Hypoxic Changes in Hepatocellular Carcinoma (HCC) Following Trans Arterial Chemo Embolization and Stereotactic Radiation: [18F]Fluoromisonidazole (FMISO) Imaging

Study Protocol & Statistical Analysis Plan

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SCHEMA

This Phase II study will investigate the utility of [^{18}F] FMISO in patients with hepatocellular carcinoma (HCC). This trial is designed to test the hypothesis that PET determined [^{18}F]FMISO uptake will indicate tumor hypoxia in patients with HCC treated with trans-arterial chemo-embolization (TACE). We anticipate that [^{18}F] FMISO PET/CT will advance our understanding of the role of hypoxia in HCC prior to treatment and that this knowledge will help design newer combination therapeutic trials for better treatment outcomes.

Adults with HCC and limited hepatic disease eligible for TACE will be enrolled in the study and imaged according to the following schema: After imaging (FMISO 1 and FMISO 2 in the diagram below), patients will be followed for two years or until disease progression. [^{18}F] FMISO PET/CT provides three parameters, tissue hypoxic volume (HV), maximum tissue to blood uptake ratio ($T:B_{max}$) and tumor blood flow. Both pre-and post-therapy images will be examined to investigate changes in these parameters during the course of TACE treatment.

The study schema is shown below.

Figure 1.

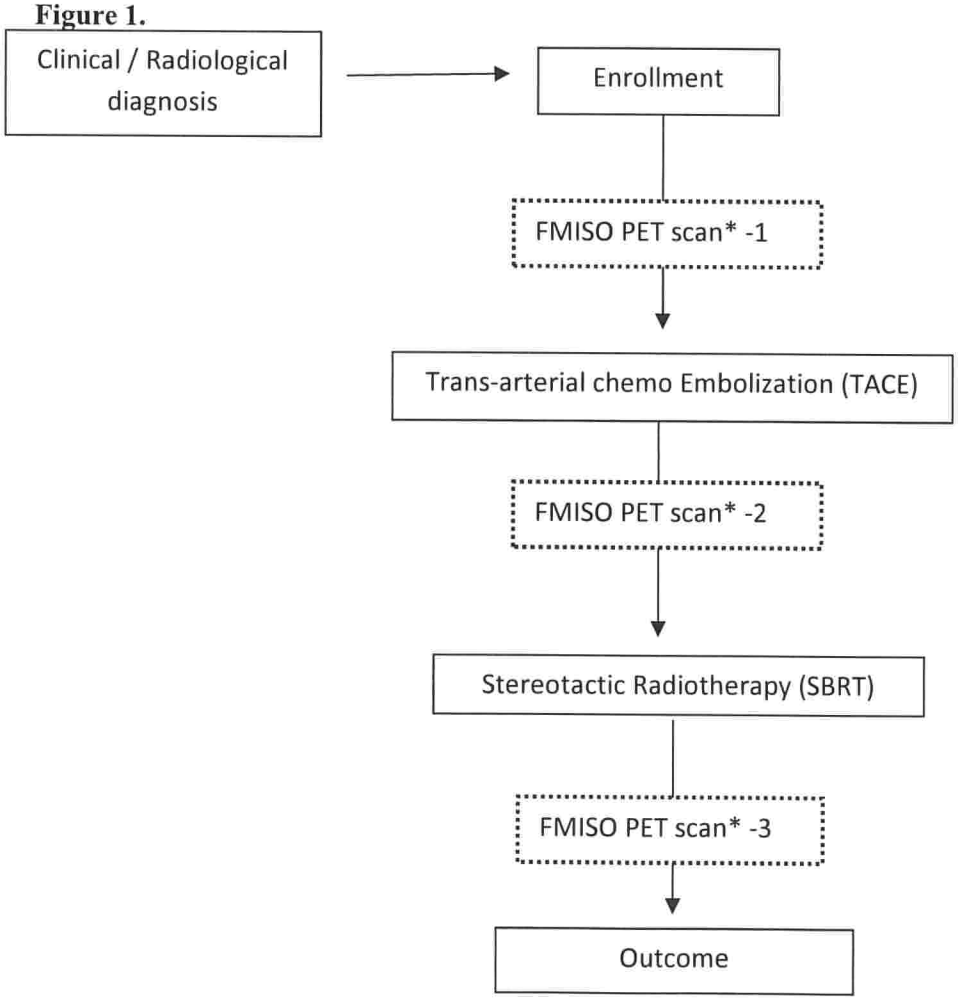

Figure 1: Diagram of patient management and timepoints where the [^{18}F] FMISO studies will be done in the course of treatment. FMISO PET #1 will be performed prior to treatment. FMISO PET #2 will be performed 1 month after all TACE procedures (in case multiple TACE procedures are performed) and prior to SBRT, and FMISO PET #3 will be performed 1 month following the end of liver stereotactic radiation therapy. 20 patients will be enrolled in this study.

