Clinical Study Protocol

RefleXion Medical

Performance and Safety of Biology-Guided Radiotherapy using the RefleXion Medical Radiotherapy System (BIOGUIDE-X)

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Protocol Synopsis

Study Objective	
Primary Objectives	To identify the Recommended RefleXion FDG Dose (RRFD) that enables the use of biology-guided radiotherapy (BgRT) on the RefleXion system. (Cohort I: RRFD) To determine whether BgRT dose distributions generated from Limited Time Sample (LTS) Positron Emission Tomography (PET) images obtained at the time of treatment delivery are consistent with the approved BgRT plan. (Cohort II: Emulated Delivery)
Study Device	RefleXion Medical Radiotherapy System IDE device
Device Description	The RefleXion Medical Radiotherapy System (RMRS) is a hybrid imaging-therapy system that is designed to facilitate delivery of biology-guided radiotherapy (BgRT). The system uses PET emissions to guide radiotherapy delivery in real-time and consists of 6 MV photon radiotherapy delivery, PET imaging, kilovoltage (kV) X-ray CT imaging, MV X-ray detection, and treatment planning subsystems.
Study Design	
Study Design	Open-label, prospective study.
Planned Number of Subjects	Cohort I - RRFD: 6 to 12 subjects (3 to 6 bone tumors, 3 to 6 lung tumors) Cohort II - Emulated Delivery: 8 to 22 subjects (4 or more bone tumors, 4 or more lung tumors)
Planned Number of Sites	1 to 3 US sites
Study Duration	Enrollment: approximately 6 months Subject participation: Cohort I – RRFD: approximately 2 weeks Cohort II - Emulated Delivery: approximately 4 weeks Total study duration: approximately 9 months
Primary Endpoints	Cohort I: 1. Recommended RefleXion FDG Dose (RRFD): The FDG dose that results in Activity Concentration necessary for BgRT functioning: 5 kBq/ml or higher. Cohort II: 2. The percent of radiotherapy fractions where the emulated BgRT dose distribution *in silico* is shown to be consistent with the approved BgRT treatment plan (i.e., 95% of $DVH_{Delivered}$ points for the BTZ and OAR fall within bounded DVH of the approved BgRT plan).
Secondary Endpoints	Cohort I: 1. Percent of cases where there is agreement between a site investigator and the agreement standard for the BgRT PET Imaging-only session localization decision (overall percent agreement). Positive percent agreement and negative percent agreement will also be reported. 2. Percent of cases where there is concordance of a positive “plan proceed” decision between the BgRT Imaging-only session PET and a cleared, third-party diagnostic PET/CT (positive percent agreement). Overall percent agreement and negative percent agreement will also be reported.

	3. Percent of cases where RefleXion PET data can be used to generate an acceptable BgRT plan such that dosimetric parameters for the target and the nearby normal anatomy are met based on investigator assessment. 4. Percent of cases where the intended dose distribution of the BgRT plan is achieved in a physical phantom, defined as meeting a standard gamma index for external beam radiotherapy quality assurance, i.e. whether 90% of pixels meet the 3mm/3% deviation standard. Cohort II: 1. Percent of fractions where there is concordance between the physical and digital phantoms of emulated BgRT delivery derived from human subject PET emissions. Concordance is defined as a standard gamma index with a goal that 90% of pixels meet the 3mm/3% deviation standard. 2. Percent of cases where there is agreement between a site investigator and the agreement standard for the BgRT PET PreScan localization decision (overall percent agreement). Positive percent agreement and negative percent agreement will also be reported. 3. Percent of cases where there is concordance of a positive localization decision between the short-duration PET PreScan and a third-party diagnostic PET/CT scan (positive percent agreement). Overall percent agreement and negative percent agreement will also be reported. 4. Safety of multiple FDG administrations and toxicity rates of bladder and bone marrow assessed by complete blood count, urinalysis and AEs specific to bladder and bone marrow determined by Common Terminology Criteria for Adverse Events (CTCAE) v5 at 72±24 hours after final FDG injection. 5. Workflow characterization: a. Percent of PET imaging sessions at RRFD that meet the Activity Concentration threshold for BgRT (5 kBq/mL) b. Percent of PET imaging sessions which lead to acceptable BgRT plans, with acceptability based upon meeting user-defined coverage goals for tumor targets and avoidance goals for OARs c. Percent of approved BgRT plans that go on to pass physics quality assurance, as defined by 90% of pixels meeting the 3mm/3% deviation standard d. Percent of PET Evaluations on the day of fraction delivery that elicit a “Pass” signal
Key Inclusion Criteria	1. Age greater than 21 years 2. A new or prior diagnosis of biopsy-proven cancer with a solid tumor (non-hematologic, non-lymphoma) 3. At least one active tumor in the bone or lung which is either the primary tumor or metastatic lesion determined either by biopsy or imaging suspicious of active disease 4. Target tumor size ≥2cm and ≤5cm 5. Target lesion in the bone or lung that is discrete and assessed by investigator to be FDG-avid (i.e. SUV_{max}≥6 on third-party diagnostic PET/CT performed within 60 days with no new intervening oncologic therapies) 6. ECOG Performance Status 0-3 7. Must have completed any other oncologic therapies at least 15 days prior to planned start of study procedures (preferably 30 days) and must have no plans to initiate systemic therapy until after study follow up is complete -OR- must be

	recorded by physician to have an active candidate lesion that is unresponsive to ongoing systemic therapy 8. Females of childbearing potential should have negative urine or serum pregnancy test within 14 days prior to initiation of study scans. 9. Demonstrate adequate organ function: determined by ANC, platelets, hemoglobin, with no gross hematuria 10. For Cohort II only: Patient is dispositioned to undergo SBRT to a bone or lung tumor
Key Exclusion Criteria	1. Clinically significant blood glucose abnormalities that preclude a satisfactory FDG PET/CT scan. 2. Previous history of external radiotherapy where prior radiotherapy fields are anticipated to overlap with the radiotherapy fields required for the present study 3. Diffuse metastatic process (leptomeningeal disease, peritoneal carcinomatosis, diffuse bone marrow involvement, etc.) 4. PET-avid structures not intended for radiation are within 2cm from target on third-party diagnostic PET/CT as assessed by investigator 5. Known allergy to FDG 6. Known psychiatric or substance abuse disorder that would interfere with conduct of the study 7. Pregnant, breast-feeding or expecting to conceive during the study 8. Patient weight exceeding the weight limit outlined per IFU. 9. For Cohort II only: Patients with pacemakers and other implantable devices who are deemed to be at high risk by the treating physician for complications secondary to radiotherapy. 10. For Cohort II only: Patients with bone lesions who are determined to be high risk by the treating physician for pathologic fracture prior to beginning radiotherapy. 11. For Cohort II only: Active inflammatory bowel disease, scleroderma, or other disorder deemed to be a risk factor for excess toxicity in the area of treatment by the treating physician.
Baseline Assessments and Follow-Up Schedule	<u>Baseline Assessments within fourteen days prior to the PET Imaging-only session:</u> 1. Demographics, medical history & physical exam 2. Complete Blood Count (CBC) 3. Comprehensive Metabolic Panel (CMP) 4. ECOG performance status 5. Blood glucose 6. Urinalysis 7. Pregnancy test (required for women of child-bearing potential only; within 14 days prior to the initiation of the study scans.) <u>Cohort I Follow-Up (at 72±24 hours after completion of PET Imaging-only session)</u> 1. AE assessment by using Common Terminology Criteria for Adverse Events (CTCAE) v5 <u>Cohort II Follow-Up (at 72±24 hours after completion of final FDG administration)</u> 1. Safety of multiple FDG administrations and toxicity rates of bladder and bone marrow assessed by complete blood count and urinalysis 2. AE assessment by using Common Terminology Criteria for Adverse Events (CTCAE) v5

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Attachment 1: IDE Study Event Schedule

List of Abbreviations

AE	Adverse Event
ALARA	As Low As Reasonably Achievable
BgRT	Biology-Guided Radiotherapy
BTZ	Biology-Tracking Zone
CT	Computed Tomography
CTV	Clinical Target Volume
CTCAE	Common Terminology Criteria for Adverse Events
FDG	F18-Fluorodeoxyglucose
GTV	Gross Tumor Volume
IB	Investigator's Brochure
IGTV	Internal Gross Tumor Volume
IPTV	Internal Planning Treatment Volume
ITV	Internal Tumor Volume
kVCT	Kilovoltage Computed Tomography
LTS	Limited Time Sample
MLC	Multi-leaf Collimator
NTS	Normalized Target Signal
OAR	Organ-at-risk
PET	Positron Emission Tomography
PTV	Planning Treatment Volume
SAE	Serious Adverse Event
SBRT	Stereotactic Body Radiotherapy
SMC	Safety Monitoring Committee
RMRS	RefleXion Medical Radiotherapy System
RRFD	Recommended RefleXion FDG Dose
UADE	Unanticipated Adverse Device Effect

1. Background and Rationale

1.1 Biology-Guided Radiotherapy: Core Principle and Clinical Benefit

The RefleXion Medical Radiotherapy System (RMRS) is a hybrid imaging-therapy system that is designed to enable delivery of biology-guided radiotherapy (BgRT), a novel radiotherapy technique that aims to improve the conformality and precision with which external beam radiotherapy is delivered to malignant lesions, including those that are in motion. This system functions by utilizing outgoing Positron Emission Tomography (PET) emissions emanating from a tumor to direct beamlets of external radiotherapy back to the lesion with sub-second latency. This core process can be repeated until a desired total dose of external radiation is delivered to a CT-defined target volume anchored in space to the PET profile of the tumor. In this way, the PET profile of the tumor acts as an endogenous biological fiducial.

BgRT is expected to improve radiation delivery for two reasons. Firstly, the use of the lesion's own PET profile as a fiducial enables increased confidence in lesion localization during therapy, which in turn allows the radiation oncologist to reduce positional margins at treatment planning that account for set-up error and patient shifts; said differently, BgRT improves the *conformality* of radiotherapy dose to the lesion by creating conditions of plan delivery that compensates for a static shift at the time of treatment. The benefit of reduced margins around gross disease extent is that the surrounding normal tissue is exposed to less radiation, including at the high ablative dose range, which may result in a lower burden of acute toxicity and long-term morbidity.

Secondly, BgRT addresses the challenge of *motion management* for mobile tumors by enabling the therapeutic beam to direct beamlets of radiation with sub-second (350-400 ms) latency. The aggregate effect of delivering a series of radiation beamlets this way is to effectively deliver a tracked dose distribution. This is a beneficial departure from current internal target volume (ITV) techniques that require ablation of the entire envelope of a tumor's motion path plus margin in order to ensure tumor coverage at all times[1–3]. The fact that ITV approaches require more ablation of normal tissues to ensure coverage during tumor motion comes at the cost of clinically-evident patient toxicities arising from those injured tissues[4–7].

In summary, by using a tumor's own biology as a fiducial signal, BgRT has the potential to improve the therapeutic index of radiotherapy through improved conformality and by enabling better motion management via tracked dose distributions that compensate for natural processes like respiration.

1.2 The Biology-Guided Radiotherapy Algorithm

In traditional treatment planning, the desired dose to the target and constraints to normal tissue are met by optimizing for a set of machine-deliverable radiotherapy fluences that are delivered from many pre-determined angles around the patient. The downside of this rigid approach is that the ablative radiotherapy must be directed to significant margins around the target or even the target's entire path of motion in case there is variation in the target's position or motion at the time of treatment. BgRT improves upon this technique by enabling radiotherapy dose to be tailored to the target's position and motion at the time of treatment delivery, which in turn may reduce the amount of normal anatomy that must be incidentally subjected to ablative radiotherapy. For achieving this outcome, the BgRT algorithm enables a linear accelerator system to capture sub-second PET acquisitions from a target, which are

termed limited-time sample (LTS) PET images, and to respond with beamlets of radiotherapy (partial fluences). This cycle is then repeated until the full intended dose of external beam radiotherapy is delivered to the target. Importantly, the portion of the LTS PET images used to guide beamlets is confined to a physician-defined anatomic region, termed the Biology-tracking zone (see section 1.3.2), that corresponds to the motion envelope of the target tumor. This feature prevents radiotherapy beamlets from being directed at PET emissions originating away from the tumor.

Given the reactive nature of the beam delivery, the BgRT algorithm cannot optimize for machine-deliverable fluence directly because rigid predetermination of machine instructions would not be capable of adjusting to a fluctuating LTS PET profile. Instead, the BgRT algorithm optimizes for fluence *indirectly* by calculating a transfer function, termed the firing matrix, that can translate a target's PET profile into the desired fluence. To achieve this, the BgRT algorithm relies on a full PET dataset captured ahead of BgRT delivery to calculate an operator, termed a "firing filter" - that can convert this full PET image into a complete radiotherapy fluence that meets the physician's objectives for target coverage and organ-at-risk avoidance. This optimization is similar to intensity-modulated radiotherapy (IMRT) algorithms that rely on a standard cost function that can iterate among candidate firing filters until those physician-defined objectives are best optimized.

After the firing matrix is calculated using a full PET image, it can then be applied to LTS PET images to generate partial fluences. Importantly, adding all the partial fluences generated from a series of sequentially obtained LTS images is mathematically expected to sum to the complete fluence goals of the physician. This follows from the fact that the firing matrix is confined by the algorithm to take the form of a linear, shift-invariant operator, which in turn means that the principle of linear superposition applies to the conversion of LTS PET images to partial fluences. This principle ensures that, just as the sequential LTS images sum to a full PET image, the partial fluences will sum to the complete intended fluence. *Figure 1* is a visual representation of this principle.

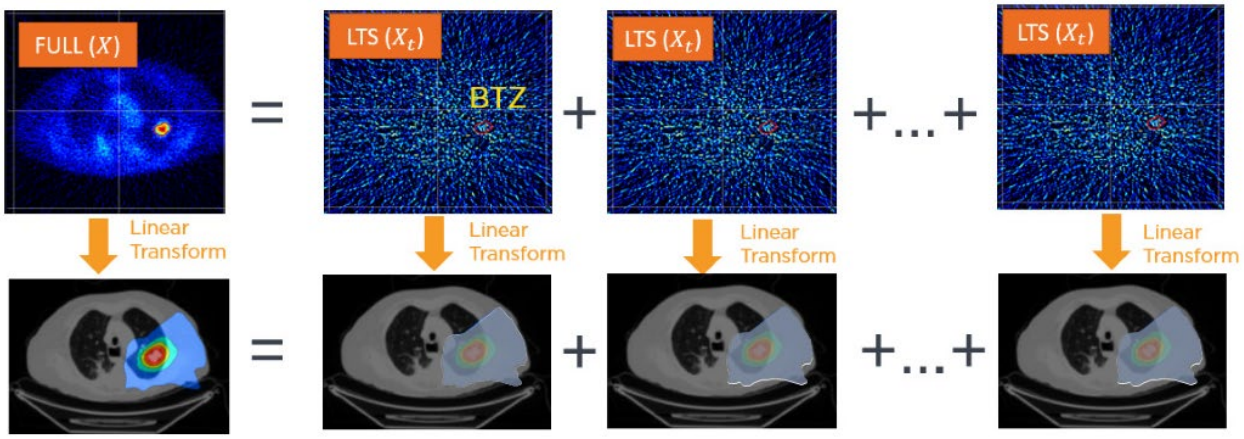

Figure 1. BgRT principle of linear superposition. Just as the limited-time sample (LTS) images, Xt, sum to the full PET image X, the derived partial fluences sum to the complete intended fluence. BTZ: Biology-tracking zone

Finally, a tumor's PET profile and, by extension, its constituent LTS images can vary from one radiotracer administration to the next due to a variety of biological, physiological, and logistical factors. Therefore, BgRT treatment planning algorithm generates a range of possible radiotherapy dose distributions to reflect these possible variations in tumor motion and tumor contrast. As described below, prior to every radiotherapy delivery, the system checks that the PET profile on that day (and the resulting fluence) fall within that spectrum, or otherwise the treatment must be aborted. Of note, preliminary evidence suggests that variations in PET profile over the course of standard ablative radiotherapy are expected to be modest, at least in the case of FDG[8].

1.3 BgRT Workflow

The basic workflow for conventional radiotherapy acts as a foundation for BgRT. In essence, the BgRT workflow includes the typical composition of processes observed in radiation oncology along with additional steps to accommodate the introduction of PET information. These processes include the core steps of prescription, simulation, treatment planning, and treatment delivery[9–12]. For context BgRT treatment delivery directed at a patient is described in section 1.3.3 below as part of the envisioned BgRT product, but the study investigation replaces this step with emulated delivery, which establishes anatomic dose distribution using offline tools, as later described.

Summary of core workflow steps used in conventional radiotherapy:

1. Prescription: The radiation oncologist determines that a patient requires radiotherapy as part of their cancer care. The clinician then selects the target, radiation dose, fraction size, and schedule for the treatment. The prescription process also includes specifying the tolerance limits for normal tissues near the target, which are also known as organs-at-risk (OAR).

2. Simulation: The patient is placed in a CT simulator in a desired position along with immobilization devices like customized cradles or head-masks to reduce shifts. CT images are obtained in that position and used to plan treatment. In modern radiotherapy, four-dimensional (4D) CT image sets are often acquired to capture the differing positions of tumor across natural processes such as the inhalation-exhalation respiratory cycle.

3. Treatment Planning: The radiation oncologist outlines targets and OARs on the simulation CT image, a process also known as "contouring". Contouring is followed by radiation delivery planning which entails *in silico* modeling of external beam delivery. This modeling includes the selection of differing particle types, particle energies, beam angles and beam intensities to achieve desired goals for target coverage and OAR avoidance. The approved *in silico* plan is then verified independently in a process called "quality assurance" (QA). During QA, the linear accelerator actually delivers the approved treatment plan into a physical phantom that can measure the delivered dose and distribution of radiation. The measured dose and distribution is checked to ensure that it is concordant to an *in silico* model of plan delivery into a digital facsimile of the physical phantom.

4. Treatment Delivery: The patient is set-up and immobilized on the linear accelerator couch in the same position as the CT simulation. Alignment is verified using external markers and on-board imaging, and then the approved radiation plan is delivered from the linear accelerator. Each such treatment is called a fraction. This step is repeated until all planned fractions of the radiotherapy course have been delivered. Every five fractions, the radiation oncologist typically conducts an interview with the patient to address patient concerns and manage toxicities.

