA-ECL1i has proven to be safe in our ongoing clinical trials with favorable radiation dosimetry (0.064 rem/ mCi) and is compatible with other imaging tracers such as $^{18}$F-Fludeoxyglucose (FDG)

## 6.2.0 Objectives

We are planning to perform a trial with goals to evaluate tumor uptake of $^{64}$Cu-DOTA-ECL1i in patients with squamous cell head and neck cancer with at least one measurable lesion of ≥ 1.5 cm. We also will correlate the tumor uptake of $^{64}$Cu-DOTA-ECL1i with immunohistochemical staining for CCR2 receptors in the tumor specimens.

Study objectives:

6.2.1 To determine the diagnostic quality and CCR2 expression detection rate of $^{64}$Cu-DOTA-ECL1i-PET/CT images.

6.2.2 To evaluate the relationship between $^{64}$Cu-DOTA-ECL1i uptake and immunohistochemical staining for various types of chemokine receptors such as CCR2.

6.2.3 To assess the association of CCR2 expression and HPV status. CCR2 immunohistochemical staining in HPV+ and HPV- squamous cell cancer of the head and neck. To better assess the analysis of tissue prospectively we will retrospectively review a limited number of identified samples obtained from the Department of Pathology at Washington University (20 total samples).

## 6.3.0 Statement of Qualifications

The study will be done under eIND (#137620). Robert J. Gropler, M.D., Washington University School of Medicine, St. Louis, Missouri is the physician-investigator of the IND.

## 6.4.0 Inclusion Criteria/ Exclusion Criteria

Adult patients (men or women) with confirmed new diagnosis of squamous cell head and neck cancer who are being evaluated for surgical resection or definitive therapy are eligible to participate in this study.

Inclusion Criteria:

- Adult patient 18 years of age or older
- Cytology or biopsy-proven squamous cell head and neck cancer scheduled to be treated with standard of care surgery. Patients who are not surgical candidates should have adequate tissue from tumor biopsy for analysis of CCR2.
- Lesion size of at least 1.5 cm (treatment naïve)
- Able to give informed consent
- Not currently pregnant or nursing: Subject must be surgically sterile (has had a documented bilateral oophorectomy and/or documented hysterectomy), post-menopausal (cessation of menses for more than 1 year), non-lactating, or of

childbearing potential for whom a urine pregnancy test (with the test performed within the 24 hour period immediately prior to administration of $^{64}$Cu-DOTA-ECL1i is negative

Exclusion Criteria:

- Patients with other invasive malignancies, with the exception of non-melanoma skin cancer, who had (or have) any evidence of the other cancer present within the last 2 years
- Unable to tolerate approximately 90 min (total time) of PET/CT imaging.

### 6.4.1 Inclusion of Women and Minorities

Both men and women are eligible. Members of all races and ethnic groups are eligible for this trial.

### 6.4.2 Registration Procedures & Screen Failures

Initial eligibility will be confirmed by the study coordinator. The PI, Co-PI or other engaged study investigator will review and sign an eligibility checklist to confirm the patient is eligible to participate on study. All enrolled subjects will be given a unique study specific patient identification and registered through the Siteman Cancer Center OnCore database at Washington University. Screen failures are those subjects who consent to and undergo any study related procedures. A subject who signs consent in clinic but declines to participate after further discussion of the imaging protocol and study requirements with the study coordinator is not considered a screen failure and will not be registered to the protocol since no study procedures took place.

## 6.5.0 METHODS

### 6.5.1 Study Design

A single center, open-label, baseline-controlled diagnostic imaging study designed to assess the diagnostic quality and CCR2 expression detectability of $^{64}$Cu-DOTA-ECL1i imaging with PET/CT. In addition, data will be collected to build the ongoing safety profile of $^{64}$Cu-DOTA-ECL1i and uptake will be correlated to in vitro assays of chemokines receptor from clinically obtained biopsy/surgical specimen.