1. OBJECTIVES

This Phase II study will investigate the ability of [^{18}F] FMISO (FMISO) to identify hypoxia in HCC and development of increased tumor hypoxia in tumors treated with TACE.

Quantitative assessment of baseline hypoxia in HCC

HCC are often large volume tumors at presentation and are known to have focal hypoxia. This tissue condition is a significant driver of tumor aggressive recurrent behavior and invasiveness which lead to a poor long-term response to therapy. Low tissue O_2 allows the hypoxia-inducible factor 1 HIF1 transcription factor to up regulate production of a host of proteins that promote survival of tumor cells in an environment of diffusion-limited or chronic hypoxia. Because hypoxia increases tumor treatment resistance to chemo- and radiation therapy, it is important to identify this risk factor, as hypoxic cells can survive chemotherapy while retaining their potential to proliferate. Though FMISO has been used in a limited fashion to evaluate hypoxia in HCC, quantitative measurement and 3-dimensional tumor mapping of hypoxia in HCC has not been performed. In this study, we aim to evaluate the extent of hypoxia within HCC and changes in tumor hypoxic volume in response to combination TACE + stereotactic hepatic radiation therapy using FMISO PET imaging. In this combination treatment, hypoxia is postulated as a factor in treatment resistance and tumor recurrence.

A host of factors influence glucose metabolism within HCC including the extent of anaerobic glycolysis which exists within hypoxic tissue and the influence of HIF-1 α expression on glucose metabolism. Therefore, the level and extent of HCC [^{18}F] fluorodeoxyglucose (FDG) uptake in does not correlate with significant treatment risk factors related to the tumor level of hypoxia. FMISO is a Nitroimidazole compound. These compounds are lipophilic and passively diffuse through the cell membrane. In a hypoxic environment, they are converted to R-NO2 radicals by the nitroreductase enzyme. Under continued hypoxic conditions, R-NHOH compounds are produced in tissue that bind covalently to intracellular molecules which is the mechanism of tissue uptake and retention for FMISO. Because of these differences in tissue uptake and retention between FDG and FMISO, it is widely accepted that a combination of FDG and FMISO PET derived image data provide complementary tumor biologic information in tumors where significant volumes of hypoxia are present. Utility of FMISO has been studied extensively in gliomas, and cancers of the lung, head and neck and prostate. In patients with brain tumors, FMISO can be used to differentiate glioblastoma multiforme from other low grade glioma due to their inherent differences in tumor hypoxia. In addition, hypoxic volume determined by FMISO correlates well with the presence of disrupted vasculature (an indicator for potential hypoxia) on gadolinium-enhanced T1-weighted MRI. Findings on FMISO imaging in head and neck cancers have been shown to be predictive of patient outcome. In addition, tumor hypoxia mapping using FMISO PET has been utilized successfully to intensify local therapy.

The objective of this study is to perform an imaging study of FMISO in patients with HCC. These data will help characterize the extent of hypoxia in HCC. Additional exploratory study findings will relate tumor response to TACE treatment, including radiotherapy, which is our current management for HCC patients. The results will be used to evaluate the use of FMISO PET hypoxia imaging for guiding therapy and evaluating treatment response in a large-scale trial.

1.1 Primary Aims:

1.1.a Quantitate HCC tumor hypoxia at baseline using FMISO PET.

1.1.b Determine changes in HCC tumor hypoxia and blood flow after TACE, prior to stereotactic radiotherapy.

1.1.c Determine changes in treated HCC tumor hypoxia following TACE and radiotherapy.

1.2 Exploratory Aims:

1.2.a Test FMISO HCC tumor uptake and blood flow as independent predictors of patient outcome prior to TACE.

1.2.b Test FMISO post combination treatment HCC tumor uptake and blood flow as predictors of response in of patients receiving TACE+ stereotactic hepatic radiation therapy.