BgRT adds to this conventional workflow by introducing PET into two key steps of the workflow: The PET Imaging-Only Session during treatment planning and the PET PreScan and LTS image acquisition at treatment delivery. These BgRT modifications to the conventional radiotherapy workflow are detailed below.

1.3.1 Prescription

At the time of the patient's initial encounter with a radiation oncologist, the patient must be deemed a candidate for biology-guided radiotherapy. As with conventional radiotherapy, this requires that the radiation oncologist first make a determination that radiotherapy itself is indicated. The radiation oncologist also specifies the usual parameters of the prescription: Dose, target volumes, coverage goals, and avoidance parameters for OARs. The next decision point for biology-guided radiotherapy is for the clinician to determine whether BgRT might confer therapeutic advantages to the patient via improved conformality and motion management compared to conventional radiotherapy. If so, the radiation oncologist can review any previously obtained diagnostic PET/CT images to determine *qualitatively* whether the target lesion appears to have adequate FDG activity over background, such that a more rigorous, *quantitative* assessment of BgRT candidacy on the RefleXion system itself is warranted.

General guidance for adequate FDG avidity for BgRT candidacy, as observed on a previously-obtained diagnostic PET/CT, is that the maximum tumor SUV is 6 or higher. The physician can also consider qualitative factors such as the relative positions of OARs with FDG uptake to the tumor and the contrast of tumor to the background tissue. Again, it is important to emphasize that this assessment is meant to act as a first screen of candidates, and that formal assessment of BgRT candidacy is made on the RefleXion device.

1.3.2 Simulation and Treatment Planning

If BgRT candidacy is considered, the patient must then undergo a simulation process for radiotherapy treatment planning that collects necessary PET emission data on the RefleXion system. In the workflow, this requires that the patient first undergo a traditional CT simulation which is then followed by a new PET imaging step on the RefleXion system, labeled the PET Imaging-Only Session. In this step, RMRS PET emission data is collected in the same treatment position as was established during the anatomic CT simulation.

The sequencing of CT simulation prior to RMRS PET Imaging-Only Session is necessary because the PET Imaging-Only Session requires an overlay of (4D)CT-defined contours, which act to narrow down the anatomic location for PET emission collection. Specifically, a Biology-tracking zone (BTZ) defined as the motion envelope of the tumor on the (4D)CT image set plus a margin must be delineated ahead of the

PET Imaging-Only Session in order to localize where in the body the target tumor is located. In addition to the BTZ, other CT-defined tumor targets (GTV, CTV, PTV) and nearby organs-at-risk (OARs) are also contoured based upon the CT simulation images.

At the PET Imaging-Only Session itself, the patient is injected with FDG and, after an approximate 1-hour uptake period, is setup in the RefleXion system in the same treatment position as was used during CT simulation. The same immobilization devices used during the CT simulation are likewise deployed. Next, the patient's alignment is fine-tuned with CT-guidance using RefleXion's on-board fanbeam kVCT, and the patient is moved on the treatment couch such that the PET detector arcs on the RefleXion system are aligned with the region of interest containing the target tumor.

Next, the PET detector arcs capture PET emission data and generate a PET image. Of note, the time window for imaging relative to the injection must be controlled so that the activity is not too high, which could saturate the detectors, or too low secondary to FDG decay, which would result in inadequate PET activity for BgRT. After the PET emission data is collected, several steps occur to formally assess for BgRT candidacy and, if appropriate, to generate a high quality BgRT treatment plan.

At the RMRS Device:

1. The patient's (4D)CT-defined BTZ is overlaid on the RMRS PET image. This overlay is enabled by the prior CT alignment step, which indexes the CT anatomy obtained in the treatment position on the day of the PET Imaging-Only Session to the CT anatomy obtained previously at CT simulation. Once the PET image is formed, the radiation oncologist confirms that the tumor in the PET image can, in fact, be visualized and localized to within the BTZ as expected.

   Notably, the BTZ overlay is a volume based upon (4D)CT images generated on a commercial, third-party Sim CT device. Therefore, in addition to capturing PET information for the BgRT algorithm, this step also acts to verify the location accuracy of the RMRS PET image as compared to an external reference standard.

   The patient may leave the department once the RMRS PET image is acquired and validated by the radiation oncologist. Provided that localization/visualization of the tumor PET image within the BTZ is confirmed by the radiation oncologist, the PET data is then transmitted to the treatment planning system (TPS) for the next steps.

At the Treatment Planning System Console:

2. Specific quantitative parameters of the tumor PET image are calculated by the treatment planning software to determine whether BgRT is a viable option for BgRT planning and treatment delivery.

   These quantitative criteria for BgRT candidacy include:

   a. Activity Concentration: Greater than 5 kBq/ml

b. Tumor signal-to-background ratio as measured by the Normalized Target Signal (NTS): Greater than 2.7

Notably, these values are indexed to RefleXion's software and hardware architecture as well as the patient's radiotherapy treatment position. Therefore, they cannot be extrapolated from a third-party PET/CT.

3. The physician also qualitatively assesses the following:

   a. FDG-avid OARs are outside the BTZ

   b. No FDG-avid structures aside from the tumor are in the BTZ.

If all of the qualitative and quantitative criteria are met, the PET emission data from within the BTZ can be incorporated into the treatment planning software for the BgRT algorithm's dose calculations. As described in detail in the Investigator Brochure (IB) and summarized in section 1.2, the algorithm acts to optimize a firing matrix that converts PET projections to a machine-deliverable fluence such that the fluence in turn meets traditional radiotherapy dose objectives, such as coverage goals for the target and avoidance metrics for OARs.

An important feature of a BgRT treatment plan is that it presents the clinician with a bounded dose volume histogram (DVH), which is significantly more detailed than a traditional dose volume histogram used in conventional radiotherapy systems. In a bounded DVH (bDVH), the nominal plan based on the tumor's PET appearance on the day of the imaging session is indicated graphically by solid lines as per the usual DVH format. However, the bDVH also includes bands around these lines, which are calculated in order to visualize how dose and coverage would change in response to variations in tumor PET signal or tumor motion encountered on the day of treatment.

To complete treatment planning, the clinician is recommended to create a back-up conventional SBRT plan based only on the CT simulation images, which can be used in scenarios where BgRT is unable to be delivered on the day of treatment (as described in the IB).

As with conventional workflows, both completed treatment plans – BgRT and back-up SBRT - are then finalized by a dosimetrist and reviewed by a radiation oncologist for final approval. The standard practice of reviewing anatomic isodose lines and dose volume histograms for target coverage and OAR avoidance is done with the additional nuance that BgRT requires review of a bounded DVH as described above and in the IB.

Next, standard quality assurance (QA) is performed by a qualified medical physicist, which consists of the approved plans undergoing a dry run on the linear accelerator with a physical phantom placed on the couch to measure the dose and distribution of actually delivered therapeutic radiation[13]. The measured dose is compared to *in silico* modeled delivery of the BgRT plan into a digital scan of the test phantom. For BgRT, confirmation that the physical delivery and modeled delivery are concordant is assessed using standard gamma index methods. Of note, the phantom test utilizes the PET information used by the BgRT plan and assumes the target is static. Once quality assurance is complete, the BgRT plan receives a final

approval from the medical physicist and radiation oncologist. The patient is then scheduled to return to the radiation oncology department to initiate treatment.

1.3.3 Treatment Delivery

At each treatment fraction, the patient is injected with FDG, and the proper uptake period must elapse. The patient is then set up in the correct treatment position with deployment of the same immobilization devices used during both the CT simulation and the PET Imaging-Only Session. The patient's alignment is fine-tuned using CT guidance with RefleXion's on-board fanbeam kVCT. As was the case during the imaging session, this alignment is made possible by indexing the on-board CT images to the images acquired at CT simulation. The (4D)CT-defined BTZ is also again overlaid on the indexed images.

Next, a PET PreScan is obtained, which represents a second new step introduced by the BgRT workflow. The PET PreScan image analysis, termed the PET Evaluation, is a critical safety interlock wherein the RefleXion system checks whether the pattern and quantitative characteristics of PET emissions observed in the BTZ on the day of treatment are consistent, within a tolerance threshold, to the PET emission profile observed during the PET Imaging-Only Session (upon which the BgRT plan is based). As with the PET Imaging-Only Session criteria, the checked parameters are calibrated to the RefleXion system hardware and software and cannot be meaningfully extrapolated from a different vendor's system.

The specifics of the PET PreScan process are as follows. Firstly, the PreScan itself, which is a short-duration RMRS PET acquisition, is performed to generate a PET image of the anatomic region containing the target. The user reviews the image set to visually confirm that the tumor PET image is localized within the (4D)CT-defined BTZ. The user can also assess whether FDG-activity from neighboring OARs remains outside of the BTZ. Both of these localization features need to be verified in order to continue with BgRT. In addition to the PET image review, CT images from the alignment process can provide further confirmation of the relative positions of targets and OARs as is done in conventional radiotherapy.

Next, the RefleXion system quantifies characteristics of the PET emission data from the PET PreScan to assure consistency with the PET imaging session. This is a critical safety interlock that occurs prior to activating the linear accelerator for BgRT delivery. These quantitative criteria include:

1. Confirmation of Activity Concentration greater than 5 kBq/ml

2. Confirmation that the tumor NTS larger than a threshold of 2, which allows for a value that is 25% lower than the NTS observed at PET Imaging-Only Session

3. Predicted DVH: The RefleXion system calculates a BgRT dose distribution that is anticipated to be delivered based upon the short-duration emission profile of the PET PreScan. This Predicted DVH of the BTZ and OAR distribution ($DVH_{Predicted}$)is confirmed to be "within the bounds" of the bDVH in the approved BgRT plan. This calculation involves confirming that 95% of the points on the $DVH_{Predicted}$ for the BTZ and OARs fall within the approved bDVH.

If, in conjunction with visual localization of the tumor within the BTZ, all of these quantitative requirements for PET emission quality are met, then the user is allowed to proceed with BgRT delivery.

This means that the PET detectors are instructed to collect LTS PET emission data, and the linear accelerator and multi-leaf collimator (MLC)'s are instructed to direct partial fluences to the target in response to those LTS images in a continuous closed-loop system directed by the BgRT algorithm.

If any of the above quantitative parameters are not met, then the user is locked out of using BgRT during that session and has the choice of either rescheduling therapy or substituting another mode of radiotherapy (i.e. SBRT) for that fraction. The reasons for not meeting the parameters may be due to intrinsic changes in the PET profile of the tumor or surrounding OARs, but may also be due to process issues, examples of which include misadministration of FDG at the time of injection or administration of a batch of FDG with poor activity due to manufacturing errors upstream at the FDG vendor [14].

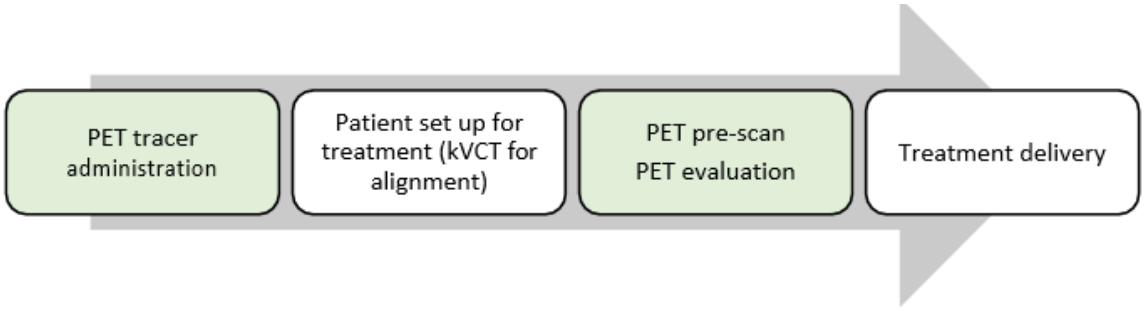

Figure 2. BgRT delivery workflow.

To complete the course of treatment, the patient undergoes as many fractions of biology-guided radiotherapy as prescribed by the radiation oncologist. The PET PreScan is required before each fraction of therapy. A weekly on-treatment visit is also recommended as per standard radiotherapy practice.

As mentioned previously, this description refers to the envisioned BgRT product where treatment delivery is directed at the patient. This IDE study does not direct the external radiation at the patient because *in vivo* measurement of dose delivery accuracy is not feasible. Instead, as detailed in sections 4 and 6, the study utilizes PET datasets from enrolled patients to predict and measure anatomic dose distribution using offline software tools and phantoms, an investigational technique that is termed "emulated delivery".

1.4 FDG Administration in the Context of BgRT

The initial radiotracer for use in the RefleXion system is FDG, because this agent is a widely available and well-understood diagnostic tracer that accumulates in many cancer cell types due to their shared biological feature of abnormal glucose uptake. As described, the BgRT workflow utilizes FDG at multiple steps including at the PET Imaging-Only Session in the context of simulation and treatment planning as well as during each fraction of radiation delivery as characterized by the PET PreScan and the active functioning of BgRT external beam delivery. Because biology-guided radiotherapy will be used to deliver ablative doses of radiation, which typically require 1 to 5 fractions of therapy, one can expect that BgRT regimens will require 2 to 6 injections of FDG: 1 injection for the PET imaging session and 1 injection for each treatment fraction.

1.5 BgRT Evidence Landscape: Technical and Clinical Evidence

In general, radiotherapy device validation evidence can be either Technical or Clinical.

The technical evidence includes experiments conducted outside a clinical context, such as dosimetric and physics investigations, that validate performance and functioning of the radiotherapy device. This body of evidence is essential because it entails measurement of critical quantities that cannot otherwise be measured. The most important example is the use of physical phantoms, which enable the measurement of the actual dose and distribution of therapeutic radiation delivered by a linear accelerator, metrics that cannot be measured directly *in vivo*. Comparison of the actually delivered dose distribution in a phantom to a corresponding modeled dose distribution in a digital facsimile of the same phantom constitutes the core methodology for assuring linear accelerator performance.

For BgRT, technical validation follows this precedent: The measurement of actually delivered radiation dose and distribution can be evaluated directly in an FDG-avid physical phantom ("FDG-phantom") that mimics the conditions of an FDG-avid tumor. Furthermore, the accuracy of this delivery can be confirmed via comparison to modeled delivery in a digital facsimile of the FDG-phantom. Experiments such as these form the foundational base of evidence for assessing the performance of radiotherapy techniques in general, and such technical evidence remains necessary and indispensable for BgRT.

At the same time, this technical evidence can be supplemented by well-conceived clinical evidence obtained in a real-world practice setting. To that end, the present investigational device exemption (IDE) study aims to clinically validate RefleXion BgRT performance by evaluating end-to-end system functioning using human subject data obtained in the context of radiation oncology practice. The study's first primary objective is to test the PET subsystem and to identify the Recommended RefleXion FDG Dose(RRFD) that enables the use of BgRT. The second primary objective is to assess end-to-end BgRT performance by determining the final accuracy of BgRT external beam dose distributions that are based upon tumor PET emissions obtained from human subjects in the radiotherapy treatment position. Additional objectives are to comprehensively characterize all key features in the BgRT process including PET imaging performance; BgRT plan creation, approval, and quality assurance; physical deliverability of BgRT fluence; workflow performance; and safety associated with multiple FDG administrations.

Importantly, clinical validation of BgRT across the entire scope of radiotherapy indications is not feasible. Therefore, in choosing a study population, advantageous features are that the population (1) entails a degree of homogeneity in clinical characteristics to inform useful conclusions while (2) still being reasonably generalizable to a broad range of malignant conditions, especially when this clinical evidence is combined with a strong foundation of technical validation. To that end, the proposed IDE investigation focuses on two anatomic locations – the lung and osseous compartments – which together are representative of a comprehensive spectrum of scenarios for which biology-guided radiotherapy can be utilized.

Tumors in the lung are an important, representative use case for motion management in radiotherapy because they undergo excursion with respiration in all directions. Furthermore, conformality is necessary in these tumors because of their close proximity to multiple organs at risk in the thorax and upper

abdomen. Finally, lung anatomy in the context of PET imaging is generally characterized by relatively high contrast between FDG-avid tumors and the background tissue (lung parenchyma). Given these characteristics, lung tumors represent one end of the range for motion and PET contrast against which the viability of a radiotherapy modality like BgRT should be demonstrated.

In contrast, tumors in the bone are not subject to internal anatomic motion, but instead are subject to motion derived from set-up error and patient shifts during treatment. Also, the background tissue of bony lesions, which consists of soft tissues and normal bone, has on average more inherent FDG activity than lung parenchyma, which reduces the contrast between osseous tumors and their surrounding tissue. Therefore, demonstrating utility of BgRT in bony lesions addresses the opposite end of the motion management and PET contrast spectrum as lesions in the lung. Conformality remains of great importance for bone tumors as radiosensitive normal structures throughout the patient's anatomy are in proximity to the skeleton. Indeed, in modern radiotherapy, conformality is a desired feature across all anatomic geographies and clinical contexts.

In light of these features, clinical evidence derived from patients with lung and bone tumors represents a versatile set of real-world conditions with respect to motion and PET environment. In combination with technical and benchtop experiments, this IDE seeks to provide robust evidence for BgRT functioning and accuracy that can be applied to the spectrum of malignant conditions for which radiotherapy is indicated.

2. STUDY OBJECTIVES

2.1 Cohort I: RRFD Cohort

2.1.1 Primary Objective

To identify the Recommended RefleXion FDG Dose (RRFD) that enables the use of BgRT on the RefleXion system.

2.1.2 Secondary Objective

To assess the performance of the BgRT PET Imaging-only session, treatment planning and quality assurance at the studied dose level.

2.2 Cohort II: Emulated Delivery Cohort

2.2.1 Primary Objective

To determine whether BgRT dose distributions generated from Limited Time Sample (LTS) PET images obtained at the time of treatment delivery are consistent with the approved BgRT plan.

2.2.2 Secondary Objective

To emulate and confirm deliverability of the fluence associated with the BgRT dose distribution generated from LTS PET images obtained at the time of treatment delivery as well as to assess imaging, process and safety characteristics of the end-to-end workflow.