### 6.5.2 Patient Population

Twenty adult individuals with new diagnosis of squamous cell of the head and neck cancer who are scheduled for definitive therapy (surgery for fresh tissue collection or other standard of care therapy who have adequate tissue from tumor biopsy for analysis of CCR2) and have not received treatment for their cancer will be studied. Written informed consent will be obtained prior to the PET imaging session. $^{64}$Cu-DOTA-ECL1i-PET/CT imaging consisting of a dynamic scan centered at the level of the known tumor followed by a limited body scan of the head/neck and upper chest will be performed in each patient. $^{64}$Cu-DOTA-ECL1i-PET/CT images will also be correlated with all available imaging studies to assess lesion localization.

To aid prospective tissue analysis a total of 20 samples (10 each: HPV+ and HPV-) will be obtained from the Department of Pathology at Washington University and immunohistochemical staining of CCR2 will be performed.

### 6.5.3 PET Imaging

All subjects will have a minimum of 24 hours to review the consent form before agreeing to participate in this research project. All PET imaging will be performed with a PET/CT scanner with preference given to scanners located in the Center for Clinical Imaging Research Center (CCIR) located on the 10th floor West Pavilion of Barnes-Jewish Hospital.

All patients will undergo routine clinical staging as dictated by the treating physician. The results of $^{64}$Cu-DOTA-ECL1i-PET/CT will not be provided to the patient or the treating oncologist/surgeon unless, in the judgment of the principal investigator, the images demonstrate an unsuspected abnormality that may warrant further evaluation.

Serum Stability and Metabolite Analysis (whenever possible): $^{64}$Cu-DOTA-ECL1i has been shown to be stable up to 60 min in preclinical studies. Our preclinical studies showed 95% of the tracer was intact after 1 hour incubation with mouse serum. In order to assess serum stability and for metabolite analysis of $^{64}$Cu-DOTA-ECL1i in this patient population, 2 venous samples (approximately 1 mL each) will be obtained (1 within the first 5 min of imaging and 1 at the completion of the first hour of imaging). Additional blood samples may be obtained based on results of the 2 samples to increase the time points, if necessary.

For each PET/CT study, when collected, high performance liquid chromatography (HPLC) analysis of the serum stability and metabolite profile will be performed by Dr. Liu Lab [37,38]. Whole blood will be centrifuged; plasma and packed red cells will be separated and the radioactivity in each portion will be recorded. A 200 µL aliquot of plasma will be diluted with 200 µL MilliQ water and passed through a 0.22 micron filter prior to HPLC injection. Eluent fractions will be collected every 1 minute and analyzed by gamma counting.

#### 6.5.3.1 PET Imaging Procedure

Following administration of 8-10 mCi of the radiotracer $^{64}$Cu-DOTA-ECL1i-PET/CT, all twenty subjects who enter on study will undergo a 60 minute dynamic scan centered over the site of the known tumor. An additional limited scan (top of head to upper/mid-chest) will be performed approximately 1-3 hours after administration.

For dynamic imaging, the subject will be placed supine on the imaging table with arms secured comfortably by the side of the body. A low dose spiral CT scan for attenuation correction will be obtained centered on the primary tumor site. The CT will consist of a 5-20 second topogram for determining correct anatomical positioning followed by a spiral CT at a maximum of 50 mAs. Care dose will be calculated for each scan and the care dose imaging parameters will be used if less than the maximum 50 mAs is calculated. CT imaging will be acquired with a standard 120 kVp. Average spiral CT scan time is 5-30 seconds. Scans are acquired

using a 3-5 mm slice thickness. Immediately after the attenuation CT scan, subjects will be injected with 8 – 10 mCi $^{64}$Cu-DOTA-ECL1i. The dose will be followed with a normal saline flush of 10-30 ml. A 60 min list mode dynamic imaging acquisition will follow study drug injection. At the end of the imaging session, subjects will be encouraged to void.