2. BACKGROUND

2.1 Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma is a leading cause of mortality and morbidity worldwide. The incidence of this malady is on the rise, largely as a consequence of widespread infection with Hepatitis B and C viruses. Surgical management, which is the only curative option, is not suited for all patients. In fact, a large proportion of patients are only eligible for palliative or life-prolonging treatments. HCC is relatively resistant to most classic local therapies such as conventional radiation.

2.1.a Role of Hypoxia in tumor proliferation and treatment responses of HCC

Rapidly proliferating solid tumors are under-perfused at the central zone due to micro-environment changes and abnormal angiogenesis. These hypoxic areas derive oxygen predominately through the process of diffusion, which is often insufficient. Tumor cells surviving within hypoxic zones are therefore capable of anaerobic metabolism or other adaptive survival mechanisms. Preferential proliferation of cells adapted to hypoxia can thus result in increasing volumes of hypoxia within a growing solid tumor. Tumor hypoxia is a known prognostic indicator of response to anticancer treatments. Hypoxic cells are classically more resistant to radiation (RT).

In HCC, hypoxia is a significant contributor of tumor progression and treatment resistance. HIF-1α levels are significantly elevated in HCC and act as the trigger for a number of down-stream events leading to tumor progression, invasion and metastases. Furthermore, hypoxia induced HIF-1 promotes gene expression that enhances cell survival under hypoxic conditions. In addition, it is also known that RT responses are enhanced in tumors deficient in the function of hypoxia-inducible factor-1. As with many other cancers, treatment planning can be tailored and intensified if tissue markers can be used to identify a patient at higher risk for treatment failure and poor outcome. PET imaging with a hypoxia specific imaging agent (FMISO) will be an effective means of identifying at risk HCC patients, and will provide critical information for combination treatment planning.

2.1.b. Use of hypofractionated radiotherapy and trans arterial chemoembolization (TACE)

Hypoxic cell sensitizers and hypofractionated radiotherapy have been used to circumvent radio-resistance of hypoxic tumors. The latter exerts its effect through activation of the immune system, endothelial cell death and re-oxygenation. High dose hypofractionated radiation is increasingly being used in the treatment of solid tumors, made possible by the availability of sophisticated computing and systems capable of delivering focused radiation with very high fidelity. This treatment has the advantage of ensuring rapid fall off of radiation away from the target, which helps to reduce the incidence of normal tissue complications.

Trans Arterial Chemoembolization (TACE) is another local therapy currently recommended for most unresectable HCC due to clinical results that show improved survival when compared to best supportive care alone. TACE exerts its effect by interrupting arterial supply, predominantly to the center of the tumor (Lo 2002, Llovet 2002). Though up to a 63% survival rate is reported at 2-years following TACE, it is still considered a life-prolonging palliative therapy, as the procedure seldom completely eliminates the tumor. The impact of TACE in inducing tumor hypoxia is not clearly known. Theoretically, TACE triggers expression of proteins associated with tumor progenitor cell differentiation. In a study by Lai et al, the cell hypoxia marker CAIX, and cholangiocytic/progenitor markers CK19 and EpCAM were increasingly expressed in areas of hypoxia within HCC following TACE procedure (Lai 2015).

A number of strategies are currently used to circumvent TACE induced hypoxia in HCC. These include the addition of hypoxia-activated prodrugs (such as TH-302) or multi-kinase inhibitors (such as Sorafenib) following TACE. At the University of Alabama at Birmingham (UAB), we have adopted a strategy of combining TACE with highly focused hypofractionated radiotherapy (Stereotactic Body Radiotherapy -SBRT). SBRT combined with TACE offers a number of theoretical advantages such as radio-sensitization, spatial targeting and hypoxic targeting, which can potentially improve tumor control. In a study from our institution, Jacob et al observed significantly improved survival rates among HCC patients treated using a combination of TACE-SBRT compared with those treated with TACE alone. This treatment related survival advantage was more pronounced among patients with tumors over 3 cm, implying that inherent or TACE-induced hypoxia is likely a treatment resistance risk factor in larger tumors.