3. STUDY ENDPOINTS

3.1 Cohort I: RRFD Cohort

3.1.1 Primary Endpoint

RefleXion Recommended FDG Dose (RRFD): The FDG dose that results in Activity Concentration necessary for BgRT functioning: 5 kBq/ml or higher.

3.1.2 Secondary Endpoints

1. Percent of cases where there is agreement between a site investigator and the agreement standard for the BgRT PET Imaging-only session localization decision (overall percent agreement). Positive percent agreement and negative percent agreement will also be reported.

2. Percent of cases where there is concordance of a positive "plan proceed" decision between the BgRT Imaging-only session PET and a cleared, third-party diagnostic PET/CT (positive percent agreement). Overall percent agreement and negative percent agreement will also be reported.

3. Percent of cases where RefleXion PET data can be used to generate an acceptable BgRT plan such that dosimetric parameters for the target and the nearby normal anatomy are met based on investigator assessment.

4. Percent of cases where the intended dose distribution of the BgRT plan is achieved in a physical phantom, defined as meeting a standard gamma index for external beam radiotherapy quality assurance, i.e. whether 90% of pixels meet the 3mm/3% deviation standard.

3.2 Cohort II: Emulated Delivery Cohort

3.2.1 Primary Endpoint

The percent of radiotherapy fractions where the emulated BgRT dose distribution *in silico* is shown to be consistent with the approved BgRT treatment plan (i.e., 95% of $DVH_{Delivered}$ points for the BTZ and OAR fall within bounded DVH of the approved BgRT plan).

3.2.2 Secondary Endpoints

1. Percent of fractions where there is concordance between the physical and digital phantoms of emulated BgRT delivery derived from human subject PET emissions. Concordance is defined as a standard gamma index with a goal that 90% of pixels meet the 3mm/3% deviation standard.

2. Percent of cases where there is agreement between a site investigator and the agreement standard for the BgRT PET PreScan localization decision (overall percent agreement). Positive percent agreement and negative percent agreement will also be reported.

3. Percent of cases where there is concordance of a positive localization decision between the short-duration PET PreScan and a third-party diagnostic PET/CT scan (positive percent agreement). Overall percent agreement and negative percent agreement will also be reported.

4. Safety of multiple FDG administrations and toxicity rates of bladder and bone marrow assessed by complete blood count, urinalysis and AEs specific to bladder and bone marrow determined by Common Terminology Criteria for Adverse Events (CTCAE) v5 at 72±24 hours after final FDG injection.

5. Workflow characterization:

    a. Percent of PET imaging sessions at RRFD that meet the Activity Concentration threshold for BgRT (5 kBq/mL)

    b. Percent of PET imaging sessions which lead to acceptable BgRT plans, with acceptability based upon meeting user-defined coverage goals for tumor targets and avoidance goals for OARs

    c. Percent of approved BgRT plans that go on to pass physics quality assurance, as defined by 90% of pixels meeting the 3mm/3% deviation standard

    d. Percent of PET Evaluations on the day of fraction delivery that elicit a “Pass” signal

4. STUDY DESIGN

4.1 Overview and Justification for Study Design

In light of this new technology (BgRT) and its potential to improve radiotherapy dose distribution, we propose a single arm prospective study to optimize FDG dosing, assess the performance of the PET imaging subsystem for BgRT treatment planning and delivery, including its role as an interlock, and to validate the dose delivery performance of the end-to-end BgRT workflow.

As described in section 1.5, this clinical evidence is intended to supplement and enhance technical validation studies of BgRT delivery which include, but are not limited to, *in silico* simulations and physical phantom measurements. As such, the patient population selected for this investigation is meant to optimally represent the spectrum of cases, with respect to motion and radiographic environment, that a radiation oncologist may encounter in practice. As described previously, patients with lung and bone tumors are specifically selected so that diverse omnidirectional motion profiles and different tumor-to-background PET contrasts can be evaluated in the investigation.

This is a single-arm, open-label, prospective study. The study will be divided into sequential cohorts of patients with one targetable metastatic lesion in either the lungs or bone (*Figure 3*). Patients with multiple metastases can be accrued, but these investigations will focus on only one lesion per patient.

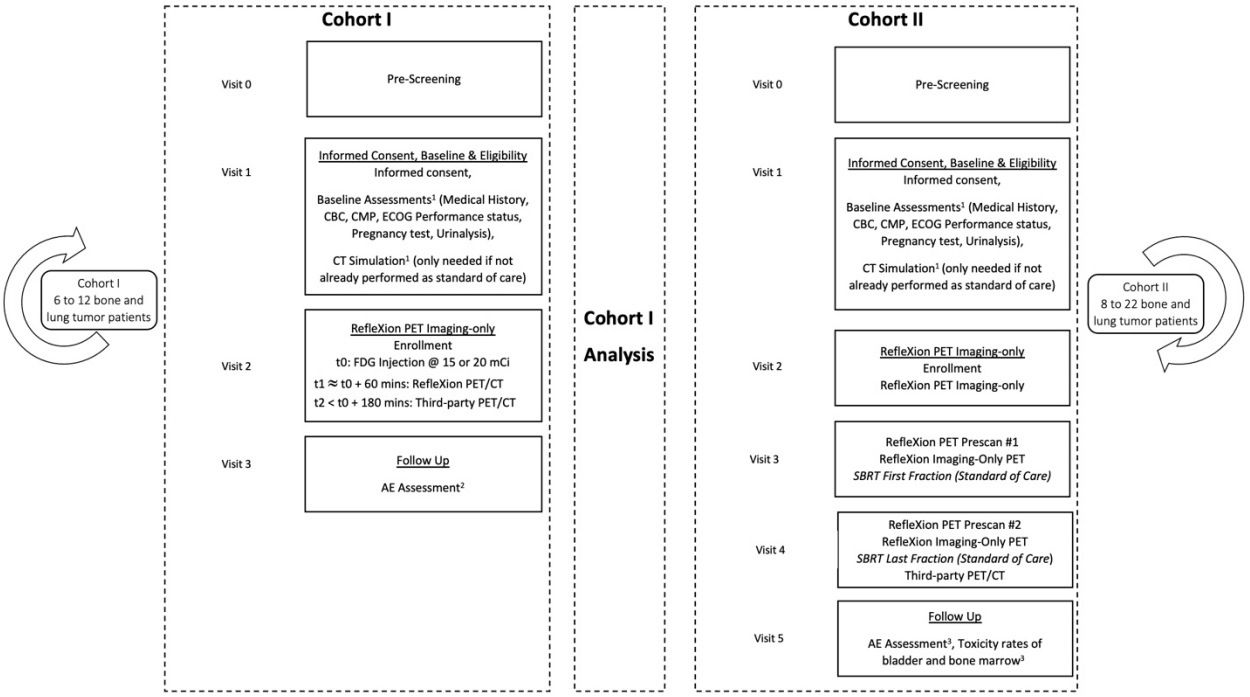

Figure 3: Study Flowchart

[1]Baseline assessments and CT Simulation should be collected within two weeks prior to the RefleXion PET Imaging-only session (Visit 2). Baseline assessments and CT Simulation completed as SOC within this period do not need to be repeated for study purposes.
[2] within 72±24 hours after the PET Imaging-only session
[3] within 72±24 hours after final FDG administration

4.2 Cohort I: RRFD Cohort

This cohort will seek to identify the Recommended RefleXion FDG dose (RRFD), which is the dose of administered FDG – within a range concordant with the American College of Radiology and Society of Pediatric Radiology Practice Parameter for Performing PET/CT in Oncology (ACR-SPR Practice Parameter) – that allows for functioning of the RefleXion system[15]. Keeping ALARA principles in mind, this phase of the investigation seeks to optimize the balance between minimizing patient exposure to the radiotracer and achieving satisfactory performance of the RMRS PET subsystem for BgRT. This cohort will also seek to assess RMRS PET imaging performance in comparison to a third-party diagnostic PET/CT.

To that end, dose levels of 15 mCi and 20 mCi (if required) will be assessed sequentially in an escalation protocol. Patients with at least one known FDG avid tumor (i.e. SUV_{max}≥6 on diagnostic PET/CT) in the bone or lung will be enrolled into this cohort. These patients will undergo a CT simulation in an acceptable radiotherapy treatment position and with immobilization devices as needed. After acquisition of (4D)CT images, contours for targets, OARs, and BTZ will be generated by the investigator. Next, the patient will undergo back-to-back PET scans on the RefleXion device and a third-party diagnostic PET/CT device after a single injection of FDG at the studied FDG dose level (in the unlikely event that the third-party diagnostic PET/CT scan cannot be initiated within 180 minutes of the FDG injection, the scan can be completed with a separate FDG injection 24 to 96 hours after the initial FDG injection). Quantitative metrics will be collected (described below) for each lesion in order to assess the performance of the RMRS PET subsystem

at that dose. This sequence of events is reflected in *Figure 4*. Actual delivery of radiotherapy to the patient is not part of this investigation.

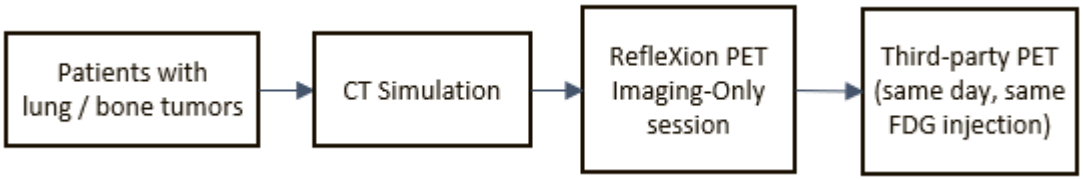

Figure 4. Cohort I schematic

Primary Objective:

The objective of this cohort study is to determine the lowest dose among candidate doses of administered [18F]-FDG that results in the Activity Concentration necessary for BgRT functioning: 5 kBq/ml or higher. An adequate Activity Concentration is the key parameter necessary for BgRT performance and is also the most sensitive quantity to FDG injection. It is calculated from the BTZ volume and includes activity from both the target and the enclosed background. To determine the RRFD, a modified 3+3 design will be utilized wherein meeting the Activity Concentration threshold – not dose-limiting toxicity as is typically used – will be the relevant criteria for escalating from one dose to the next (*Figure 5*).

The first subjects will receive 15 mCi [18F]-FDG. If the Activity Concentration in 2 of the first 3 patients is <5kBq/ml, accrual to this cohort will stop and the next cohort will receive 20 mCi [18F]-FDG. If the Activity Concentration in 2 or more of the first 3 patients is ≥5kBq/ml, accrual in the 15 mCi cohort can continue. If the Activity Concentration in at least 5 of 6 patients at this cohort is ≥5kBq/ml, 15 mCi will be considered the RRFD. If the Activity Concentration in two or more subjects at this cohort is <5kBq/ml, the subsequent group will receive 20 mCi [18F]-FDG, and an identical accrual schema will be followed for this group. Each dose-level will aim to have equal numbers of bone and lung tumors once all 6 subjects are accrued.

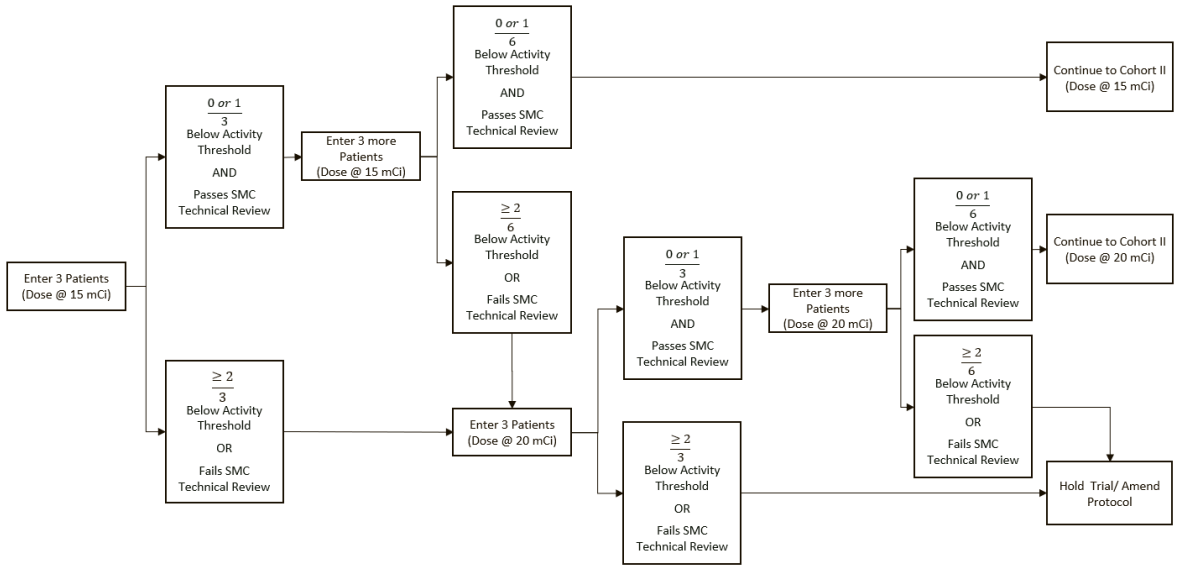

Figure 5. Modified 3+3 Design

The initial FDG injection dose of 15 mCi was selected based on the internal analysis of prospective trial data sponsored by RefleXion Medical[8], design specifications, and phantom testing results. This dose provides a good margin for handling different patient body sizes and for assessing the validity of PET evaluation criteria. In order to facilitate the enrollment of a representative and relevant patient population, the inclusion and exclusion criteria will not impose any limitations on BMI and participant weight will only be limited by device's table weight limits.

Secondary Objectives: Additional analyses of the RefleXion PET images will be performed to further assess the performance of the BgRT PET Imaging-only session, treatment planning and quality assurance at the studied dose level. Implied in these secondary objectives is that all other quantitative criteria for BgRT candidacy (i.e. NTS level) are met, and that the BgRT algorithm is then able to use the aggregate PET data to generate an acceptable BgRT plan that subsequently passes physics quality assurance. In other words, these endpoints comprehensively characterize the workflow through the steps that precede BgRT treatment delivery.

These secondary endpoints will include the following:

1. Percent of cases where there is agreement between a site investigator and the agreement standard for the BgRT PET Imaging-only session localization decision (overall percent agreement). Positive percent agreement and negative percent agreement will also be reported.

2. Percent of cases where there is concordance of a positive "plan proceed" decision between the BgRT Imaging-only session PET and a cleared, third-party diagnostic PET/CT (positive percent agreement). Overall percent agreement and negative percent agreement will also be reported.

3. Percent of cases where RefleXion PET data can be used to generate an acceptable BgRT plan such that relevant dosimetric parameters for the target and the nearby normal anatomy are met based on investigator assessment.

4. Percent of cases where the intended dose distribution of the BgRT plan is achieved in a physical phantom, defined as meeting a standard gamma index for external beam radiotherapy quality assurance, i.e. whether 90% of pixels meet the 3mm/3% deviation standard.

The first secondary endpoint measures inter-observer agreement for the localization decision made after the RMRS PET imaging session. As such, the decision made by the site investigator will be assessed against an agreement standard made by a panel of independent reviewers using the same RMRS PET image with overlaid BTZ. The two criteria required for a positive localization decision (tumor signal within BTZ, OAR signal outside BTZ) will be captured and reported for all observers for each case. Overall percent agreement will constitute the main analysis, but positive percent agreement and negative percent agreement will also be reported. Details of this analysis are described in section 6.2.3.

The next secondary endpoint seeks to assess the RMRS PET image in relation to a third-party diagnostic PET/CT device. Importantly, the hardware architecture of the RMRS PET is not designed to have the same level of diagnostic performance as a dedicated, diagnostic PET/CT [16]. This is an expected outcome as the

RMRS PET design utilizes two 90° arcs of detectors as opposed to a full 360° ring; additionally, the RMRS PET has a significantly shorter field of view in the patient superior-inferior direction compared to a diagnostic PET/CT device. These hardware limitations in the RMRS PET subsystem may result in a scenario where some tumors may not be visible in the RMRS PET image. However, this scenario does not pose a radiotherapy health risk to the patient as it would simply result in deciding that the patient is not a candidate for BgRT.

Given these observations, the relevant question is whether a RMRS PET image that results in a positive plan proceed decision – which would result in proceeding on with the BgRT workflow – is concordant with a third-party diagnostic PET/CT obtained on the same encounter (positive percent agreement). Although, only cases with positive plan proceed decisions are clinically relevant from a BgRT workflow perspective, overall percent agreement and negative percent agreement will be reported to allow a more comprehensive view of plan proceed decision between the BgRT Imaging-only session PET and a cleared, third-party diagnostic PET/CT. Details of this analysis are provided in section 6.2.4.

Dose Selection: As shown in *Figure 5*, the RRFD will be primarily based upon the lower FDG dose level where 0 to 1 failure events in the primary endpoint occur in 6 patients. If 2 or more failure events occur at the 15 mCi dose level, then accrual to that dose level will be stopped and subsequent patients will be enrolled at the next dose level (20 mCi). In the event that 2 failure events occur at the 20 mCi dose level, then accrual to the trial will be held and discussions with the sponsor will take place to determine if there is a feasible protocol amendment that would allow trial continuation. The 15mCi and 20mCi data will be used to extrapolate the next dose, if necessary.

Adjudication will be done by the Safety Monitoring Committee (SMC) after every 3 patients or fewer are enrolled. The SMC will include individuals with pertinent expertise, including but not limited to a non-site radiation oncologist, site radiation oncologist and/or physicist, a RefleXion medical physician, and RefleXion technical staff. Finally, even if the 15 mCi dose level meets the primary endpoint for performance, the SMC can choose to close accrual to the 15 mCi dose level and enroll at the 20 mCi dose level if secondary objectives are found to be deficient at the 15 mCi dose level. Similarly, the SMC can choose to close accrual to the 20 mCi dose level if secondary objectives are found to be deficient. These analyses will be considered concurrently with the assessment of the primary endpoint.

If an RRFD is established by meeting the statistical parameters outlined above, and there are no objections arising from the other analyses, then the SMC will have the discretion to open Cohort II to accrual.