Subjects will be allowed to rest outside of the scanner until such time subject indicates they are willing to start the 2nd scan (1 – 3 hours post injection). Scan #2 will consist of a limited body scan from the vertex to upper/mid chest region. Following topogram and low dose CT scan for attenuation correction (maximum 50 mAs with care dose imaging parameters) a 2 bed limited scan from vertex to upper / mid chest will be obtained at 3-5 minutes per bed position.

To add to the existing safety profile of $^{64}$Cu-DOTA-ECL1i, all subjects will undergo vital sign measurement, clinical laboratory testing, and ECG testing as specified in sections 6.5.5, 6.5.6 and 6.5.7 of the protocol.

### 6.5.4 PET Image Processing and Analysis

List mode dynamic PET images will be reconstructed using current scanner manufacturer recommendations as follows (frames x seconds) 12x10, 6x30, and 11x300. PET images will be evaluated qualitatively (graded on a five-point scale regarding the presence or absence of abnormal uptake: 1 = definitely negative; 2 = probably negative; 3 = equivocal; 4 = probably positive; and 5 = definitely positive) and then, semi-quantitatively (standardized uptake value (SUV), tumor-to-normal tissue ratio (T/N) or tumor-muscle-ratio (T/M)) with the knowledge of the location (correlating with all available imaging studies). SUV is a decay-corrected measurement of activity per volume of tissue (μCi/mL) divided by the average activity per unit mass in the entire body. The overall PET image quality also will be graded (using 4-point scale with 1 being the worse and poor quality, not acceptable for diagnostic interpretation and 4 being good image quality, similar to routine clinical studies. PET analysis will not be performed in real time, but performed in batches or after all subjects have been enrolled, however, the images will be evaluated qualitatively for incidental findings.

The pharmacokinetic of the lesion time-activity curve using graphical analysis and tracer kinetic compartmental modeling will be determined. Classical receptor-ligand models will be employed to describe the kinetics of the tracers and different models to optimize goodness-of-fit will be evaluated. Goodness-of-fit will be judged by a nested F-test for the number of parameters, Akaike Information Criterion (AIC), and the Schwarz Criteria.1-tissue with two kinetic rate constants and 2-tissue with 4 rate constants compartmental reversible models will be used to fit the data using the blood input function as input function. The blood time activity curve will be determined noninvasively from a large 3D ROI traced over the aorta (OR carotid artery) and corrected for metabolites per above analyses, if available. The measured lesion time-activity curves will be fitted to the 1- or 2-compartment model prediction and added to the plasma component weighted by the tissue blood fraction $V_b$. Best fit will be used to determine the distribution volume (DV) from the 1-tissue model (with $DV = K_1/k_2$), and we will determine the DV and the non-displaceable binding potential (BP) from the 2-tissue model (with $DV = (K_1/k_2)(1+BP)$ and $BP = k_3/k_4$). The graphical analysis using Logan and Patlak analysis to determine the volume of distribution (DV) also will be performed. We will further use both the plasma time activity curve and a reference region to determined DV and DVR, respectively with non-displaceable BP = DVR -1. A possible region of reference will be a suitably located

muscle in the same field of view as head and neck cancer. All quantitative and semi-quantitative metrics of $^{64}$Cu-DOTA-ECL1i-PET/CT will be correlated with CCR2 receptor expression and binding affinity of each biopsy-proven tumor specimen.

### 6.5.5 Vital Signs

All vital signs will be recorded on the case report form. Vital signs may be obtained with the subject in the supine or upright position. Care will be taken to obtain subsequent recordings with the subject in the same position (supine or upright). Although allergic or other immediate adverse reactions are not anticipated, subjects will be monitored for at least 60 min post injection. Vital signs will be obtained at 3 time points pre-injection (anticipated to be within 30 mins prior to injection of $^{64}$Cu-DOTA-ECL1i although it might be longer if delays in quality control testing result in delay of study drug injection), at the completion of dynamic imaging and at study discharge. Vital signs will include the following: heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate, and body temperature. The following changes from baseline will be considered noteworthy:

| | |
|---|---|
| Heart rate: | > 30 beats per min |
| Systolic blood pressure | > 40 mm Hg |
| Diastolic blood pressure | > 30 mm Hg |

Noteworthy changes will be documented on the Principal Investigator Data Safety Review Form. The Principal Investigator will indicate on the form whether or not the changes in vital signs are clinically significant. If clinically significant, the principal investigator will assess the causality of the change to the injection of $^{64}$Cu-DOTA-ECL1i.. If a clinically significant change of a vital sign is noted and assessed as being attributable to $^{64}$Cu-DOTA-ECL1i injection by the Principal Investigator on the Data Safety and Review CRF, it will be reported on the adverse event log.