2.1.c. Biologic Factors Influencing Outcome in Solid Tumors

Hypoxia Influences Response to Treatment: In the absence of oxygen, the free radicals formed by ionizing radiation in tissues recombine without producing the desired cellular damage thus requiring as much as three times more radiation to cause the same cytotoxic effect (Coleman 1988, Hockel 1993). Using [^{60}Cu]-ATSM PET, Dehdashti et al have shown a negative influence of tissue hypoxia (as inferred by the level of uptake of this radiopharmaceutical) on response to radiation. (Dehdashti 2003) Hypoxia is also known to negatively affect response to chemotherapy, including cisplatin by a number of different biological mechanisms. (Amellem 1991, Sutherland 1998, Comerford 2002)

2.2. Imaging of Hypoxia

2.2.a [^{18}F] FMISO as a PET Hypoxia Imaging Agent

2-nitroimidazole agents which preferentially bind to hypoxic cells within tissues have been evaluated both as hypoxic markers for use in cancer imaging as well as hypoxic-cell radiosensitizers. ^{18}F-labeled misonidazole (FMISO), ^{123}I-labeled iodoazomycinarabinoside (^{123}I-IAZA) and ^{62}Cu labeled diacetyl-bis (N^4-methylthiosemicarbazone) (Cu ATSM) have been used to image tumor hypoxia. Of these, [^{18}F] FMISO has most commonly been used; the half-life of the ^{18}F isotope mirrors the biological clearance of misonidazole making this combination ideal for tumor imaging. [^{18}F] FMISO PET studies have the advantage of short half-life and positron emission, which help in reducing absorbed radiation dose in tissues, while providing high resolution imaging. There is significant discordance between the fluoro-deoxy-glucose (FDG) and FMISO uptake within the same tumor tissue due to prevalence of hypoxic areas which are likely to be less active via classic glucose metabolic pathway.

Estimation of tumor hypoxia has several clinical applications such as prognostication, intensification of therapy, use of alternate therapies (which are likely to be more effective in hypoxic tissue) or for combining differing treatment modalities. As a proof of principle, Hendrickson et al utilized [^{18}F] FMISO PET imaging and Intensity Modulated Radiotherapy (IMRT) planning to escalate radiation dose to hypoxic sub-volumes within locally advanced Head & Neck cancers. (Hendrickson 2011) A significant improvement in outcome was predicted with additional dose delivered using a simultaneous integrated boost technique.

[^{18}F]FMISO is a radiolabeled imaging agent that has been used for investigating tumor hypoxia with positron emission tomography (PET). The evaluation of FMISO was developed at the University of Washington under the authority of FDA IND 32,353. FMISO is an azomycin-based hypoxic cell sensitizer that when reduced by hypoxia, binds covalently to cellular molecules at rates that are inversely proportional to intracellular oxygen concentration, rather than by any downstream biochemical interactions. (Prekeges 1991, Casciari 1995, Rasey 2000) [^{18}F]FMISO consists of fluoromisonidazole labeled with ≤10 mCi of radioactive ^{18}F at a specific activity of greater than> 125 Ci/mmol @ time of injection. The drug, <15 μg injected, is the only active ingredient and it is used as an investigational radiopharmaceutical. The sterile doses of the drug are formulated in <10 mL of 4% ethanol in saline for intravenous injection. The radiochemical purity of the injected FMISO is ≥95%.

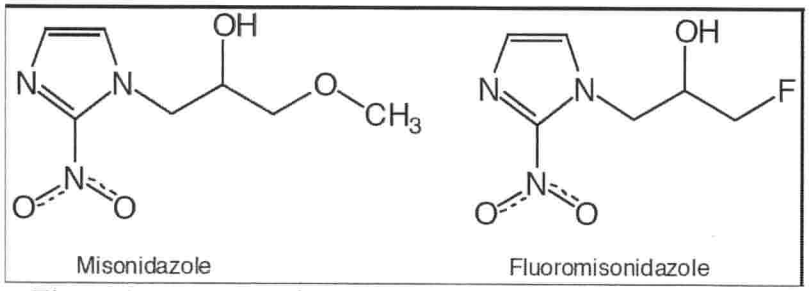

Figure 2. Structures of misonidazole and fluoromisonidazole.