4.3 Cohort II: Emulated Delivery Cohort

The BgRT product design is such that when used to guide treatment delivery, the hardware instructions for the linear accelerator, gantry, and MLCs are determined during each fraction based upon the dynamic input of PET LTS images on that day. Therefore, each fraction of treatment delivery is expected to utilize different hardware instructions than the others. Nonetheless, each of these delivered variations and their individual dose volume histograms ($DVH_{Delivered}$) should be reflected in the approved BgRT plan, which represents the spectrum of delivery possibilities (within an allowed threshold of variation) in the bounded dose volume histogram (bDVH).

The chief objective of this cohort is to confirm that the machine-deliverable fluence generated by applying the BgRT firing filter to PET LTS images obtained at the time of a radiotherapy delivery does in fact result in an anatomic dose distribution that is consistent with the approved BgRT plan. A secondary objective is to extend this analysis by also confirming that the linear accelerator subsystem hardware is able to deliver the received machine instructions. Importantly, this investigation comprehensively emulates and assesses (without actually delivering the radiation therapy to the patient) the entire end-to-end BgRT workflow from simulation to treatment planning to, finally, dose delivery. This design also provides an opportunity to assess imaging, workflow, and the toxicity, if any, associated with multiple administrations of FDG.

To do this, 8 to 22 subjects dispositioned to undergo conventional SBRT for a single bone tumor or a single lung tumor will be enrolled. As noted previously, patients with multiple metastases can be accrued but the investigation will focus only one targeted lesion per patient. For each patient, RMRS PET collections will be added to the SBRT workflow at 3 timepoints representing the steps when the RMRS PET subsystem would be utilized during the BgRT workflow. Specifically, these timepoints will include a RMRS PET imaging-only session prior to the start of SBRT delivery that will be used to create a BgRT plan as well as RMRS PET collections before the first and final fractions of their planned course of SBRT (*Figure 6*). A single comparison third-party diagnostic PET/CT image will also be obtained (utilizing the same FDG injection) on the day of the final fraction.

The two fractional timepoints are selected because they will capture any day to day PET variations as well as potential interference on the tumor PET signal arising from the radiotherapy itself. A PET collection prior to the start of the treatment course will not be influenced by the SBRT treatment itself, whereas the PET collection preceding the final fraction will be subject to the full impact of SBRT on the tumor PET profile.

In order to emulate BgRT delivery, each of these fractional PET collections will consist of two phases:

- Short-duration PET collection that corresponds to the duration of a PET PreScan Evaluation
- Long-duration PET collection that corresponds to the duration of an active BgRT delivery fraction

The long-duration PET collection data mimics RefleXion LTS PET image acquisition during live BgRT and will be used to determine the hardware instructions that would be transmitted to the RefleXion delivery hardware (linear accelerator, gantry, MLC, etc.) based upon the algorithmic interaction between the incoming LTS images and the approved BgRT treatment plan.

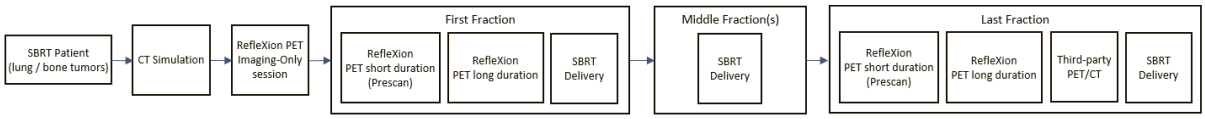

Figure 6. Cohort II Patient Encounters

Primary Objective: The quality of each specific instance of emulated BgRT dose delivery will be validated in two ways. Firstly, the dose distribution resulting from the hardware instructions triggered by fraction timepoint LTS images will be modeled *in silico* on the patient's CT anatomy. This radiotherapy dose distribution will be checked against the expectations of the approved BgRT plan. As such, the primary endpoint is the percent of radiotherapy fractions where the emulated BgRT dose distribution *in silico* is shown to be consistent with the approved BgRT treatment plan (i.e., 95% of $DVH_{Delivered}$ points for the BTZ and OARs fall within the bounded DVH of the approved BgRT plan).

Secondary Objectives: The second test of dose distribution quality is to confirm that the machine instruction-set generated by the BgRT algorithm can be effectively and accurately carried out by the linear accelerator hardware to deliver the intended dose and distribution.

To that end, the fluence resulting from the hardware instruction set will be delivered into a physical phantom in order to emulate delivery during BgRT. The dose and distribution measured in the phantom will be judged against *in silico* delivery of the same instruction set to a digital facsimile of the phantom, as per standard physics quality assurance methodology.

As such, the first secondary endpoint will be the following:

1. Percent of fractions where there is concordance between the physical and digital phantoms of emulated BgRT delivery derived from human subject PET emissions. Concordance is defined as a standard gamma index with a goal that 90% of pixels meet the 3mm/3% deviation standard.

Collectively, these primary and secondary analyses emulate end-to-end BgRT delivery insofar as they enable visualization of anatomic dose distributions resulting from varying PET LTS conditions and confirm quality assurance and delivery of those dose distributions by the linear accelerator hardware. In this way, these analyses address the known limitation of human subjects research in radiotherapy device validation (see section 1.5), which is that dose distributions are not directly measurable *in vivo*.

These two analyses of emulated delivery are graphically characterized in *Figure 7* below:

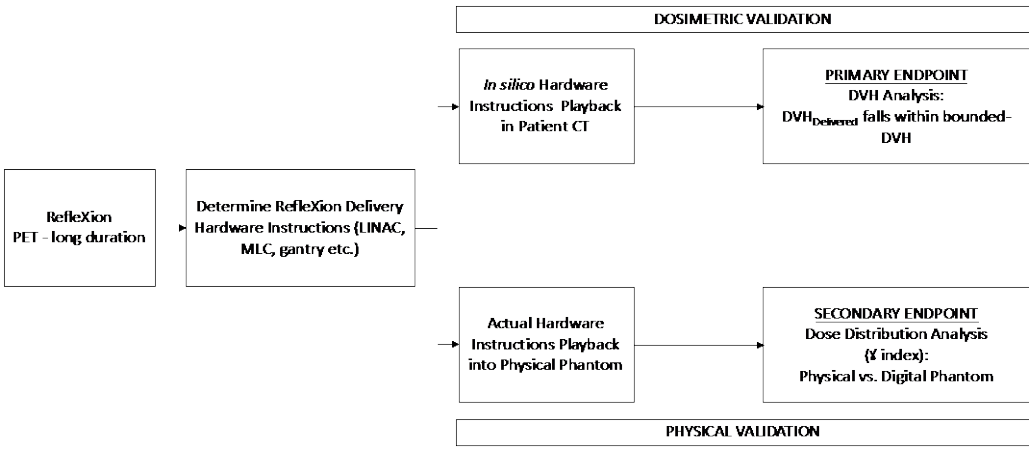

Figure 7. Physical and Dosimetric Validation of BgRT Plan Delivery Based upon Human-Subject Derived PET Data on the RefleXion System

To reflect clinical conditions, these analyses of BgRT dose distribution are necessarily limited to those fractions where BgRT would have been delivered in practice. Therefore, a dose distribution is evaluable for the primary endpoint if, preceding the long-duration PET collection, (1) an acceptable BgRT plan was approved and (2) the PET PreScan evaluation delivered a "Pass" signal. In order to obtain a total of 16 evaluable dose distributions for assessing delivery quality in Cohort II, a minimum of 8 subjects (4 bone tumors and 4 lung tumors) and up to 22 subjects can be accrued. For an enrolled subject, if a PET PreScan delivers a "Fail" signal prior to long-duration PET collection, then the investigator can choose to repeat FDG administration at a subsequent treatment fraction for that subject (which again reflects clinical practice). If a data point is ultimately non-evaluable, then an additional patient with the same anatomic type of tumor can be accrued. Once a total of 16 evaluable dose distributions are acquired, Cohort II accrual will stop.

The next two secondary endpoints assess the imaging performance of the PET PreScan. The first of these endpoints seeks to assess the inter-observer agreement for the localization decision made after each RMRS PreScan. A positive or negative decision on the PET PreScan will be indexed against current standard of care for radiotherapy tumor localization: CT-based localization. As such, whether the tumor PET signal is within the BTZ – which is a rigid volume defined on and registered to the patient's CT anatomy – will underpin this determination. The localization decision made by the site investigator will be assessed against an agreement standard made by a panel of independent reviewers using the same RMRS PreScan images with the overlaid BTZ:

2. Percent of cases where there is an agreement between a site investigator and the agreement standard for the BgRT PET PreScan localization decision (overall percent agreement). Positive percent agreement and negative percent agreement will also be reported.

The next imaging secondary endpoint seeks to validate the qualitative visualization of the tumor and OARs on the PreScan against an established reference standard. To establish this concordance, a third-party diagnostic PET/CT scan following the RMRS PET acquisitions obtained prior to the last fraction.

3. Percent of cases where there is concordance of a positive localization decision between the short-duration PET PreScan and a third-party diagnostic PET/CT scan (positive percent agreement). Overall percent agreement and negative percent agreement will also be reported.

Details of these two imaging analyses are described in section 6.3.8 and 6.3.9. Of note, these endpoints will not be limited to only those cases that are evaluable for the primary endpoint. Nonetheless, interpretation of the results of this analysis should bear in mind that the clinically meaningful localization decisions are the positive ones because they would prompt BgRT external beam delivery; negative localization decisions at the RMRS PET PreScan would result in aborting BgRT delivery in clinical practice.

A fourth secondary endpoint is to measure toxicity arising from multiple administrations of FDG. As described in detail in the IB, the theoretical risk is minimal given first principles of radiation exposure and reassuring animal and human studies of very high-dose FDG administrations. This secondary endpoint will seek to further confirm safety by assessing the organs most likely to be affected by repeat FDG administrations:

4. Safety of multiple FDG administrations and toxicity rates of bladder and bone marrow assessed by complete blood count, urinalysis and AEs specific to bladder and bone marrow determined by Common Terminology Criteria for Adverse Events (CTCAE) v5 at 72±24 hours after final FDG injection.

Finally, characterization of the different steps in the workflow and the robustness of the PET Evaluation pre-delivery will be assessed to better characterize RefleXion system functioning. This data will be collected for all enrolled subjects regardless of the evaluability of their BgRT dose distributions:

5. Workflow characterization:

    a. Percent of PET imaging sessions at RRFD that meet BTZ activation concentration threshold for BgRT (5 kBq/ml)

    b. Percent of PET imaging sessions that lead to acceptable BgRT plans, with acceptability based upon meeting user-defined coverage goals for tumor targets and avoidance goals for OARs

    c. Percent of approved BgRT plans that go on to pass physics quality assurance as defined by 90% of pixels meet the 3mm/3% deviation standard.

    d. Percent of PET PreScan Evaluations on the day of fraction delivery that elicit a "Pass" signal.

For each of these conversion assessments, the constituent metrics underling the outcomes will be recorded. For example, the underlying measurements for the PET PreScan Evaluation (i.e., Activity Concentration, NTS level, predicted DVH, predicted 3D dose distribution, and calculated agreement between the predicted DVH and bounded DVH) will be recorded for each assessment.

Additional exploratory analysis for Cohort II may include pairwise dosimetric comparison of the BgRT and SBRT plans for each lesion. When additional time-paired third-party images are available, they may be compared to RMRS PET images and a qualitative assessment may be performed.

5. STUDY POPULATION

5.1 Selection Criteria

5.1.1 Inclusion Criteria

1. Age greater than 21 years

2. A new or prior diagnosis of biopsy-proven cancer with a solid tumor (non-hematologic, non-lymphoma)

3. At least one active tumor in the bone or lung which is either the primary tumor or metastatic lesion determined either by biopsy or imaging suspicious of active disease

4. Target tumor size ≥2cm and ≤5cm

5. Target lesion in the bone or lung that is discrete and assessed by investigator to be FDG-avid (i.e. $SUV_{max} \geq 6$ on third-party diagnostic PET/CT performed within 60 days with no new intervening oncologic therapies)

6. ECOG Performance Status 0-3

7. Must have completed any other oncologic therapies at least 15 days prior to planned start of study procedures (preferably 30 days) and must have no plans to initiate systemic therapy until after study follow up is complete -OR- must be recorded by physician to have an active candidate lesion that is unresponsive to ongoing systemic therapy

8. Females of childbearing potential should have negative urine or serum pregnancy test within 14 days prior to initiation of study scans.

9. Demonstrate adequate organ function:

    a. Absolute neutrophil count (ANC) > 1,500 /mcL

    b. Platelets > 50,000 / mcL

    c. Hemoglobin > 8 g/dL

    d. No gross hematuria

10. For Cohort II only: Patient is dispositioned to undergo SBRT to a bone or lung tumor

5.1.2 Exclusion Criteria

1. Clinically significant blood glucose abnormalities that preclude a satisfactory FDG PET/CT scan.

2. Previous history of external radiotherapy where prior radiotherapy fields are anticipated to overlap with the radiotherapy fields required for the present study.

3. Diffuse metastatic process (leptomeningeal disease, peritoneal carcinomatosis, diffuse bone marrow involvement, etc.)

4. PET-avid structures not intended for radiation are within 2cm from target on third-party diagnostic PET/CT as assessed by investigator

5. Known allergy to FDG

6. Known psychiatric or substance abuse disorder that would interfere with conduct of the study

7. Pregnant, breast-feeding or expecting to conceive during the study

8. Patient weight exceeding the weight limit outlined per IFU

9. For Cohort II only: Patients with pacemakers and other implantable devices who are deemed to be at high risk by the treating physician for complications secondary to radiotherapy

10. For Cohort II only: Patients with bone lesions who are determined to be high risk by the treating physician for pathologic fracture prior to beginning radiotherapy

11. For Cohort II only: Active inflammatory bowel disease, scleroderma, or other disorder deemed to be a risk factor for excess toxicity in the area of treatment by the treating physician.

5.2 Withdrawal and Replacement of Subjects

While study withdrawal is discouraged, subjects may withdraw their consent at any time, with or without reason and without prejudice to further treatment. Subject's withdrawal of consent must be documented. Withdrawn subjects will not undergo any additional follow-up, nor will they be replaced.

Subjects whose imaging sessions do not adhere to the sequence and timing defined in the protocol (see section 6. Study Procedures) may be replaced at the discretion of the investigator in consultation with the SMC. The reasons for not adhering could be due to patient choice or due to scheduling issues or device operation delays with the RefleXion or third party PET/CT equipment. All subjects will be accounted for in the study tables and all subject data will be reviewed by the SMC.

5.3 Enrollment Controls

Enrollment will be monitored to ensure that no more than the maximum planned number of subjects is enrolled. An electronic data capture system will be used, and the system will be set to automatically notify the Project Manager and/or CRA of all subject enrollments being entered within the system. Study sites will be notified as enrollment nears maximum allowed.

6. STUDY PROCEDURES

6.1 Written Informed Consent

Written Informed Consent must be obtained for all subjects who are screened and meet the general inclusion/exclusion criteria prior to enrollment.

The subject or the subject's Legally Authorized Representative (LAR) will be asked to sign the Informed Consent form before any study-specific tests or procedures are performed. The Informed Consent Form (ICF) must be approved by the study Institutional Review Board (IRB). Electronic informed consent procedures may be utilized if approved by the IRB and consistent with FDA guidance on use of electronic informed consent in clinical investigations. Study personnel should explain that even if a subject agrees to participate in the study and signs an Informed Consent Form, imaging or other tests may demonstrate that the subject is not a suitable candidate for study participation/treatment. All subjects that have signed

the ICF, met all study eligibility criteria (and none of the exclusion criteria), and present for the RefleXion Imaging-only session will be considered enrolled at the time of FDG administration.

A Screening and Enrollment Log will be maintained by the site to document basic information such as date screened and reason for screen failures for subjects who fail to meet the study eligibility criteria. Screen-failed subjects and their reason(s) for screen failure will be documented and may be entered into the electronic database, but they will not be followed beyond the screening visit, and no further data will be collected/recorded.

Investigator and/or study personnel at the site will review the study requirements with the subject to maximize compliance with the follow-up schedule. Study personnel will instruct subjects to return for study assessments per Study Event Schedule for the applicable cohort the patient is enrolled in.

6.2 Cohort I: RRFD Cohort

6.2.1 Baseline Visit

The following baseline data should be collected within two weeks prior to the Imaging-only PET session for all subjects enrolled in Cohort I and documented in the case report form (CRF):

- Confirmation that all inclusion criteria and none of the exclusion criteria are met.
- Demographics, medical history and physical exam
- Complete Blood Count (CBC)
- Comprehensive Metabolic Panel (CMP)
- ECOG performance status
- Blood glucose
- Urinalysis
- Pregnancy test (required for women of child-bearing potential only; within 14 days prior to the initiation of study scans.)

For patients undergoing systemic therapy at the time of enrollment, decisions regarding whether to continue the systemic therapy or undergo a wash-out will be made by the treating physician in accordance with standard of care for combining radiation and drug therapy.

Additionally, a Sim CT should be obtained within two weeks prior to the PET Imaging-only session if not already planned to be obtained as standard of care. Any additional institutional guidelines must be followed regarding negative pregnancy testing in patients about to undergo radiation therapy.

6.2.2 PET Imaging-Only Session, BgRT Treatment Planning, and Quality Assurance

All subjects enrolled in Cohort I will undergo a PET Imaging-only session including a single injection of the applicable FDG dose. Standard-of-care Sim CT images of subjects who are dispositioned to have SBRT will be collected and utilized for the PET Imaging-only session. For subjects who do not have a Sim (4D)CT within two weeks prior to the PET Imaging-only session and are not planned to undergo a Sim-CT for SBRT (e.g. follow-up patients), a Sim (4D)CT obtained as a part of the study protocol within two weeks prior to the PET Imaging-only session will be utilized. The images from the Sim-CT will be used to outline structures necessary for treatment planning including target volumes, organs-at-risk, and the biology-tracking zone.

Once the structures have been delineated and imported into the RefleXion treatment planning system, a BgRT plan is initiated, and the patient can return to the site to undergo passive PET collection on the RMRS system (the PET Imaging-only session). The patient will undergo FDG injection in accordance with standard diagnostic PET/CT protocols. Prior to FDG injection, patients should be fasting for 4-6 hours. A blood glucose measurement will be obtained prior to FDG injection to confirm a level less than or equal to 200 mg/dL. Patients will be rescheduled should the blood glucose value exceed this threshold. After injection the patients should be resting in the uptake area for 60 minutes (±15min).