### 6.5.6 Clinical Laboratory Testing

Laboratory tests will consist of the following: standard CBC, and comprehensive metabolic panel obtained at the following time points:

- Baseline: Baseline labs may be obtained anytime within 21 days or immediately prior to $^{64}$Cu-DOTA-ECL1i injection and can be done as standard of care or for research purposes.
- Discharge: At least 60 minutes post injection or prior to study discharge.

Approximately 15 mL of blood will be collected for clinical laboratory testing, serum stability / metabolite analysis.

Labs obtained at baseline prior to injection of $^{64}$Cu-DOTA-ECL1i will be compared to labs obtained at study discharge. The following changes from baseline clinical laboratory values are considered to be noteworthy and require assessment as to clinical significance when they fall outside of normal limits. Clinically significant changes in laboratory values which are attributed to $^{64}$Cu-DOTA-ECL1i injection should be followed up until they return to baseline or normal levels, or until follow-up is no longer warranted. Laboratory values that are abnormal at baseline but move into normal range will not be considered clinically significant. If a clinically significant change of a laboratory value is noted, it will be reported on the PI Data Safety and Review Form so that the PI can assess the change as being attributable to $^{64}$Cu-DOTA-ECL1i injection. All clinically significant laboratory changes as identified below

which are identified by the PI as being attributable to $^{64}$Cu-DOTA-ECL1i will be recorded on the adverse event log.

**Clinically Significant Laboratory Values**

| Analyte | Change from baseline |
|---|---|
| Hemoglobin | > 2g/dL |
| WBCs | > 1 K/mm$^3$ |
| Neutrophils | > 10 % |
| Lymphocytes | > 10% |
| Platelets | > 50 K/mm$^3$ |
| Creatinine | > 0.75 mg/dL |
| BUN | > 20 mg/dL |
| Calcium | > 1mg/dL |
| Sodium | > 5 mmol//L |
| Potassium | > 0.5 mmol/L |
| $CO_2$ | > 4 mmol/L |
| ALT (SGPT) | > 150 IU/L |
| AST (SGOT) | > 100 IU/L |
| Alkaline Phosphatase | > 150 IU/L |
| Total Bilirubin | > 0.5 mg/dL |
| Albumin | > 1g/dL |

### 6.5.7 Electrocardiograms (ECGs)

A standard 12-lead ECG will be obtained on all subjects at baseline (within approximately 30 mins prior to injection of $^{64}$Cu-DOTA-ECL1i although it might be longer if delays in quality control testing result in delay of study drug injection), and at least 60 minutes post injection or prior to study discharge.

The following table lists criteria for normal limits and clinically notable limits for ECGs in adults.

**Criteria for Normal Limits and Clinically Notable Limits for ECGs in Adults**

| | **Normal Limits (msec)** | | **Notable Limits (msec)** | |
|---|---|---|---|---|
| ECG Variables | Low | High | Low | High |
| PR interval | 120 | 200 | <120 | >200 |
| QRS interval | 50 | 100 | < 50 | >100 |
| RR interval | 600 | 1000 | <600 | >1000 |
| QT interval | No lower limit | ⩾ 460 | No lower limit | ⩾ 460 |

ECG's which have limits noted to be outside of the table above will be recorded on the PI Data Safety and Review Form where the PI will review and assess as being attributable to $^{64}$Cu-DOTA-ECL1i injection. Any ECG recordings identified by the PI as being attributable to $^{64}$Cu-DOTA-ECL1i will be recorded on the adverse event log.