2.2.b. Pharmacology of FMISO

Summary of mechanism of action

[^{18}F]FMISO is a nitro-imidazole derivative that has an electron affinity nearly as high as O_2. It continually reacts in an environment of enzymatic electron transport to give a 1-electron reduction product. The latter is a radical anion that can either lose its e^- to something with a higher e^- affinity, commonly O_2, or accept another e^-. The 2-electron reduction product ultimately binds to intracellular macromolecules through a bioreductive alkylation mechanism. The reaction with O_2 returns the radical anion reduction product to its nitroimidazole parent form in a futile cycle. Thus, in viable cells there is a steady state concentration of the radical anion intermediate that is subsequently trapped in the cell at a rate that is inversely proportional to the concentration of O_2. FMISO is not trapped in necrotic tissue because mitochondrial electron transport is absent. It has an octanol : water partition coefficient of 0.41, so that it would be expected to reflect plasma flow as an inert, freely-diffusible tracer immediately after injection, but later images reflect its tissue partition coefficient in normoxic tissues.

In vivo under normal oxygen tension, MISO is metabolized primarily in the liver to its demethylated form; ~7% (in man) to 14% (in mice) is conjugated to glucuronide, and small amounts (<5%) are converted to aminoimidazole. Substantial amounts of MISO are recoverable in feces. Fecal bacteria are able to reduce misonidazole only in the absence of oxygen. The plasma half-lives of both FMISO and MISO range from 8 – 17.5 hours with treatment doses. (Josephy 1985) The parent molecule and glucuronide metabolites are primarily excreted in the urine. (Flockhart 1978, Flockhart 1978)

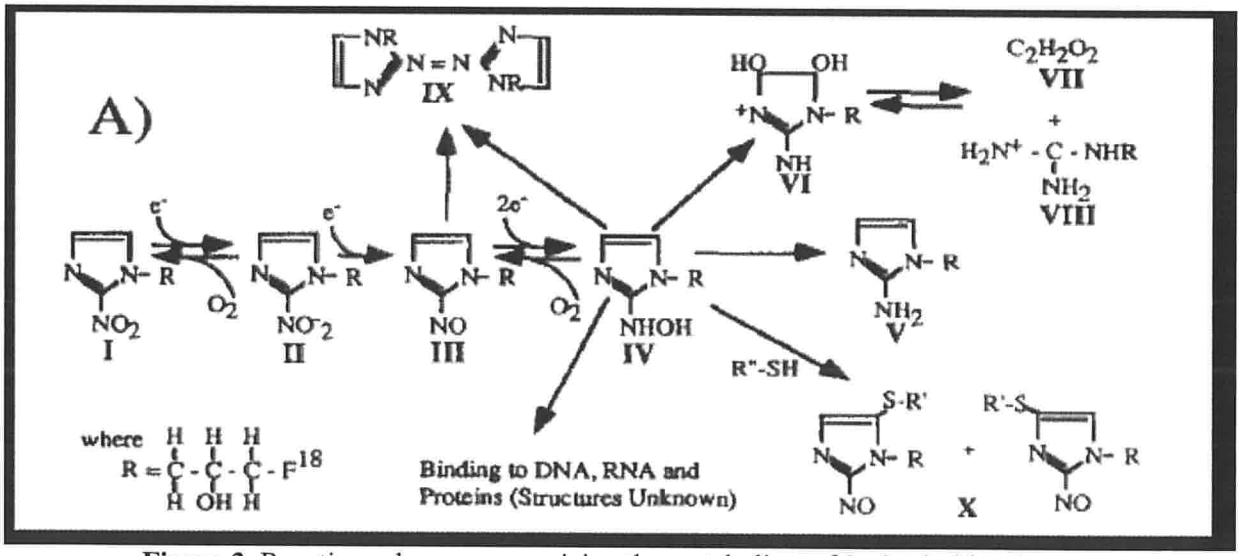

Figure 3. Reaction scheme summarizing the metabolism of 2-nitroimidazoles.

2.2.c. Human Radiation Dosimetry for [^{18}F] FMISO

^{18}F is a positron emitter with a half-life of 110 minutes. Intravenously injected [^{18}F]FMISO distributes throughout the total body water space, crossing cell membranes, including the blood-brain-barrier, by passive diffusion. [^{18}F]FMISO is bound and retained within viable hypoxic cells in an inverse relationship

to the O_2 concentration. The uptake of [^{18}F]FMISO in normal human tissues has been measured and used to estimate the radiation absorbed dose associated with the imaging procedure. Dosimetry studies were performed at the University of Washington and have been peer-reviewed and published in the Journal of Nuclear Medicine. (Graham 1997)