After the uptake period, patient set-up will include positioning patients on the RefleXion system couch in the treatment position, followed by immobilization (if necessary) and rough alignment using lasers and tattoos. A localization kVCT scan will be performed for refining anatomical alignment in the region of interest and to localize the treatment target area. Sim CT images will be registered with the kVCT to propagate contoured volumes. Specifically, the BTZ will be overlaid digitally on the kVCT images once positioning is completed. A RMRS PET Imaging-only session will then be performed over the 12-20 minutes depending on the size of region of interest. The resulting PET dataset will be transmitted to the RefleXion treatment planning workstation.

Immediately following the RMRS PET imaging session, subjects will be imaged again with a third-party PET/CT in the same treatment position. A second dose of FDG will not be administered for the scan immediately following the RMRS PET Imaging-only session. Sites will make every effort to minimize the time between two scans. To ensure adequate FDG activity, third-party PET scan will be initiated within 180 minutes after the FDG injection. In the unlikely event that third-party PET scan cannot be initiated within 180 minutes, third-party scan can be completed with a separate FDG injection 24 to 96 hours after the initial FDG injection in the same treatment position as per the RMRS PET scan.

Data collected during the RMRS PET Imaging-only and third-party PET/CT scans will be documented on the appropriate CRF and will include the time of FDG injection, start and end times for RMRS PET and third-party diagnostic PET/CT scans. Sites will be provided with instructions for how images should be collected and submitted to RefleXion Medical within five days of the PET Imaging-only session. AEs will be assessed by using Common Terminology Criteria for Adverse Events (CTCAE) v5 at 72±24 hours after the end of PET Imaging-only session.

After the Imaging-only PET dataset is transmitted to the RefleXion treatment planning workstation, BgRT treatment planning can be completed with dose calculation and optimization employing the BgRT

algorithm as described in the BgRT User Manual. Scoring of a successful BgRT plan requires positive localization at the RMRS device during the PET Imaging-only session; reaching the necessary quantitative thresholds (i.e. Activity Concentration and NTS); and meeting coverage goals for target and avoidance goals for OARs. In Cohort I, physician intent, which includes radiotherapy dose and coverage goals, should match institutional practice for ablative radiation for the tumor type and anatomic region being targeted. Dose constraints are recommended to follow NRG Oncology guidelines. As in conventional radiotherapy planning, dose, fractionation, and volume modifications are allowed if such changes are in accordance with institutional practice for the clinical scenario. Similarly, changes to the BTZ volume are allowed at the time of planning. However, the original BTZ must be utilized for the primary endpoint and secondary imaging endpoints described in section 6.2.3 and 6.2.4 since those endpoints precede treatment planning. The BgRT plan is considered to be successfully completed when approved by physics and the treating physician.

The BgRT physics quality assurance process verifies that the treatment planning system calculated dose in a phantom is equal to the measured dose in the phantom. Once the BgRT plan is ready (optimization and dose calculation are completed and the plan quality meets desired treatment objectives) a Verification Plan is generated in the treatment planning system as described in the BgRT User Manual. As is common practice, this QA plan is generated by taking the calculated treatment plan fluence and projecting the fluence in a forward dose calculation onto the CT image of an ArcCHECK phantom. The QA plan is then delivered to the ArcCHECK phantom. Upon completion of the dose delivery, the accuracy of the plan delivery can be assessed by comparing the dose in the phantom calculated by the treatment planning system with the actual measured dose in the phantom using gamma analysis software provided with the ArcCHECK phantom.

Table 1: Cohort I- Study Event Schedule

	Screening &Baseline[1]	**PET Imaging-Only Session**	**Follow-Up** (72±24 hours after the PET Imaging-only session)
Informed Consent[2]	**x**		
Demographics, Medical History & Physical Exam	**x**		
CBC	**x**		
CMP	**x**		
Blood glucose	**x**	**x**	
Urinalysis	**x**		
ECOG performance status	**x**		
Pregnancy test[3]	**x**		
Sim CT[4] **(if not done as standard of care)**	**x**		
FDG injection[5] **(15mCi or 20mCi)**		**x**	
RMRS PET w/ kVCT localization[4,5]		**x**	
Third-party diagnostic PET/CT[4,5]		**x**	
AE Assessment[6]		**x**	**x**

[1] *Baseline Assessments should be completed within 14 days prior to the PET Imaging-only session*

[2] *Informed consent is not considered a baseline assessment and has no requirement to be completed within a specific time window from study visits*

[3] *Women of child-bearing potential only; within 14 days prior to the initiation of the study scans.*

[4] *Images should be de-identified before submitting to RefleXion Medical.*

[5] *In the event that third-party PET scan cannot be initiated within 180 minutes of original FDG injection, then the third-party scan can be completed with a separate FDG injection 24 to 96 hours after the initial FDG injection.*

[6] *AEs to be assessed using Common Terminology Criteria for Adverse Events (CTCAE) v5*

6.2.3 Schema for Cohort I Secondary Endpoint #1: RMRS Image Inter-Observer Agreement

6.2.3.1 The first read will occur at the clinical study site and will be the localization decision by a site Investigator as part of the planned clinical workflow. This comparison will check the decision that is made after investigator review of the RMRS PET image against an agreement standard.

6.2.3.1.1 The site investigator (radiation oncologist) will be presented with the RMRS PET image obtained at the PET Imaging-only session. The contours of the sim CT-defined biology-tracking zone (BTZ) will be presented on the RMRS PET image, which was acquired after anatomic registration of the sim CT with the RMRS localization kVCT. The site investigator will make a localization decision in accordance with the BgRT workflow.

6.2.3.1.2 The localization decision will be "Yes" if (a) the PET tumor signal is fully within the BTZ and (b) OAR and non-target PET signal(s) are outside the BTZ. Each of these two criteria will be recorded for each case. If either criterion is not met, then the localization decision will be "No". The site investigator localization decision will be logged for comparison to an agreement standard.

6.2.3.2 The second read will be to create the determination of an Agreement Standard to match against the localization decision of a site Investigator. This will be conducted offline at an independent center and will be done by a panel of readers.

6.2.3.2.1 Three radiation oncologists will be selected. All the readers will be trained using identical training data set and image review software programs as detailed in the image review charter (IRC). Readers will be blinded to reader evaluations, patient diagnosis and other clinical data.

6.2.3.2.2 The RMRS PET will be transferred to a workstation for image viewing and image registration. Readers will be presented with the RMRS PET image obtained at the Imaging-only session. The contours of the biology-tracking zone (BTZ) radiotherapy structure will be presented throughout the reading process using the same registration process as 6.2.3.1.

6.2.3.2.3 Each reader makes a localization decision as "Yes" if (a) the PET tumor signal is fully within the Biology Tracking Zone (BTZ) and (b) Organ-at-risk (OAR) and non-target PET signal(s) are outside the BTZ. Each of these two criteria will be recorded. If either criterion is not met, then the localization decision will be "No".

6.2.3.2.4 If reader 1 and reader 2 both rate the localization decision the same, then no third reader is required.

6.2.3.2.5 If reader 1 and reader 2 are in a disagreement, then a third reader who is masked to the first two reads will perform an independent read as a tie breaker. Two agreeing out of the three read results will be selected as the Agreement Standard.

6.2.3.3 Inter-observer agreement between the site investigator (SI) and the agreement standard (AS) will be calculated as follows:

1. If both SI and AS localization decisions are “yes” then the rating should be “A”
2. If SI decides “yes” for localization but AS decides “no” then the rating should be “B”.
3. If SI decides “no” for localization but AS decides “yes” then the rating should be “C”.
4. If both SI and AS localizations decisions are “no” then the rating should be “D”.

6.2.3.4 Based on the rating performed in above section for inter-observer agreement, an overall, positive and negative % agreement will be calculated.

Percent overall agreement = (A+D)/(A+B+C+D) {main analysis}

Percent positive agreement = A/(A+C)

Percent negative agreement = D/(D+B)

6.2.4 Schema for Cohort I Secondary Endpoint #2 - RMRS PET vs Diagnostic PET/CT Comparison:

6.2.4.1 The comparison of the PET image from the RMRS imaging-only session and the third-party diagnostic PET/CT will be conducted and descriptive statistics including positive and negative percent agreement will be provided as outlined below.

The positive percent agreement analysis seeks confirmation of RMRS findings in clinically relevant cases based on an affirmative scoring of two questions which correspond to the plan proceed decision. For the two questions, FDG-avid targets or organs are defined qualitatively as discrete structures with visually discernable FDG-PET signal above immediate background. The two questions are:

- Q1 (target avidity): Is there an FDG-avid target that correlates to the tumor location on the registered CT image?
- Q2 (confounding non-target signal): Are FDG-avid organs an adequate distance away (≥1 cm) from the FDG-avid tumor for biology-guided radiotherapy?

It should be noted that cases that do not meet these criteria on the RMRS PET imaging-only session do not create a radiotherapy health risk, since the result is that the physician will not choose to use BgRT as the treatment modality. Nonetheless, the overall percent agreement and negative percent agreement will also be reported to allow for comprehensive imaging comparison with the third-party, diagnostic PET/CT.

Although calculation of the agreement endpoint will be based on the qualitative questions above (Q1 and Q2), data on target avidity and non-confounding target signal in relationship to the CT-defined, overlaid biological tracking zone (BTZ) will also be collected:

- Q3: Does the FDG-avid target fall within the BTZ?
- Q4: Do all FDG-avid OARs fall outside the BTZ?

6.2.4.2 As described previously, after each PET Imaging-only session on the RMRS PET, the patient undergoes sequential PET imaging on a cleared, 3rd party diagnostic PET/CT system.

6.2.4.3 The RMRS PET, localization kVCT, Sim CT & third-party diagnostic PET/CT images will be transferred to a workstation after the completion of imaging sessions for offline image viewing. These images will be reviewed by two separate groups of image reviewers blinded to the other device.

6.2.4.4 **RMRS PET review:** Three radiation oncologists will assess the RMRS PET images independently. All the readers will be trained using an identical training data set and image review software programs as detailed in the image review charter (IRC). Readers will be blinded to patient diagnosis, reader evaluations, images from the compared device (i.e. third-party diagnostic PET/CT), but not to the localization kVCT obtained on the RMRS device on the same day, Sim CT and other clinical data.

6.2.4.4.1 Each reader answers the same set of questions for rating whether the patient is suitable for BgRT planning. A positive plan proceed decision requires a "yes" to both Question 1 and Question 2 above. A negative decision is any other combination of answers to the two questions.

6.2.4.4.2 Individual read results will be compared to the agreement standard. A case rating will also be collected based upon agreement of two or of the three read results.

6.2.4.5 **Third-party Diagnostic PET/CT review:** Three radiologists and/or nuclear medicine physicians with significant PET/CT experience will assess the third-party diagnostic PET/CT images independently. All the readers will be trained using an identical training data set and image review software programs as detailed in the image review charter (IRC). Readers will be blinded to patient diagnosis, reader evaluations, images from the compared device (i.e. RMRS PET and RMRS kVCT for localization) but not Sim CT and other clinical data.

6.2.4.5.1 Each reader answers the same set of questions for rating whether the patient is suitable for BgRT planning. A positive decision requires a "yes" to both Question 1 and Question 2 above. A negative decision is any other combination of answers to the two questions.

6.2.4.5.2 Two or more agreeing out of the three read results will be selected as the case rating. Individual read results will also be provided.

6.2.4.6 Based on the decisions performed in the above sections, the following table will be constructed for each RMRS PET reader individually and for the case rating collectively. Tables will be used to provide descriptive statistics for agreement as described below

Plan Proceed Decision	RMRS PET Positive	RMRS PET Negative
Diagnostic PET Positive	A	B
Diagnostic PET Negative	C	D

Percent positive agreement = A/(A+C) {Main analysis for clinically relevant cases}

Percent negative agreement = D/(B+D)

Percent overall agreement = (A+D)/(A+B+C+D)

6.3 Cohort II: Emulated Delivery Cohort

The primary objective of this cohort is to determine whether BgRT dose distributions generated from Limited Time Sample (LTS) PET images obtained at the time of treatment delivery are consistent with the approved BgRT plan. To achieve this objective, RMRS PET scans will be added to the SBRT workflow at timepoints representing some of the instances when the RMRS PET subsystem would be utilized during a BgRT workflow. Specifically, subjects will undergo RMRS PET collections at the time of planning, and then before the first and final fractions of their planned course of SBRT treatment (*Figure 8*).

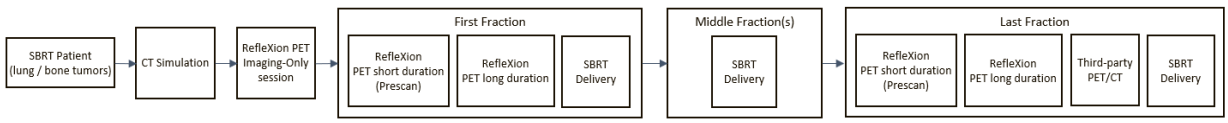

Figure 8. Cohort II Patient Encounters (Duplicated from above).

6.3.1 Baseline Visit

The following baseline data should be collected within two weeks prior to RMRS PET Imaging-only session for all subjects enrolled in Cohort II and documented in the case report form (CRF):

- Confirmation that all inclusion criteria and none of the exclusion criteria are met.
- Demographics, medical history and physical exam
- Complete Blood Count (CBC)
- Comprehensive Metabolic Panel (CMP)
- Blood glucose
- Urinalysis

- ECOG performance status
- Baseline assessment of bladder and bone marrow with CTCAE grading
- Pregnancy test (required for women of child-bearing potential only, if applicable within 14 days prior to the initiation of the study scans.)

For patients undergoing systemic therapy at the time of enrollment, decisions regarding whether to continue the systemic therapy or undergo a wash-out will be made by the treating physician in accordance with standard of care for combining radiation and drug therapy.

Additionally, a Sim CT should be obtained within two weeks prior to the PET Imaging-only session if not already planned to be obtained as standard of care. Any additional institutional guidelines must be followed regarding negative pregnancy testing in patients about to undergo radiation therapy.

6.3.2 Radiation Therapy Intent and Planning

Each patient enrolled in Cohort II will have been deemed an SBRT candidate by the investigating physician. Physician intent including selection of radiation prescription and treatment planning for SBRT will be done as per institutional practice. To create a parallel BgRT plan, patients will also undergo an RMRS PET Imaging-only session as described in Section 6.3.4; this entails a visit that includes a single FDG injection of RRFD; setting up the patient on the RMRS couch; undergoing one kVCT scan to ensure alignment with the patient's Sim CT; and then a passive collection of the PET emissions by the RMRS. The final step of the planning process is physics quality assurance of the SBRT and BgRT plans, respectively. This sequence is detailed below.

6.3.2.1 Radiation Therapy Guidelines

Radiotherapy will be performed according to best practices. Machine-level quality assurance should be performed per its guidelines for any linear accelerator system used in the conduct of this clinical trial. Stereotactic body radiotherapy (SBRT), when utilized, must be performed on a cleared, commercial linear accelerator system.

6.3.2.2 Radiation Prescription: Dose and Fractionation

Radiotherapy dose planned in Cohort II will be in the range delineated below. A minimum of 24 hours is required between fractions and 40 hours is recommended.

Fractions	**Recommended Dose**
3 Fractions	15-45 Gy
5 Fractions	25-50 Gy

6.3.2.3 CT Simulation

Patients will undergo CT simulation, immobilization, and image set acquisition using standard procedures and with the assistance of patient-customized devices for the anatomy being treated. The devices used for patient immobilization and setup must be compatible with the RMRS couch, as the scans on the RMRS device must occur with the patient in the same position as the CT simulation.

For lung tumors, this includes a thoracic cradle, and if tolerable, a T-bar for arm positioning above the head. Image acquisition during CT simulation must include capture of a four-dimensional (4D) image set across a full respiratory cycle as well as average and maximum intensity projections (MIP) image sets. The use of IV contrast is encouraged when medically feasible and applicable per the investigating physician.

For bony sites, immobilization should use a cradle at the site of the lesion. The use of (4D)CT and contrast (IV or oral) contrast are encouraged when medically feasible and deemed applicable by the investigating physician. For example, (4D)CT may be desired in the setting of a rib lesion; oral contrast may be desirable for bowel delineation in the setting of treatment for a pelvic osseous lesion.

The slice thickness for the simulation CT scan used for treatment planning should follow the site's standard clinical practice for SBRT to the specific treatment location. Uniform CT slice thickness must be used.

6.3.2.4 Target Delineation and Treatment Planning

The treatment volume will generally consist of the Gross Tumor Volume (GTV) expanded per the treating physician by 3-10 mm to generate a Planning Target Volume (PTV).

For lung tumors in the context of BgRT planning, the GTV and PTV contouring should be performed on a single phase of respiration. The GTV is contoured on this single phase and then expanded by 5 mm to generate the PTV. For the BTZ, GTV contours from all phases of respiration are first summed to generate an Internal GTV (IGTV) and then expanded by 8 to 20 mm to generate a BTZ. In contrast, for SBRT planning, GTV contours from all phases of respiration will be summed to generate the IGTV and then expanded by 3-10 mm to generate the final prescription volume, called the internal planning treatment volume (IPTV).

For bone tumors in the context of BgRT planning, the GTV and PTV contouring should be performed on a static image set or a single phase of respiration if a (4D)CT set is obtained. Again, a 5 mm expansion of the GTV will be used for the PTV. The BTZ will be a 8 to 20 mm expansion of the static GTV or IGTV if (4D)CT is used. For SBRT planning, either a static GTV contour or an IGTV, if applicable, will be expanded by 3-10 mm to generate the final prescription volume, called the internal planning treatment volume (IPTV).

SBRT dose calculation and optimization will be done as per institutional practice using validated treatment planning software. The SBRT plan must also be evaluated with a robust quality assurance protocol as per institutional practice with necessary procedures performed and verified by physics and the treating physician. Likewise, BgRT dose calculation and quality assurance are performed as described in section 6.2.2 with one clarification: In order to be consistent with normal BgRT workflow, if the BTZ is modified during treatment planning then the modified BTZ should be used for timepoints that occur after treatment planning (i.e., time points described in 6.3.5, 6.3.6, and 6.3.7) .