### 6.5.8 Safety & Clinical Follow-Up

To continue collecting data for the safety profile of $^{64}$Cu-DOTA-ECL1i a follow up phone call or in person visit to assess for adverse events will occur at least 24 hours following administration of $^{64}$Cu-DOTA-EL1i. Taking into consideration both the physical half-life (13 hours) and the much quicker biological half-life optimal follow up time is 48-120 hours post injection but follow up as soon as 24 hours and as late as 14 days post administration are allowed. As this is a verbal secondary assessment follow up phone call or visit beyond 14 days will be accepted with proper documentation of delay(s) such as subject underwent surgical resection and unavailable due to surgery and subsequent recovery. It is anticipated that some subjects may be scheduled for surgery as soon as 12 hours following $^{64}$Cu-DOTA-ECL1i administration and imaging so care will be taken to separate surgical related signs and symptoms from those which might be attributed to $^{64}$Cu-DOTA-ECL1i administration and PET/CT imaging.

Clinically, subject's medical record will be followed to document surgical resection or the start of definitive therapy.

### 6.5.9 Immunohistochemical Evaluation of Chemokinetic Receptors

To evaluate the relationship between $^{64}$Cu-DOTA-ECL1i uptake and expression of CCR2 receptors in head and neck tumor samples, IHC staining in all patients who have available tissue will be performed using techniques routinely performed in Dr. Liu's Laboratory [37,38]. Intensity of CCR2 signal in IHC will be assessed by study pathologist, Dr. Chernock and categorized into 4 categories (0=absent/faint, 1=weak, 2=moderate, 3=strong). Any cells that stained positively with CCR2 will be assessed and the final score will be based on an overall assessment of CCR2 IHC on all these cells. It is anticipated that most subjects will have tumor available from planned surgical resection or prior biopsy however standard of care clinical analysis of resected tumor will take priority over research tissue testing. Tumor testing will occur in batches or at the end of study recruitment depending on availability of testing supplies.

To better understand how CCR2 expression correlates with HPV status and to correlate with samples obtained prospectively a small retrospective tissue analysis study will also be performed. We will obtain tissue from 10 HPV+ and 10 HPV- squamous cell head and neck cancer subjects from the Department of Pathology at Washington University. These samples will be analyzed, as described above, to establish baseline expectations on the correlation of CCR2 expression to HPV status.

### 6.6.0 Drug Preparation

$^{64}CuCl_2$ will be produced by the Cyclotron Facility at Washington University School of Medicine, Mallinckrodt Institute of Radiology. $^{64}$Cu-DOTA-ECL1i will be produced in the Biologic Therapy Core Facility (ISO 7, class 10,000) at Washington University School of Medicine following SOPs and batch production records. To produce $^{64}$Cu-DOTA-ECL1i, $^{64}$CuCl2 (20-70 mCi) will be buffered with 20 mM sodium acetate stabilized with gentisic acid and then added to DOTA-ECL1i (30 μg) to bring the total volume to 500 μL (we do not expect any interference with CCR2/5 inhibitor therapeutic effect, because we use a trace amount of

DOTA-ECL1i in comparison to 600 mg therapeutic dose, which is 20,000 higher than the imaging dose). This reaction is incubated in a 45ºC heated shaker (ThermoMixer, Eppendorf) for 45 minutes at 950 rpm during which time the DOTA chelator binds with $^{64}$Cu. The product will be evaluated by pre-release specifications and post-release specifications to control the quality of the radiotracer for clinical study. This study will be done under an eIND (#137620) for $^{64}$Cu-DOTA-ECL1i

### 6.7.0 Statistics

Demographic and clinical characteristics of the enrolled patients will be summarized using descriptive statistics with frequency and percentages used for categorical variables, median (25$^{th}$, 75$^{th}$) for ordinal variables and mean (standard deviation) for continuous variables. The diagnostic quality of images and CCR2 expression detection will be assessed qualitatively and semi-quantitatively for all participants and these measures (described above) will be summarized for a lesion using descriptive statistics. The association between $^{64}$Cu-DOTA-ECL1i uptake and tissue signal intensity for CCR2 and other chemokine receptors will be evaluated using a Spearman correlation coefficient. Collection of additional data for the safety profile will not undergo formal statistical review.