Sixty subjects were involved in the study, men and women. The majority, 54, had cancer, three had a history of myocardial ischemia, two were paraplegic and one had rheumatoid arthritis. After injecting 3.7 MBq/kg (0.1 mCi/kg), urine and normal tissues distant from each subject's primary pathology were imaged repeatedly to develop time-activity curves for target tissues. All tissues demonstrated a rapid uptake phase and first-order near-logarithmic clearance curves. All tissues receive a similar radiation dose, reflecting the similarity of biodistribution to that of water. Total tissue uptake data were normalized for a 1.0 MBq injection into a 70 kg man. (Graham 1997) From this human data, radiation absorbed doses to organs was calculated using the MIRD schema and the results are shown in **Table 1.** from (Graham 1997).

Table 1. Radiation absorbed doses for [^{18}F] FMISO

organ	Mean (mGy/MBq)	Mean (mrad/mCi)	Total / 7 mCi (mrads)
adrenals	0.0166	61.4	430
brain	0.0086	31.8	223
breasts	0.0123	45.5	319
gall bladder wall	0.0148	54.8	383
lower large intestine	0.0143	52.9	370
small intestine	0.0132	48.8	342
stomach	0.0126	46.6	326
upper large intestine	0.0140	51.8	363
heart wall	0.0185	68.5	479
kidneys	0.0157	58.1	407
liver	0.0183	67.7	474
lungs	0.0099	36.6	256
muscle	0.0142	52.5	368
ovaries	0.0176	65.1	456
pancreas	0.0179	66.2	464
red marrow	0.0109	40.3	282
bone surface	0.0077	28.5	199
skin	0.0048	17.8	124
spleen	0.0163	60.3	422
testes	0.0146	54.0	378
thymus	0.0155	57.4	401
thyroid	0.0151	55.9	391
urinary bladder wall	0.0210	77.7	544
uterus	0.0183	67.7	474
eye lens	0.0154	57.0	399
Total body	0.0126	46.6	325

While the maximum radiation absorbed dose was to the bladder wall, this varied with voiding intervals from 0.021 to 0.029 mGy/MBq.

The calculated total body dose for a 70 Kg man injected with 3.7 MBq/kg was 0.013 mGy/MBq; for a 57 Kg woman it was 0.016 mGy/MBq. Effective dose equivalents were 0.013 mSv/MBq for men and 0.014 mSv/MBq for women. Ninety-seven percent of the injected radiation was homogenously distributed in the body, leaving only 3% for urinary excretion. Doses to smaller organs not directly determined by visualization, such as the lens, were calculated assuming average total-body concentrations. The absence of tracer visualized in images of those organs indicated that accumulation there was not increased.

The radiation exposure from FMISO is equal to or lower than from other widely used nuclear medicine studies. Increasing the frequency of voiding can reduce radiation dose to the normal organ receiving the highest radiation absorbed dose, the bladder wall. Potential radiation risks associated with this PET study are within generally accepted limits.

2.2.d. Previous Human Experience with FMISO

Hypoxia imaging in cancer was reviewed in several publications (Rasey 1999, Rajendran 2005, Koh 1995, Rajendran 2004). [^{18}F]FMISO is a robust radiopharmaceutical useful in obtaining images to quantify hypoxia using PET imaging (Graham 1997, Silverman 1998). It is the most commonly used agent for hypoxia PET imaging (Rasey 1999, Koh 1995, Rajendran 2004, Valk 1992, Eschmann 2005, Hicks 2005). While its biodistribution properties do not result in high contrast images, they result in images at 2 hours after injection that unambiguously reflect regional PO_2 and hypoxia in the time interval after the radiopharmaceutical was administered.

Positron emission scanning with FMISO has been studied over the past ten years in Australia, Switzerland, Denmark, Germany and in the United States under RDRC approval or its equivalent. All the listed studies from the United States are from the University of Washington in Seattle and were conducted under IND 32,353. Since 1994 a total of 304 [^{18}F]FMISO PET scans have been done in Seattle on 232 patients. [^{18}F]FMISO has also been used to image ischemic stroke, myocardial ischemia and a wide variety of malignancies. A total of 280 patients* are represented in the listed studies below. Administered doses ranged from approximately 3 to 30 mCi (100 to 1100 MBq). No adverse events were noted in any of these papers. These studies are summarized in Table 2.