The coverage goal for both BgRT and SBRT plans is for 95% of the prescription volume (PTV or IPTV) to receive 95% of the prescription dose. Dose modifications can be employed in either the SBRT or BgRT plan if necessary to meet OAR constraints provided that the resulting dose remains in accordance with institutional practice. Quality assessment of the treatment plan, both with anatomically correlated isodose lines and with DVH (SBRT) or bDVH (BgRT), is mandatory.

6.3.2.5 OAR Dose Constraints

Dose constraints should adhere to NRG guidelines for 3 and 5 fraction SBRT as described in NRG BR002. Planning should avoid hot spots (defined as 105% of the prescription dose) in adjacent OARs. These are hard constraints, and dose de-escalation is required whenever necessary to meet these constraints. For non-adjacent OARs, prudent planning based on the principle of ALARA should be observed.

6.3.3 Image Guidance Requirement

At each imaging session or prior to any PET collection on the RMRS PET, the patient's initial positioning based upon external markers must be confirmed and adjusted with the use of the on-board fan-beam kVCT capability of the RMRS PET.

6.3.4 PET Imaging-Only Session including FDG Administration

Prior to the imaging-only session, the simulation CT and contoured structures will be imported into the RefleXion treatment planning system. The physician intent and plan setup for the BgRT plan will then be created in the treatment planning systems. Once this has been done, the BgRT plan can be initiated, and the patient can return for the PET Imaging-only session.

On the day of the imaging-only session, the patient will undergo FDG injection at the RRFD dose in accordance with standard diagnostic PET/CT protocols. Prior to FDG injection, patients should be fasting for 4-6 hours. A blood glucose measurement will be obtained prior to FDG injection to confirm a level less than or equal to 200 mg/dL. Patients will be rescheduled should the blood glucose value exceed this threshold. After injection the patients should be resting in the uptake area for 60 minutes (±15min).

After the uptake period, patient set-up will include positioning patients on the RefleXion system couch in the treatment position, followed by immobilization (if necessary) and rough alignment using lasers and tattoos. A localization kVCT scan will be performed for refining anatomical alignment in the region of interest and to localize the treatment target. Sim CT images will be registered with the kVCT to propagate contoured volumes. Specifically, the BTZ will be overlaid digitally on the kVCT images once positioning is completed. A RMRS PET imaging session will then be performed over the 12-20 minutes depending on the size of region of interest. The resulting PET dataset will be transmitted to the BgRT treatment planning workstation. BgRT dose calculation performed on the RMRS treatment planning system and physics quality assurance are then performed as described in section 6.2.2 to generate a final BgRT plan that must then be approved by physics and the treating physician.

Data collected during the RMRS PET imaging-only session will be documented on the appropriate CRF and will include the time of FDG injection and start and end times for RMRS PET scans. Sites will be provided with instructions for how images and image data (e.g. contours) should be collected and submitted to

RefleXion Medical within five days of the PET Imaging-only session.

6.3.5 First Fraction of SBRT: Short-Duration PET PreScan and Long-Duration PET Collection

Subjects enrolled in Cohort II will undergo conventional SBRT treatment on a commercial linear accelerator system. The investigation seeks to emulate BgRT patient set-up and treatment delivery as this course of SBRT is ongoing. The study procedure requires that an approved BgRT plan was generated previously for the relevant lesion.

Passive collection of PET emission data will be performed prior the first SBRT fraction. On the day of PET data collection, the patient will undergo FDG injection at the RRFD dose in accordance with standard diagnostic PET/CT protocols. Prior to FDG injection, patients should be fasting for 4-6 hours. A blood glucose measurement will be obtained prior to FDG injection to confirm a level less than or equal to 200 mg/dL (note: patients can be rescheduled to undergo their PET data collection at the next fraction should the blood glucose value exceed this threshold). After FDG is injected, the patients should be resting in the uptake area for 60 minutes (±15min).

After the uptake period, patient set-up will include positioning patients on the RefleXion system couch in the treatment position, followed by immobilization (if necessary) and rough alignment using lasers and tattoos. A localization kVCT scan will be performed for refining anatomical alignment in the region of interest and to localize the treatment target. Sim CT images will be registered with the kVCT to propagate contoured volumes. Specifically, the BTZ will be overlaid digitally on the kVCT images once positioning is completed.

In the BgRT delivery workflow, a short-duration PET PreScan is first conducted prior to BgRT delivery to confirm consistency (within tolerance thresholds) of the PET emission profile when compared to the original PET imaging-session used for planning. For study purposes in Cohort II, a short-duration PET PreScan will be obtained to emulate this workflow step. The scan will be acquired over the axial extent of the planned treatment region over approximately 3-5 minutes depending on BTZ size. The quantitative measures that would guide the PET PreScan Evaluation to be considered a “pass” or “fail” signal as described in section 1.3 will be collected. Next, a long-duration PET acquisition will be obtained consisting of four passes of the planned treatment region through the gantry over approximately 12-20 minutes, which emulates LTS PET data collection during live BgRT. An additional kVCT for localization may be performed immediately prior to the long-duration PET acquisition.

The time window to collect this data is within 96 hours prior to the start of the first SBRT fraction; ideally this would be done on the same day as the SBRT fraction. Data collected before the first fraction of SBRT will be documented on the appropriate CRF and will include the time of FDG injection as well as start and end times for RMRS PET scans, and the results of the quantitative calculations based on the short duration PET scan.

6.3.6 Final Fraction of SBRT: Short-Duration PET PreScan, Long-Duration PET Collection and third-party Diagnostic PET

Before the last fraction of conventional SBRT, subjects enrolled in Cohort II will undergo RMRS PET scans in similar fashion as prior to the first SBRT fraction (section 6.3.5), with the addition of an scan on a third party Diagnostic PET/CT system. As such, steps will include setting up the patient on the RMRS couch; undergoing kVCT scan(s) to ensure alignment with the patient's Sim CT; and then a passive collection of the PET emissions by the RMRS.

Following the short-duration PET PreScan and long-duration PET collection, subjects will be imaged again with a third-party PET/CT in the same treatment position. A second dose of FDG will not be administered for the scan following the RMRS PET session. Sites will make every effort to minimize the time between two scans. To ensure adequate FDG activity, third-party diagnostic PET/CT scan should be initiated within 180 minutes after the FDG injection. In the unlikely event that third-party PET scan cannot be initiated within 180 minutes, third-party scan can be completed with a separate FDG injection 24 to 96 hours after the initial FDG injection in the same treatment position as the RMRS PET scan.

The time window to collect this data is within 96 hours prior to the start of the final SBRT fraction, and ideally would be done on the same day. Data collected at the last fraction of SBRT will be documented on the appropriate CRF and will include the time of FDG injection, start and end times for RMRS PET and third-party diagnostic PET/CT scans, and the results of the quantitative calculations based on the short duration PET scan. Sites will be provided with instructions for how images should be collected and submitted to RefleXion Medical within five days of the RMRS PET and third-party diagnostic PET/CT scans.

Additionally, the safety of multiple FDG administrations and toxicity rates of bladder and bone marrow will be assessed. Complete blood count, urinalysis and AEs specific to bladder and bone marrow determined by Common Terminology Criteria for Adverse Events (CTCAE) v5 will be collected at 72±24 hours after the final FDG injection.

6.3.7 Emulated Delivery and Analysis of collected PET Data

Several of the endpoints require *in silico* and phantom analysis of the collected RMRS PET information and resulting BgRT external beam dose distribution that is generated by this PET information. The sponsor will set up a workstation at the clinical site and provide validated tools, training and procedures that will allow the site staff (most likely the site medical physicist) to perform the required analyses. Depending on the complexity, the sponsor may contract with an independent expert to conduct the analyses at a central location. In addition, the collected images and detailed machine log files will be provided to the sponsor for off-line analysis after de-identification. Sites will be provided with instructions for how images should be collected and submitted to RefleXion Medical within five days of the last fraction of SBRT.

For the *in-silico* dose distribution analysis, a long-duration PET scan is obtained on the day of a radiotherapy treatment. This PET scan is subdivided by the BgRT software into sequential LTS PET images. The beam station location and number of rotations required for sampling these LTS images are determined by the approved BgRT plan. Next, the LTS image set is inputted into the delivery algorithm to calculate the machine instructions for external beam delivery. The machine instructions are used to model delivery of their associated fluences *in silico* to emulate dose distribution in the patient's CT anatomy in the treatment position. This emulated dose distribution is then checked to ensure that it is concordant

with the originally approved BgRT plan, which is established by demonstrating that 95% of the $DVH_{Delivered}$ points for the BTZ and surrounding OARs fall within the BgRT plan's bounded DVH (see cohort II primary endpoint). A 3D visualization of the emulated dose distribution on the patient's CT anatomy will also be generated.

For physical validation, the emulated fluence (dose) from the primary endpoint analysis is utilized. RefleXion offline tools will convert the machine instructions into a deterministic radiotherapy (RT) plan. Deliverability of the plan is confirmed by comparing its resultant dose distribution, measured in a physical phantom, to a model of plan delivery in a digital representation (CT image) of the same phantom. A standard gamma index will be utilized for this confirmation with a goal that 90% of the pixels meet the 3mm/3% deviation standard (see cohort II secondary endpoint 1). The ArcCHECK platform will be utilized to perform this assessment.

Table 2: Cohort II - Study Event Schedule

	Screening & Baseline[1]	Planning / PET Imaging-only session (within 7 days prior to the first fraction of SBRT)	First Fraction of SBRT	Last Fraction of SBRT	Follow-Up (72±24 hours after final FDG administration)
Informed Consent[2]	x				
Demographics, Medical History & Physical Exam	x				
CBC	x				x
CMP	x				x
Blood glucose	x	x	x	x	x
Urinalysis	x				x
ECOG performance status	x				x
Pregnancy test[3]	x				
FDG injection[4] (RRFD based on Cohort I)		x	x	x	
RMRS PET w/ kVCT localization [4,5]		x	x	x	
Third-party diagnostic PET/CT[4,5]				x	
AE assessment[6]	x	x	x	x	x

[1]Baseline Assessments should be completed within 14 days prior to the PET Imaging-only session

[2]Informed consent is not considered a baseline assessment and has no requirement to be completed within a specific time window from study visits

[3]Women of child-bearing potential only, if applicable within 14 days prior to the initiation of the study scans.

[4]At the last SBRT fraction timepoint, if the third-party PET scan cannot be initiated within 180 minutes of original FDG injection, then the third-party scan can be completed with a separate FDG injection 24 to 96 hours after the initial FDG injection.

[5]Images should be de-identified before submitting to RefleXion Medical. Type of RMRS PET acquisition (short-duration, long-duration, etc.) as per written protocol.

[6]AEs to be assessed using Common Terminology Criteria for Adverse Events (CTCAE) v5. In Cohort II, directed grading for bladder and bone marrow toxicity will be completed at baseline and 72±24 hours after final FDG administration.

6.3.8 Schema for Cohort II Secondary Endpoint #2: RMRS PreScan Image Inter-Observer Localization Agreement

6.3.8.1 The first read will occur at the clinical study site and will be the localization decision by a site Investigator. This comparison will check the decision that is being made by the investigator after review of the RMRS PET PreScan image against an agreement standard.

6.3.8.1.1 The site investigator (radiation oncologist) will be presented with the RMRS PET PreScan images. The contours of the sim CT-defined biology-tracking zone (BTZ) will be presented on the RMRS PET image, which was acquired after anatomic registration of the sim CT with the RMRS localization kVCT. The site investigator will make a localization decision in accordance with the BgRT workflow.

6.3.8.1.2 The localization decision will be "Yes" if (a) the PET tumor signal is fully within the BTZ, and (b) OAR and non-target PET signal(s) are outside the BTZ. Each of these two criteria will be recorded for each case. If either criterion is not met, then the localization decision will be "No". The site investigator localization decision will be logged for comparison to an agreement standard.

6.3.8.2 The second read will be to create the determination of an Agreement Standard to match against the localization decision of a site Investigator. This will be conducted at an independent center and will be done by a panel of readers.

6.3.8.2.1 Three radiation oncologists will be selected. All the readers will be trained using identical training data set and image reviewed software programs as detailed in the image review charter (IRC). Readers will be blinded to reader evaluations, patient diagnosis and other clinical data.

6.3.8.2.2 The RMRS PET will be transferred to a workstation for image viewing and image registration. Readers will be presented with the RMRS PET PreScan images. The contours of the biology-tracking zone (BTZ) radiotherapy structure will be presented throughout the reading process using the same registration process as 6.3.8.1.1.

6.3.8.2.3 Each reader makes a localization decision as "Yes" if (a) the PET tumor signal is fully within the BTZ, and (b) OAR and non-target PET signal(s) are outside the BTZ. Each of these two criteria will be recorded for each case. If either criterion is not met, then the localization decision will be "No". The site investigator localization decision will be logged for comparison to an agreement standard.

6.3.8.2.4 If reader 1 and reader 2 both rate the localization decision the same, then no third reader is required.

6.3.8.2.5 If reader 1 and reader 2 are in a disagreement, then a third reader who is masked to the first two reads will perform an independent read as a tie breaker. Two agreeing out of the three read results will be selected as the Agreement Standard.

6.3.8.3 Inter-observer agreement between the site investigator (SI) and the agreement standard (AS) will be calculated as follows:

6.3.8.3.1 If both SI and AS localization decisions are “yes” then the rating should be “A”

6.3.8.3.2 If SI decides “yes” for localization but AS decides “no” then the rating should be “B”.

6.3.8.3.3 If SI decides “no” for localization but AS decides “yes” then the rating should be “C”.

6.3.8.3.4 If both SI and AS localizations decisions are “no” then the rating should be “D”.

6.3.8.4 Based on the rating performed in above section for inter-observer agreement, an overall, positive and negative % agreement will be calculated.

Percent overall agreement = (A+D)/(A+B+C+D) {main analysis}

Percent positive agreement = A/(A+C)

Percent negative agreement = D/(D+B)

6.3.9 Schema for Cohort II Secondary Endpoint #3: RMRS Short-Duration PreScan PET vs. Diagnostic PET Concordance Agreement

6.3.9.1 The comparison of the PET PreScan image after short-duration acquisition and the third-party diagnostic PET/CT image will be conducted and descriptive statistics including positive and negative percent agreement will be provided as outlined below.

The positive percent agreement analysis seeks confirmation of RMRS findings in clinically relevant cases based on an affirmative scoring of two questions which qualitatively correspond to a positive localization decision, which is necessary in conjunction with passing quantitative criteria for a PreScan “Pass” signal. For the two questions, FDG-avid targets or organs are defined qualitatively as discrete structures with visually discernable FDG-PET signal above immediate background. The two questions are:

- Q1 (target avidity): Is there an FDG-avid target that correlates to the tumor location on the registered CT image?

- Q2 (confounding non-target signal): Are FDG-avid organs an adequate distance away (≥1 cm) from the FDG-avid tumor for biology-guided radiotherapy?

It should be noted that cases that do not meet these criteria on the PreScan do not create a radiotherapy health risk, since the result is that BgRT delivery would be aborted. Nonetheless, overall percent agreement and negative percent agreement will be reported for a comprehensive imaging comparison with third-party, diagnostic PET/CT.

Although calculation of the agreement endpoint will be based on the qualitative questions above (Q1 and Q2), data on target avidity and non-confounding target signal in relationship to the CT-defined, overlaid biological tracking zone (BTZ) will also be collected:

- Q3: Does the FDG-avid target fall within the BTZ?
- Q4: Do all FDG-avid OARs fall outside the BTZ?

6.3.9.2 As described previously, after the last-fraction RMRS scans which include the PET PreScan, the patient undergoes third-party diagnostic PET/CT imaging.

6.3.9.3 The RMRS short-duration PET image, localization kVCT, Sim CT, and third-party diagnostic PET/CT images will be transferred to a workstation for image viewing. These images will be reviewed by two separate groups of image reviewers blinded to the other device.

6.3.9.4 **RMRS Short-Duration PET review:** Three radiation oncologists will assess the RMRS PET images independently. All the readers will be trained using an identical training data set and image review software programs as detailed in the image review charter (IRC). Readers will be blinded to patient diagnosis, reader evaluations, images from the compared device (i.e. third-party diagnostic PET/CT), but not to the localization kVCT obtained on the RMRS device on the same day, Sim CT, and other clinical data.

6.3.9.4.1 Each reader answers the same set of questions for rating whether the patient is suitable for BgRT planning. A positive localization decision requires a “yes” to both Question 1 and Question 2 above. A negative decision is any other combination of answers to the two questions.

6.3.9.4.2 Individual read results will be compared to the agreement standard. A case rating will also be collected based upon agreement of two or of the three read results.

6.3.9.5 **Third-party Diagnostic PET/CT review:** Three radiologists and/or nuclear medicine physicians with significant PET/CT experience will assess the third-party diagnostic PET/CT images independently. All the readers will be trained using an identical training data set and image review software programs as detailed in the image review charter (IRC). Readers will be blinded to patient diagnosis, reader evaluations, images from the compared device (i.e. RMRS short-duration PET and localization kVCT) but not Sim CT, and other clinical data.

6.3.9.5.1 Each reader answers the same set of questions for rating whether the patient is suitable for BgRT planning. A positive localization decision requires a “yes” to

both Question 1 and Question 2 above. A negative decision is any other combination of answers to the two questions.

6.3.9.5.2 Two or more agreeing out of the three read results will be selected as the case rating. Individual read results will also be provided.

6.3.9.6 Based on the decisions performed in the above sections, the following table will be constructed for each RMRS PET reader individually and for the case rating collectively. Tables will be used to provide descriptive statistics for agreement as described below:

Localization Decision	Short-duration PET Positive	Short-duration PET Negative
Diagnostic PET Positive	A	B
Diagnostic PET Negative	C	D

Percent positive agreement = A/(A+C) {Main analysis for clinically relevant cases}

Percent negative agreement = D/(B+D)

Percent overall agreement = (A+D)/(A+B+C+D)

7. DATA MANAGEMENT

7.1 Data Collection and Processing

Subject data will be collected in a secure electronic data capture (EDC) system via the Internet. All pertinent data will be entered by the study site personnel into the electronic Case Report Forms (eCRFs). A unique subject ID number will be assigned to each subject. Every reasonable effort should be made to complete data entry within five days of data collection. Any data discrepancies may be queried during ongoing review of data by RefleXion Medical personnel or its designees, or may be identified and queried during routine monitoring visits. Data monitoring will be performed to verify data accuracy and ensure queries are resolved. The Principal Investigator or Sub-investigator must ensure the accuracy and completeness of the recorded data and then provide his/her electronic signature on the appropriate CRFs. Changes to data previously submitted to the sponsor will require a new electronic signature by the investigator to acknowledge/approve the changes.