The primary goal of this single center, open-label, baseline-controlled diagnostic imaging study is to assess the diagnostic quality and CCR2 expression detectability of $^{64}$Cu-DOTA-ECL1i in head and neck cancer patients. The study is primarily descriptive and the sample size is based on feasibility of recruitment. However, a sample size of 20 achieves 80% power to detect a correlation of 0.61 using a two-sided hypothesis test with a significance level of 0.05.

### 6.8.0 Risks and Benefits

Targeting CCR2 is emerging as a promising therapeutic strategy in many types of cancer, but accurate patient selection for this strategy is critical. The potential risks from this imaging protocol are expected to be very low. Expected risks include discomfort from the placement of intravenous catheter(s) for injection of $^{64}$Cu-DOTA-ECL1i, radiation exposure from the injection of $^{64}$Cu-DOTA-ECL1i and CT for attenuation correction scans, and discomfort from lying still on the imaging table. A temporary altered taste sensation may be associated with the IV injection of $^{64}$Cu-DOTA-ECL1i. Blood samples will be obtained for safety profile, whenever possible additional samples are collected for serum stability and metabolites testing. There is a slight risk of bruising and a remote risk of infection from the placement of the intravenous catheter(s). Although unlikely, there is a rare chance of having patients suffer an allergic or other immediate adverse reaction to $^{64}$Cu-DOTA-ECL1i. To minimize the risks to subjects, intravenous catheters will only be placed by trained personnel. All studies will take place under the supervision of the Principal Investigator or a collaborating physician. Additionally, the PET scanner is located within the hospital where advanced life support personnel and equipment are available for immediate use.

Radiation Exposure: This research project involves radiation exposure to participants from the i.v. injection of $^{64}$Cu-DOTA-ECL1i. The total amount of radiation exposure received from one 10 mCi injection is 0.64 rem. Additional radiation exposure from 2 CT scans for attenuation correction will result in an additional 0.27 rem of exposure (including dose from positioning topogram – 0.09 rem from 1 bed dynamic scan + 0.18 rem from 2 bed limited scan) Total radiation exposure from one 10 mCi injection of 64Cu-DOTA-ECL1i and 2 limited low dose CT scans for attenuation correction is 0.91 rems or approximately 18% of the annual dose for a radiation worker.

The patients entered on this study will not directly benefit from $^{64}$Cu-DOTA-ECL1i-PET/CT imaging, the overall and long-term benefits to future patients with head and neck cancer far outweigh the minimal risks anticipated. The minimal risks should be far outweighed by the

potential benefit to society. It is anticipated that this study will contribute to the understanding of the performance of the $^{64}$Cu-DOTA-ECL1i-PET/CT in evaluating CCR2 expression in head and neck cancers that will have important impact on the selection of the patients for appropriate mode of therapy. Ultimately, $^{64}$Cu-DOTA-ECL1i-PET/CT may provide clinically important information to predict/assess the effectiveness of CCR2-targeted therapy.

The results of the PET imaging studies to be performed as part of the proposed research are not intended to be used in the diagnostic evaluation or treatment planning of individual patients. The results of the PET studies will not be provided to the patient or the treating oncologist or surgeon unless the CT images demonstrate an unsuspected, potentially life-threatening abnormality that warrants further investigation and/or urgent therapy. Dr. Dehdashti will assess all imaging results in light of other clinical and radiologic data and will inform the responsible attending physician as soon as possible if the information derived from the experimental imaging studies is judged to be of critical importance to the care of individual patients.

### 6.9.0 Record Keeping

It is anticipated that 20 subjects will be entered into this study. Initial review of images for quality and uptake will occur in real time with additional analysis performed in batches or after enrollment has been completed. The pathological analysis of the CCR2 will be performed after study enrollment has been completed on the standard of care specimens obtained at surgical resection or from diagnostic biopsy. Data collected to ensure the safety profile of $^{64}$Cu-DOTA-ECL1i is accurate will be assessed from the time prior to study drug injection through imaging discharge.

A patient file will be kept in the Research Coordinators' suite located on the 10$^{th}$ floor of the Mallinckrodt Institute of Radiology in Barnes-Jewish Hospital South Campus. All digital data associated with $^{64}$Cu-DOTA-ECL1i-PET/CT is maintained on the PET acquisition computer. Raw data files are backed up on password protected encrypted external hard drive(s). All imaging data may also backed up on DVD after it has been transferred to a desktop PC. Processed data will be stored on the Principal Investigator's password protected desktop. CRF's and patient files will be stored in the study coordinators' office. Entrance to the PI's and coordinators' offices requires admission through two separate locked doors. Standard laboratory assessment (CBC + CMP) will be processed locally with results added to the subjects electronic medical record. ECG's are obtained for study purposes only and are not included in the electronic medical record.

### 6.10.0 Regulatory and Reporting Requirements

The entities providing oversight of safety and compliance with the protocol require reporting as outline below. Please refer to Appendix A for definitions and Appendix B for grid of reporting timelines. Adverse events will be tracked from start of treatment through study discharge. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

- Baseline adverse events, which shall be recorded on the appropriate study CRF
- Events related to surgery or any testing or procedures performed in preparation for surgery

Reporting requirements for Washington University study team may be found in Section 6.10.1

### 6.10.1 Sponsor – Investigator / WU PI Reporting Requirements

#### 6.10.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University)

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions unless they meet the definition of unforeseen imaging exception as described in Appendix A must be obtained prior to implementing the change.

#### 6.10.1.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Washington University Sponsor Investigator / PI (or designee) is required to notify the QASMC of any unanticipated problems involving risks to participants or others occurring at WU or any BJH or SLCH institution that have been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

#### 6.10.1.3 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC. It is the responsibility of the Washington University principal investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix A for definitions) no later than 7 calendar days after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix A) no later than 15 calendar days after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix A) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
  - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
  - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
  - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than 15 calendar days after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than 15 calendar days after it is determined that the information qualifies for reporting.

- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within 15 calendar days after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

### 6.10.2 Exceptions to Expedited Reporting

Events that do not require expedited reporting as described in Section 1.1 include:

- planned hospitalizations
- hospitalizations < 24 hours
- respite care
- events related to disease progression
- Unforeseen imaging exceptions as described in Appendix A

Events that do not require expedited reporting must still be captured in the study files/ case report forms.

## 6.11.0 Data and Safety Monitoring

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least one patient has been enrolled) or one year after accrual has opened (if no patients have been enrolled at the six-month mark).

For imaging studies, the Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date

- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy (phase I studies only if efficacy is objective of the protocol)
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Power analysis and/or interim analysis (if described in the protocol)
- Summary of toxicities separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

### 6.12.0 Data Submission Schedule

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

| Case Report Form | Submission Schedule |
|---|---|
| Eligibility check List | Prior to registration |
| Original Consent Form | Prior to registration |
| PET Imaging Form | Real Time completion of events as they occur |
| PET Imaging Follow up Form | Real time to comply with protocol requirements |
| Adverse Event Log | After follow up has been completed |
| PI Data Safety Review | After follow up and AE forms have been completed |
| Tissue Collection Form | At time of surgery or requested after follow up from standard of care biopsy sample |
| Image Analysis Form | Per PI Schedule |