Results from independent reviewers will also be entered into the EDC system and will be electronically signed by the reviewer responsible for entering this data. Ongoing data review will be performed to

identify possible data discrepancies. Manual and/or automatic queries will be created in the EDC system and will be issued to responsible parties for appropriate resolution.

8. MONITORING PROCEDURES

8.1 Monitoring

Monitoring visits to the clinical sites will be made periodically during the study, to ensure that all aspects of the current, approved protocol/amendment(s) are followed. Original source documents will be reviewed for verification of data in the electronic database. The Investigator/institution guarantees direct access to original source documents by RefleXion Medical personnel, its designees, and appropriate regulatory authorities. In the event that the original medical records cannot be obtained for a subject that is seen by a non-study physician at a non-study institution, photocopies of the original source documents must be made available for review.

It is important that the Investigator and/or relevant study site personnel are available during the monitoring visits and that sufficient time is devoted to the process. In order to perform her or his role well, the monitor must be given access to primary subject medical records, which support the data that has been entered into the study CRFs. Access to Protected Health Information (PHI) by the study monitor will be disclosed to the subject within the Informed Consent Form.

8.2 Auditing

The study may be subject to a quality assurance audit by RefleXion Medical or its designees, as well as inspection by appropriate regulatory authorities. It is important that the Investigator and relevant study personnel are available during any audits and that sufficient time is devoted to the process.

8.3 Device Accountability

Device accountability records must be maintained at the study site. Investigational features of the RMRS system (BgRT mode) will be available after all essential documents are collected, the Site Initiation Visit and training of all required study personnel is completed, and the site is approved to enroll. In some circumstances, at the discretion of the Clinical Project Manager, the investigational device availability may precede the Site Initiation Visit, if a site is anticipated to complete all requirements to be eligible to begin enrollment during the visit.

Study personnel authorized to operate BgRT mode will be identified on the Delegation of Authority Log. The operator will be instructed and trained to utilize BgRT mode only for study subjects. Operator training and understanding of study requirements will be documented in the electronic Trial Master File (eTMF) per Study Master File Index (SMFI). The system software will ensure via login credentials that BgRT mode is only accessible to delegated study personnel. Additionally, the BgRT mode will display an investigational

device notification indicating that the use is limited by Federal law to investigational use. The operator will need to acknowledge the notification to be able to move forward with the BgRT mode.

9. ADVERSE EVENTS

9.1 Adverse Event Definitions and Classification

Term	Definition	Reference
Adverse Event (AE)	Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device. NOTE 1 This definition includes events related to the investigational medical device or the comparator. NOTE 2 This definition includes events related to the procedures involved. NOTE 3 For users or other persons, this definition is restricted to events related to investigational medical devices.	ISO 14155
Adverse Device Effect (ADE)	Adverse event related to the use of an investigational medical device. NOTE 1 This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device. NOTE 2 This definition includes any event resulting from use error or from intentional misuse of the investigational device.	ISO 14155
Serious Adverse Event (SAE)	Adverse event that 1. led to death, 2. led to serious deterioration in the health of the subject, that either resulted in a. a life-threatening illness or injury, or b. a permanent impairment of a body structure or a body function, or	ISO 14155

Term	Definition	Reference
	c. in-patient or prolonged hospitalization, or d. medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function, 3. led to fetal distress, fetal death or a congenital abnormality or birth defect. 4. NOTE Planned hospitalization for a pre-existing condition, or a procedure required by the clinical investigational plan, without serious deterioration in health, is not considered a serious adverse event.	
Serious Adverse Device Effect (SADE)	Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.	ISO 14155
Unanticipated Adverse Device Effect (UADE)	Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.	21 CFR Part 812

Underlying diseases are not reported as Adverse Events (AEs) unless there is an increase in severity or frequency during the course of the study. Death is not an adverse event but should be reflected as an outcome to another specific AE. Any AE experienced by the study subject after enrollment whether during or subsequent to the study procedures must be collected and recorded in the (e)CRF.

9.1.1 Relationship of Adverse Event to the Study Device and/or Procedures

The investigator must assess the relationship of the adverse event to the study device using the following criteria:

Unrelated: the adverse event is determined to be due to a concurrent illness or effect of another device/drug and is not related to the investigational device.

Possible: the adverse event is determined to be potentially related to the investigational product, and an alternative etiology is equally or less likely compared to the potential relationship to investigational product.

Probable: there is a strong relationship to investigational product, or recurs on re-challenge, and another etiology is unlikely

Highly probable: there is no other reasonable medical explanation for the event.

The investigator must assess the relationship of the adverse event to the study procedures using the following criteria:

Unrelated: the adverse event is determined to be due to a concurrent illness or effect of another device/drug and is not related to the study procedure.

Possible: the adverse event is determined to be potentially related to the investigational product, and an alternative etiology is equally or less likely compared to the potential relationship to the study procedure.

Probable: there is a strong relationship to study procedure, or recurs on re-challenge, and another etiology is unlikely

Highly probable: there is no other reasonable medical explanation for the event.

9.2 Potential Adverse Events

Study subjects will continue to receive standard of care treatments throughout the study, which are not part of study procedures. The treating physician will counsel the subjects on the risk of the treatments including the risk of surgery, radiation therapy and any other treatment appropriate for their type and stage of cancer. Toxicities commonly associated with such treatments include but are not limited to fatigue, nausea, vomiting, anorexia, and weight loss. Some of these symptoms can also be due to tumor progression.

Adverse events related to SBRT radiotherapy for the treatment of metastases are dependent on the location of the metastases treated, as well as from exposure of surrounding normal tissues. Fatigue and skin problems such as dryness, itching, blistering or peeling is likely to occur for all treated metastases and should be transient, lasting < 8 weeks. Patients who are receiving standard of care radiotherapy for lung tumors may experience difficulty swallowing, shortness of breath, breast or nipple soreness, shoulder stiffness and/or radiation pneumonitis characterized by cough, fever, and fullness of the chest associated with radiographic infiltrate. Anticipated adverse events for bone tumor patients who are undergoing standard of care radiotherapy may include erythema, desquamation, alopecia, fracture, and inflammation in nearby organs-at-risk (i.e. esophagitis, enteritis, pneumonitis, etc.).

Study procedure and/or FDG exposure related AE may include:

1. Allergic reaction, including itching, edema and rash, as well as brief or transient hypotension
2. Hypoglycemia and hyperglycemia, especially in patients with Diabetes Mellitus
3. Laboratory abnormalities, including increase in alkaline phosphatase, urinanalysis abnormalities, and cytopenias

Appropriate clinical assessments will be performed as indicated to identify all AEs. AEs will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5. Signs and symptoms of existing disease progression will not be considered AEs. Development of new cancer after the inclusion of subject in the study will be considered an AE.

9.3 Reporting Requirements

Event Classification	Communication Method to RefleXion Medical	Communication Timeline to RefleXion Medical
UADE*	Complete AE (e)CRF page including UADE question with all available new and updated information.	Within 24 hours of first becoming aware of the event.
SAE*	Complete AE (e)CRF page including UADE question with all available new and updated information.	Within 24 hours of first becoming aware of the event.
AE	Complete AE (e)CRF page	As soon as possible.
Device Failure and Malfunction	Complete applicable CRF fields/pages with all available new and updated information	Within 24 hours of first becoming aware of the event.

**Note: SAEs/UADEs are reported electronically via EDC. If the EDC system is not available, the paper SAE Back-up form should be used for reporting and EDC should be updated once available. The SAE Back-up form should be sent to Medpace Clinical Safety as follows:*

Email: Medpace-safetynotification@medpace.com OR Fax: +1-513-570-5196 or +1-866-336-5320.

9.4 Device Failures and Malfunctions

All RMRS device failures and malfunctions related to the investigational aspects of the device (e.g. BgRT functionality) shall be reported within 24 hours after becoming aware to RefleXion Medical personnel via data entry to appropriate eCRFs. If EDC system is not available to report device malfunction, the appropriate paper CRF needs to be completed with the information and faxed to the RefleXion Medical personnel listed in the current Study contact list provided in the Study binder. Device failures and malfunctions should also be documented in subject's medical record.

Study-applicable device failures and malfunctions should be reported on the appropriate eCRF within 24 hours after becoming aware of event, however failures and malfunctions should not be reported as adverse events in and of themselves. In the event that device failure or malfunction cause an AE, the AE and specific event causing the AE should be reported on the appropriate eCRFs.

All RefleXion Medical non-study device failures or malfunctions should be reported to the RefleXion customer service organization.

9.5 Reporting to Regulatory Authorities, Institutional Review Boards and Investigators

RefleXion Medical will code and report all verbatim adverse events to study investigators and regulatory authorities, as applicable. RefleXion Medical or its designees will utilize Medical Dictionary for Regulatory Affairs (MedDRA) to code all AEs reported in the trial. RefleXion Medical will report any UADEs to FDA as per Medical Device Reporting guidelines (21 CFR 803).

The site Principal Investigator is responsible for informing the IRB of UADE, SAE and/or AEs per local requirements. A copy of this report should be provided to RefleXion Medical study personnel.

Adverse events and protocol deviations along with the sites they occurred will be identified by RefleXion Medical in applicable reports and correspondence with FDA.

10. RISK BENEFIT ANALYSIS

Engineering evaluation of the RMRS has demonstrated that the safety features of the RMRS have been shown to be effective through testing and that the overall residual risk is considered to be within the clinically acceptable level.

10.1 Study Risk Management Procedures

Eligibility criteria have been selected that exclude patients who are at a higher risk for experiencing an anticipated adverse event in order to reduce the risks to patients who participate in this study.

Study subjects will continue to receive standard of care treatments throughout the study, which are not part of study procedures. The treating physician will counsel the subjects on the risk of the treatments including the risk of surgery, radiation therapy and any other treatment appropriate for their type and stage of cancer.

Monitoring will take place throughout the study and adverse events will be recorded in the Patient's charts and on Case Report Forms developed for the study. Investigational device training will be conducted at each initiated study center and appropriate training records will be maintained.

10.2 Known and Anticipated Risks

The study procedures may include multiple administrations of FDG. Reviews of the oncology and cardiology literature have not revealed known adverse reactions attributable to FDG administration aside from sporadic hypersensitivity reactions[17]. Even at multiple administrations, FDG is not expected to result in meaningful toxicity due to radioactivity exposure as each injection results in a total whole body dose of 20 mSV (2000 mrem) or less[18,19]. Therefore, exposure from multiple administrations in the BgRT workflow is comparable to the annual federal limit for radiation workers (50 mSv; 5000 mrem) and is significantly below that experienced by patients undergoing radio-iodine therapy for thyroid disease (200 mSv; 20,000

mrem). In fact, the radiation exposure of the multiple FDG injections is considerably less than the incidental exposures experienced by patients during other routine aspects of current radiotherapy practice; for instance, cumulative radiation exposure originating from both daily image-guidance using cone beam computed tomography and from scatter dose during high-energy beam delivery meaningfully exceed the radiation exposure that can result from multiple FDG administrations. These observations are summarized in the following table:

Table 3: ***Comparison of effective dose from multiple FDG administrations and other incidental radiation exposures from currently used procedures in conventional radiotherapy***

	Approximate Effective Dose (mSv)
2-6 FDG injections (10-20 mCi each)	10-120
Conventional Radiotherapy: 30 CBCT Image-Guidance Procedures	Up to 1000
Conventional Radiotherapy: Scatter Dose from 60 Gy Prescription	120-200

This comparison of FDG dose versus incidental dose from routine aspects of current radiotherapy also holds true for individual organs. For example, the organ most at risk from FDG exposure, the bladder, receives approximately 6-12 cGy from each FDG injection (36-72 cGy in the case of 6 injections). This exposure is an order of magnitude below the incidental radiation that the bladder receives from a course of conventional prostate radiation, which can range from approximately 500 cGy to 8100 cGy, depending on the proximity of the portion of bladder being assessed to the prostate gland.

This argument for tolerability, which is based upon first principles of radiation safety, is further supported by empiric evidence from human and animal studies. Cox et al. conducted a phase I trial of very high doses of FDG to determine whether the radiation emitted by FDG could be utilized for therapeutic effect[20]. In their study, three patients were given progressively higher doses of FDG (100 mCi/m^2, 150 mCi/m^2, and 200 mCi/m^2) with a single injection. With a median follow-up of 6 months, no dose limiting toxicities were observed. Notably, the third patient in the study received 400 mCi of FDG. Multiple investigations have utilized serial weekly FDG PET scans over a 6 week course of fractionated radiotherapy to assess early tumor response during treatment; although toxicity was not a formal endpoint in these imaging studies, nonetheless no unexpected adverse events attributable to multiple administrations of the tracer were reported[21,22]. Moadel et al. determined that the dose-limiting organ in human patients treated with high-dose FDG would be the red marrow using the Medical Internal Radionuclide Dosimetry Formalism, and further determined that the tolerance dose that would result in 5% risk of red marrow suppression at 5 years (TD 5/5) was 4760 mCi[23,24]. In addition to these human investigations, a number of animal studies of very high FDG doses (up to 6 mCi per 20 g mouse) have, to date, not resulted in any observable toxicity[22].

Although this evidence and first principles of radiotherapy safety provide reassurance that multiple FDG administrations at a diagnostic dose are unlikely to cause measurable toxicity, nonetheless this investigation seeks to provide additional evidence for the safety of multiple FDG administrations. Cohort II will seek to capture evidence regarding the tolerability of multiple FDG administrations through standard grading of toxicities during therapy. Study subjects will be observed for potential FDG administration related toxicity and AEs related to the organs at theoretical risk, which include the bladder and bone marrow. These adverse events may include anemia, thrombocytopenia, bleeding, lymphopenia, neutropenia, hematuria, bladder spasms, transient hypotension, hypo- or hyperglycemia and/or had transient increases in alkaline phosphatase.

Other potential FDG exposure related risks and mitigations include:

- Overexposure of bladder to FDG, mitigated by proper hydration prior to FDG (besides fasting) and frequent voiding for at least one hour after completion of the procedure
- Radiation exposure to nearby individuals, mitigated by limitation of exposure to others until the FDG decay is complete
- Risk of FDG overdose, mitigated by proper documentation of FDG administration and corresponding radiation exposure

In addition to FDG exposure risk, subjects will receive localization (positioning) scans with the RefleXion system integrated fan-beam kVCT imaging prior to each PET image collection session. The risks to the patient from this imaging procedure are expected to be in accordance with the general risks of low-dose radiation exposure, including the long-term risk of secondary malignancy.

11. STUDY COMMITTEES

11.1 Safety Monitoring Committee

A Safety Monitoring Committee (SMC) consisting of individuals with pertinent expertise (including but not limited to a non-site radiation oncologist, site radiation oncologist and/or physicist, RefleXion Medical physician and RefleXion Medical technical staff) will be formed. Responsibilities of the committee are outlined in the SMC charter and will include oversight of the overall conduct of the study with regard to study progress, ensuring subject safety and overall data quality and integrity. The SMC will be responsible for assessing Cohort I data to identify and confirm the RRFD in the context of the primary endpoint (achieving the minimum activity level in the treatment target area [BTZ]) according to the modified 3+3 design outlined in *Figure 5*. Additionally, the SMC will review Cohort I secondary analyses and has the discretion to consider the performance of the studied FDG dose in the context of secondary endpoints. If at the RRFD, the performance of emulated BgRT delivery meets statistical parameters for success in the primary endpoint and there are no objections arising from the other secondary analyses, then the SMC will open Cohort II to accrual. Additionally, the SMC will monitor patient safety by reviewing AEs and SAEs

including AE onset, intensity, action taken, and outcome on a regular basis for the duration of the study for both cohorts.

11.2 Independent Image Reviewers

Independent image reviewers with pertinent expertise in radiation oncology and/or radiology will assess RMRS PET and third-party diagnostic PET/CT images in order to determine the agreement standard and external image concordance secondary endpoints for Cohort I and Cohort II. All the readers will be trained using identical training data set and image review software programs as detailed in the image review charter (IRC). RMRS PET images will be read by three radiation oncologists and third-party diagnostic PET/CT images will be read by three radiologists. Readers will be blinded to patient diagnosis, reader evaluations, images from the compared device (i.e. the radiologist reviewers for the diagnostic PET/CT would be blinded to the patient's RMRS PET and RMRS kVCT for localization) but not the patient's Sim CT and other clinical data. Each reader will use the applicable schema and answer the same set of questions to generate a positive or negative decision as outlined in Section 6. Image assessments will be directly entered by the central reviewers in the dedicated imaging eCRF and signed electronically.

12. ETHICAL CONSIDERATIONS

12.1 Compliance with Good Clinical Practices (GCP)

The investigator is responsible for ensuring the study is conducted in accordance with clinical principles and practices originating from the Declaration of Helsinki. The study should be conducted in accordance to the Good Clinical Practices (GCP) and applicable local requirements, whichever provides subjects greater protection.

12.2 Institutional Review Board

A copy of the protocol, proposed ICF, other written subject information, including any proposed advertising material must be submitted to the IRB for written approval. A copy of the written IRB approval of the protocol and ICF must be received by RefleXion Medical before recruitment of subjects into the study.

The principal investigator is responsible for submitting and, where necessary, obtaining approval for all subsequent amendments to the protocol, and any changes to ICF, as well as obtaining annual IRB approval and renewal throughout the study. The principal investigator must also notify the IRB regarding protocol deviations, UADEs, SAEs occurring at the site and other UADE/SAE reports received form RefleXion Medical in accordance to the local requirements and procedures.

Copies of the investigator reports and all IRB approvals, including continuance of IRB approval, must be sent to RefleXion Medical.