### 6.13 Study Calendar

| Procedure | Baseline[(a)] | Imaging[(b)] | Follow up Phone Call/Visit [(d)] | Tissue Collection |
|---|---|---|---|---|
| **Informed Consent** | **X** | | | |
| **Medical History** | **X** | | | |
| **Radiology Reports** | **X** | | | |
| **Pathology Report(s)** | **X** | | | |
| **Blood for Laboratory/ Serum Stability & Metabolite Analysis** | **X[(c)]** | **X** | | |
| **Vital Signs[(d)]** | **X** | **X** | | |
| **ECG[(e)]** | **X** | **X** | | |
| **PET/CT Imaging** | | **X** | | |
| **AE Assessment** | **X** | **X** | **X** | |
| **SOC Tissue Collection [(f)]** | | | | **X** |

(a) Baseline is defined as period from initial subject consent until injection of $^{64}$Cu-DOTA-ECL1i Baseline for vital signs and ECG is within approximately 30 mins prior to injection of $^{64}$Cu-DOTA-ECL1i. Baseline blood for laboratory samples is within 21 days up to immediately prior to $^{64}$Cu-DOTA-EL1i administration.

(b) Imaging day is defined as period of time from injection of $^{64}$Cu-DOTA-ECLi1 through completion of imaging on that day.

(c) Baseline lab is CBC/CMP prior to study drug injection. Serum Stability /Metabolite samples are drawn post injection and post scan #1 whenever possible. Discharge labs are repeat CBC/CMP drawn at least 60 minutes post injection.

(d) Follow up will occur at least 24 hours following administration of 64Cu-DOTA-ECL1i optimal follow up time is 48-120 hours however follow up as soon as 24 hours and as late as 14 days is acceptable.

(e) To be performed prior to and at least 60 min after administration of 64Cu-DOTA-ECL1i.

(f) Fresh tissue is collect at the time of surgical resection. If other therapy is planned tissue or slides will be requested from standard of care biopsy samples.

## 6.14 References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## APPENDIX A: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event

- 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
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

**Unforeseen Imaging Exception:** Research imaging protocols which involve the injection of radioactive tracers can produce unique situations not common to standard treatment protocols. In the event a situation occurs which requires deviation from this protocol – for example less than expected tracer production, problems with the scanner, patient unable to tolerate the imaging protocol as described, the Principal Investigator will have final authority over whether or not a study is completed. Any protocol deviations will be documented on the PET imaging data form. Deviation such as less than expected tracer production can be accounted for during data analysis and will not necessarily result in cancellation of the scan. Similarly, adjusting imaging procedures to accommodate patient comfort can also be accounted for at the time images are reviewed.

Except as described above, pre-approval of all protocol exceptions must be obtained prior to the event

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

**APPENDIX B: Reporting Timelines**

| Expedited Reporting Deadlines | | | |
|---|---|---|---|
| **Event** | **HRPO** | **QASMC** | **FDA** |
| Serious AND unexpected suspected adverse reaction | | | Report no later than 15 calendar days after it is determined that the information qualifies for reporting |
| Unexpected fatal or life-threatening suspected adverse reaction | | | Report no later than 7 calendar days after initial receipt of the information |
| Unanticipated problem involving risk to participants or others | Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. | Report via email after IRB acknowledgment | |
| Major deviation | Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. | | |
| A series of minor deviations that are being reported as a continuing noncompliance | Report within 10 working days. | | |
| Protocol exception | Approval must be obtained prior to implementing the change | | |
| Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB | | | Report no later than 15 calendar days after it is determined that the information qualifies for reporting |
| Complaints | If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review. | | |

| Expedited Reporting Deadlines | | | |
|---|---|---|---|
| **Event** | **HRPO** | **QASMC** | **FDA** |
| Breach of confidentiality | Within 10 working days. | | |
| Incarceration | If withdrawing the participant poses a safety issue, report within 10 working days.<br><br>If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review. | | |

| Routine Reporting Requirements | | | |
|---|---|---|---|
| **Event** | **HRPO** | **QASMC** | **FDA** |
| Adverse event or SAE that does not require expedited reporting | If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review | Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months. | The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report. |
| Minor deviation | Report summary information at the time of continuing review. | | |
| Complaints | If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review. | | |
| Incarceration | If withdrawing the participant poses a safety issue, report within 10 working days.<br><br>If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review. | | |