12.3 Written Informed Consent Form

RefleXion Medical will provide the Study Informed Consent Form Template to the investigator to prepare for use at his/her site. The written Informed Consent documents should be prepared in the language(s) of the potential subject population.

RefleXion and the reviewing IRB must first approve the study ICF before use. The ICF must be in alignment with current guidelines as outlined by the GCP guidelines, Declaration of Helsinki and International Conference on Harmonization (ICH).

Prior to participating in the study, each subject must give written Informed Consent after the context has been fully explained to the subject in a language that is easily understood by the subject. The subject must be given enough time and opportunity to ask questions and have those questions answered to his/her satisfaction. Written Informed Consent process must be recorded appropriately by means of the subject's, or his/her legal representative's, dated signature. The consent process must be documented in the subject's medical chart.

12.4 Amending the Protocol

This protocol must be followed exactly and can be altered only by written amendments. Following appropriate approval, the revised protocol will be distributed to all participating sites. Each site must obtain IRB/EC approval before implementing the revised protocol.

12.5 Emergency Actions

RefleXion Medical accepts the rights of the Investigator to deviate from the protocol in an emergency when necessary to safeguard the life or the physical well-being of a study subject. The Investigator must give notice of any emergency deviations and justification for the deviation to RefleXion Medical and IRB as soon as possible after the deviation, in any event no later than 24 hours after the emergency.

12.6 Protocol Adherence

Prior to beginning of the study, the Investigator must sign the Investigator Agreement documenting his/her agreement to conduct the study in accordance with the protocol. The Investigator must not make any changes or deviate from this protocol, except to protect the life and physical well-being of a subject in an emergency. Each deviation from the protocol must be documented with the date and reason for the deviation and reported to RefleXion Medical as soon as possible, and to the IRB per local guidelines and regulations.

13. STATISTICAL CONSIDERATIONS

13.1 Sample Size Calculation

13.1.1 Cohort I: RRFD Cohort

This objective of Cohort I is to identify the Recommended RefleXion FDG Dose (RRFD). Dose levels of 15 and 20 mCi (if necessary) will be assessed sequentially in an escalation protocol. To that end, a modified 3+3 design will be utilized wherein meeting the activity level threshold, not dose-limiting toxicity, will be the relevant endpoint. The RRFD will be primarily based upon the lower FDG dose level where 5 to 6 out of 6 subjects have an Activity Concentration greater than 5 kBq/ml. As mentioned in section 4.2, dose escalation to the second dose level can also be triggered by secondary analysis of technical factors related to radiotherapy plan quality derived from the RMRS PET imaging data.

The modified 3+3 design with 6 patients at the 'Recommended RefleXion FDG Dose level' aims to ensure that the observed proportion of patients below activity threshold to be less than 33%. The following table shows the probability of observing at most 1 of 6 below activity threshold with various true below activity rates:

Probability of Observations at Most 1 of 6 below Activity Threshold

True below activity rate	40%	30%	20%	10%
Probability at most 1 of 6 patients below activity threshold	0.233	0.420	0.655	0.886

As such, either 6 patients will be accrued if 15 mCi FDG meets criteria or 9 or 12 total patients will be accrued if escalation to 20 mCi is required.

Descriptive statistics will be utilized for all secondary endpoints.

13.1.2 Cohort II: Emulated Delivery Cohort

The purpose of Cohort II is to emulate delivery of BgRT plans based upon clinical PET data obtained on the RefleXion system from human subjects using the RRFD established in Cohort I.

The primary endpoint is the percent of radiotherapy fractions where the emulated BgRT dose distribution *in silico* is shown to be consistent with the approved BgRT treatment plan (i.e., 95% of $DVH_{Delivered}$ points for the BTZ and OARs fall within the bounded DVH of the approved BgRT plan).

As previously described in Section 4.3, a BgRT dose distribution is considered evaluable if:

- The subject has successfully undergone BgRT plan creation, physics quality assurance, and plan approval

- A “pass” signal is achieved at the PET PreScan evaluation preceding the long-duration PET emission collection

The sample size was determined a priori to represent a sufficient number of dose distributions for a robust sampling of BgRT deliveries under clinical conditions, including both before and after start of external beam radiotherapy. A sample size of 16 evaluable dose distributions from 8 to 22 subjects will be obtained to inform the concurrence between BgRT dose distributions and approved BgRT plan. Assuming the concurrence rate by the outcomes of primary endpoint follows a binomial distribution, the sample size achieves cumulative probability of approximately 0.81 that at most 1 inconsistency out of 16 evaluable dose distributions when true inconsistency rate is 5%, as shown in below table:

Cumulative Probability at most 1 inconsistency out of 16 evaluable distributions:

True inconsistency rate	40%	30%	20%	10%	5%
Probability at most 1 non-concurrence out of 16 assessments	0.003	0.026	0.141	0.515	0.811

Given that no prior data exists for biology-guided radiotherapy, we will report descriptive statistics for secondary endpoints, as defined in section 4. The summary statistics for BgRT workflow steps will be described for all enrolled patients including those with non-evaluable dose distributions.

13.2 General Consideration

Data collected will be presented using summary tables, patient data listings, and figures (TLFs). Continuous variables will be summarized using descriptive statistics with the mean (standard deviation), median, minimum, and maximum. Categorical variables will be summarized by frequencies and percentages. TLFs will be presented separately for each Cohort. Cohort I will be summarized by dose level, if more than 1, and total. Cohorts I and II will be summarized by site of disease (bone, lung) and total.

13.2.1 Patient Disposition

A summary of patient disposition including patients who enrolled in the study, patients who completed the study, terminated study early, and the reasons for early study termination

13.2.2 Demographics and Baseline Characteristics

The demographic characteristics (e.g., age, race, ethnicity, gender, baseline height, and baseline body weight) and baseline characteristics such as ECOG PS, disease status etc. will be summarized and listed. Categorical characteristics (e.g., gender, ethnicity and race, ECOG) will be summarized by frequency and percentage.

13.2.3 Prior and Concomitant Medications

Each medication will be coded to a preferred term and an Anatomic Therapeutic Classification (ATC) code using WHO Drug dictionary. Prior concomitant medication is defined as any medication ended prior to the first day of the FDG administration. The number and percentage of patients taking each prior or concomitant medication will be displayed by medication class, preferred term and treatment group.

13.2.4 Safety Analyses

Extent of FDG exposure will be summarized by number of doses, cumulative dose, any dose interruption and dose reduction. Safety will be assessed through AEs, changes in laboratory parameters, and vital signs. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA); AE severities will be graded using the CTCAE criteria.

All AE data will be listed. Notably, actual delivery of BgRT treatment to the patient is not part of this investigation. All treatment-emergent AEs (TEAEs), defined as adverse events not present prior to FDG exposure and study procedures, will be summarized, as follows:

- All TEAEs by worst Grade
- All TEAEs related to the study device and/or FDG exposure by worst Grade
- All Grade 3/4/5 TEAEs
- All Treatment Emergent SAEs by worst Grade
- All Related Treatment Emergent SAEs related to study device and/or FDG exposure by worst Grade

Observed and changes from baseline in laboratory values will be summarized. Laboratory values outside the corresponding normal ranges will be flagged in listings.

Observed and Changes from baseline vital signs and ECOG data will be summarized similarly as laboratory values.

13.2.5 Primary and Secondary Endpoints

Continuous primary and secondary endpoints will be summarized descriptively using the mean (standard deviation), median, minimum, and maximum. Categorical primary and secondary endpoints will be summarized descriptively by frequencies and percentages.

For Cohort I secondary imaging endpoints, agreement comparison analysis between different readers will also be performed. The descriptive statistics including overall, positive and negative percent agreement will be calculated for each endpoint.

For categorical endpoints of Cohort II, the primary analysis will use the number of evaluable radiation dose distributions (Dose Volume Histograms) instead of number of subjects as the denominator under the assumption that all assessments are independent to each other. To evaluate the robustness of the primary analysis results, a sensitivity analysis will be carried out taking the within-subject correlation into account.

13.3 Statistical Analysis Plan

The Statistical analysis plan (SAP) will be finalized prior to database lock. Any analysis that is different than what is in the protocol will be described in detail in the SAP.

14. STUDY ADMINISTRATION

Sites will make every effort to ensure the study is conducted in compliance with GCP and all applicable regulatory requirements.

14.1 Pre-Study Documentation

The site must complete all essential pre-study documentation prior to enrolling a subject into the study. The following pre-study documentation should be on file with RefleXion Medical:

- Site PI's CV and current medical license
- Fully executed Clinical Trial Agreement
- IRB approval of the study protocol and ICF
- Documentation of a completed Site Initiation Visit
- Documentation of all required study training
- W-9 from site to facilitate payment

Sites need to have written authorization/approval from RefleXion Medical to start enrolling subjects into the study.

14.2 Record Retention

The Investigator will maintain all essential study documentation and source documentation that support the data in collected on study subjects in their original format and in compliance with ICH/GCP guidelines. Study documentation must be retained for at least two years after the last approval of marketing application until at least two years have elapsed after the formal discontinuation of the clinical study. These documents may be retained for a longer period of time by agreement with RefleXion Medical or by other regulatory requirements. It is RefleXion Medical's responsibility to inform the Investigator when these documents no longer needed to be maintained. Investigator will take appropriate measures to ensure study documents are not accidentally damaged or destroyed. If for any reason, Investigator withdraws responsibility for maintaining essential study documents, custody must be transferred to another individual who will assume the responsibility and RefleXion Medical must be provided a written notification of the custodial change.

14.3 Criteria for Suspension/Termination of a Study Site

RefleXion Medical holds the right to suspend or terminate the study at a study site at any time during the course of the study if enrollment rate is significantly lower than previously projected, or if the site has multiple or severe protocol violations without justification or fails to implement remedial actions. RefleXion Medical will notify appropriate regulatory agencies regarding termination of any study sites, as required.

14.4 Criteria for Suspension/Termination of Study

RefleXion Medical reserves the right to suspend or terminate the study but intends to only exercise this right for valid scientific or administrative reasons, and reasons related to protection of subjects. In the event of suspension or early termination of the study, RefleXion Medical will notify investigators and IRBs in writing.

15. REFERENCES

1. ICRU. International Commission on Radiation Units and Measurements (ICRU) Report 50 [Internet]. [cited 2020 Jan 21];Available from: https://icru.org/home/reports/prescribing-recording-and-reporting-photon-beam-therapy-report-50

2. ICRU. International Commission on Radiation Units and Measurements (ICRU) Report 62 [Internet]. [cited 2020 Jan 21];Available from: https://icru.org/home/reports/prescribing-recording-and-reporting-photon-beam-therapy-report-62

3. ICRU. International Commission on Radiation Units and Measurements (ICRU) Report 83 [Internet]. [cited 2020 Jan 21];Available from: https://icru.org/testing/reports/prescribing-recording-and-reporting-intensity-modulated-photon-beam-therapy-imrt-icru-report-83

4. Gargett M, Haddad C, Kneebone A, Booth JT, Hardcastle N. Clinical impact of removing respiratory motion during liver SABR. Radiat Oncol [Internet] 2019 [cited 2020 Jan 16];14. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6547575/

5. Al-Ward S, Wronski M, Ahmad SB, et al. The radiobiological impact of motion tracking of liver, pancreas and kidney SBRT tumors in a MR-linac. Phys Med Biol 2018;63(21):215022.

6. Karava K, Ehrbar S, Riesterer O, et al. Potential dosimetric benefits of adaptive tumor tracking over the internal target volume concept for stereotactic body radiation therapy of pancreatic cancer. Radiat Oncol 2017;12(1):175.

7. Caillet V, Keall PJ, Colvill E, et al. MLC tracking for lung SABR reduces planning target volumes and dose to organs at risk. Radiotherapy and Oncology 2017;124(1):18–24.

8. Tian S, Sethi I, Yang X, et al. Characterization of Inter-Fraction 18-FDG PET Variability During Lung SBRT: Results of a Prospective Pilot Study. International Journal of Radiation Oncology, Biology, Physics 2019;105(1):E536.

9. Potters L, Kavanagh B, Galvin JM, et al. American Society for Therapeutic Radiology and Oncology (ASTRO) and American College of Radiology (ACR) Practice Guideline for the Performance of Stereotactic Body Radiation Therapy. International Journal of Radiation Oncology • Biology • Physics 2010;76(2):326–32.

10. McShan DL. Workflow and Clinical Decision Support for Radiation Oncology. Efficient Decision Support Systems - Practice and Challenges in Biomedical Related Domain [Internet] 2011 [cited 2020 Jan 17];Available from: https://www.intechopen.com/books/efficient-decision-support-systems-practice-and-challenges-in-biomedical-related-domain/workflow-and-clinical-decision-support-for-radiation-oncology

11. Manyam B. A review of strategies for optimizing workflow, quality improvement, and patient safety within radiation oncology departments [Internet]. [cited 2020 Jan 17];Available from: https://appliedradiationoncology.com/articles/a-review-of-strategies-for-optimizing-workflow-quality-improvement-and-patient-safety-within-radiation-oncology-departments

12. Jaffray DA, Gospodarowicz MK. Radiation Therapy for Cancer [Internet]. In: Gelband H, Jha P, Sankaranarayanan R, Horton S, editors. Cancer: Disease Control Priorities, Third Edition (Volume 3). Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 2015 [cited 2020 Aug 19]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK343621/

13. Halvorsen PH, Cirino E, Das IJ, et al. AAPM-RSS Medical Physics Practice Guideline 9.a. for SRS-SBRT. Journal of Applied Clinical Medical Physics 2017;18(5):10–21.

14. Boellaard R. Standards for PET Image Acquisition and Quantitative Data Analysis. J Nucl Med 2009;50(Suppl 1):11S-20S.

15. ACR–SPR Practice Parameter for Performing FDG-PET/CT in Oncology. :17.

16. PET-CT Scanner - an overview | ScienceDirect Topics [Internet]. [cited 2020 Mar 5];Available from: https://www.sciencedirect.com/topics/nursing-and-health-professions/pet-ct-scanner

17. 021870s004lbl.pdf [Internet]. [cited 2020 Mar 5];Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021870s004lbl.pdf

18. Hays MT, Watson EE, Thomas SR, Stabin M. MIRD dose estimate report no. 19: radiation absorbed dose estimates from (18)F-FDG. J Nucl Med 2002;43(2):210–4.

19. Quinn B, Dauer Z, Pandit-Taskar N, Schoder H, Dauer LT. Radiation dosimetry of 18F-FDG PET/CT: incorporating exam-specific parameters in dose estimates. BMC Med Imaging 2016;16(1):41.

20. Cox BW, Vallabhajosula S, Rini J, et al. A Phase I Dose Escalation Study of Targeted Radionuclide Therapy of Malignant Tumors Using 18F Fluorodeoxyglucose (18F-FDG): Initial Experience and Early Results. International Journal of Radiation Oncology • Biology • Physics 2016;96(2):E510–1.

21. Hentschel M, Appold S, Schreiber A, et al. Serial FDG-PET on patients with head and neck cancer: Implications for radiation therapy. International Journal of Radiation Biology 2009;85(9):796–804.

22. Edet-Sanson A, Dubray B, Doyeux K, et al. Serial assessment of FDG-PET FDG uptake and functional volume during radiotherapy (RT) in patients with non-small cell lung cancer (NSCLC). Radiotherapy and Oncology 2012;102(2):251–7.

23. Moadel RM, Nguyen AV, Lin EY, et al. Positron emission tomography agent 2-deoxy-2-[18F]fluoro-D-glucose has a therapeutic potential in breast cancer. Breast Cancer Res 2003;5(6):R199-205.

24. Jaini S, Dadachova E. FDG for therapy of metabolically active tumors. Semin Nucl Med 2012;42(3):185–9.

Attachment 1: IDE Study Event Schedule

Event	**Screening & Baseline**[1]	**PET Imaging-only**	**Cohort I Follow-Up** ***(end of study - Cohort I)*** at 72±24 hours after PET Imaging-only session	**First Fraction of SBRT**	**Last Fraction of SBRT**	**Cohort II Follow-Up** ***(end of study - Cohort II)*** at 72±24 hours after final FDG administration
Informed Consent[2]	X					
Demographics, Medical History & Physical Exam	X					
CBC	X					X *(Cohort II only)*
CMP	X					X *(Cohort II only)*
ECOG performance status	X					X *(Cohort II only)*
Blood glucose	X	X		X *(Cohort II only)*	X *(Cohort II only)*	X *(Cohort II only)*
Urinalysis	X					X *(Cohort II only)*
Pregnancy test[3]	X					
Sim CT [4, 5]	X					
RRFD FDG injection		X[†]		X *(Cohort II only)*	X [‡]*(Cohort II only)*	
RMRS PET w/ kVCT localization [4]		X		X *(Cohort II only)*	X *(Cohort II only)*	
Third-party diagnostic PET/CT[4]		X *(Cohort I only)*			X *(Cohort II only)*	
AE Assessment[6]	X *(Cohort II only)*	X	X *(Cohort I only)*	X *(Cohort II only)*	X *(Cohort II only)*	X *(Cohort II only)*

[1]Baseline Assessments should be completed within 14 days of RMRS PET Imaging-only session

[2]Informed consent is not considered a baseline assessment and has no requirement to be completed within a specific time window from study visits

[3]Women of child-bearing potential only; urine or serum, within 14 days prior to the initiation of the study scans.

[4]Images should be de-identified before submitting to RefleXion Medical.

[5]If Sim CT is not done (or planned to be done) as a part of standard of care, a Sim CT will be obtained at baseline

[6]AEs to be assessed using Common Terminology Criteria for Adverse Events (CTCAE) v5. In Cohort II, directed grading for bladder and bone marrow toxicity will be completed at baseline and 72±24 hours after final FDG administration.

[†]At the PET Imaging-only session timepoint in Cohort I, if the third-party PET scan cannot be initiated within 180 minutes of original FDG injection, then the third-party diagnostic PET/CT scan can be completed with a separate FDG injection 24 to 96 hours after the initial FDG injection.

[‡]At the last fraction of SBRT timepoint in Cohort II, if the third-party PET scan cannot be initiated within 180 minutes of original FDG injection, then the third-party diagnostic PET/CT scan can be completed with a separate FDG injection 24 to 96 hours after the initial FDG injection.