Table 2.: Published manuscripts reporting ^{18}F-FMISO human imaging studies

Year	Clinical Condition	n	MBq injected	Specific Activity	nmoles injected	Reference
2005	Head & neck ca Non-small cell lung cancer	26 14	350-450			Eschmann (Germany)
2004	Various brain tumors	11	123-421 Avg.= 291			Bruehlmeier (Switzerland)
2004	Various cancers	49	3.7/Kg nom 260			Rajendran (USA)
2004	Head & neck cancer	16	292 ± 35			Gagel (Germany)
2003	Ischemic Stroke	19	nom 130			Markus (Australia)
2003	Soft tissue tumors	13	218-418			Bentzen

			Avg.= 400			(Denmark)
2003	Soft tissue sarcoma	29	3.7/Kg nom 260			Rajendran (USA)
2001	Brain tumors	13				Scott (Australia)
2000	Ischemic Stroke	24	nom 130			Read (Australia)
1996	Various cancers	37	3.7/Kg nom 260		0.1 nmol/Kg	Rasey (USA)
1995	Non-small cell lung cancer	7	3.7/Kg nom 260	1.5-2.2 E13 Bq/mmol	0.03 mol/Kg	Koh (USA)
1992	Various cancers	8	740-1100 (multiple studies)	400-600 Ci/mmol		Koh (USA)
1992	Glioma	3	370	1000 Ci/mmol		Valk (USA)
1991	Myocardial Ischemia	11	3.7/Kg nom 260			Revenaugh (USA)
	Total	280*				

*It is possible that some patients are represented twice.

The general conclusion from the studies summarized above is that [^{18}F]FMISO PET identifies hypoxic tissue that is heterogeneously distributed within human tumors (Rasey 1996), and shows promise to help facilitate image-guided radiotherapy and clinical trials of new hypoxia-selective cytotoxins, two ways that might help circumvent the cure-limiting effects of tumor hypoxia. In addition, [^{18}F]FMISO has identified a discrepancy between perfusion, blood-brain barrier disruption, and hypoxia in brain tumors (Bruehlmeier 2004) and a lack of correlation between FDG metabolism and hypoxia in several types of malignancies (Rajendran 2003). Hypoxic tissue does not correlate either with tumor volume or vascular endothelial growth factor (VEGF) expression (Rajendran 2005, Rajendran 2004). [^{18}F]FMISO imaging was able to identify post-radiotherapy tumor recurrence by differential uptake of tracer. The standardized uptake value (SUV) ratio between recurrent tumor and muscle was >1.6 and between tumor and normal mediastinum was >2.0 (Eschmann 2005).

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Adult (≥ 18 years of age) patients with documented HCC tumor mass ≥3cm, who are scheduled to undergo TACE with additional SIRT

3.1.2 The appropriate criteria for inclusion for this patient population are:

3.1.2.a Biopsy or radiological diagnosis of HCC (defined as Organ Procurement and Transplantation Network (OPTN*) Category 5 lesion either on CT or MRI)

3.1.2.b. Scheduled for TACE (using doxorubicin-eluting beads) + SBRT

3.1.2.c. Willingness to undergo PET/CT

3.1.2.d. Able to lie on the imaging table for up to 1 hour.

3.1.2.e. Able to provide signed informed consent.

3.1.2.f. Women with childbearing potential must have a negative urine β-hCG test day of procedure

3.2 Exclusion Criteria

3.2.1 Estimated life expectancy <12 months or serious medical co-morbidities that would preclude definitive local therapy.

3.2.2 Unable to lie on the imaging table

3.2.3. Age less than 18 years.

3.2.4. Pregnancy or lactation

3.2.5. Inability or unwillingness to provide informed consent.

3.2.6. Weight >500 lbs (the weight limit of the tomograph gantry table)

*See Appendix A for OPTN class 5 description

4. REGISTRATION PROCEDURES

Patients who are identified as potential candidates for the study will be identified in the Kirklin Clinic or at the Hepatobiliary Tumor Conference. They will be contacted to explain the nature of the study, to obtain written informed consent and to be enrolled in the study. The study coordinator will then schedule the imaging, and complete any subsequent forms.

5. STUDY PLAN

5.1 FMISO Administration

Fluorine-18 labeled misonidazole (FMISO) will be administered as an intravenous injection of less than or equal to: 15 μg of FMISO; 10 mCi of radioactivity; and 10 mL in volume. FMISO will be prepared in UAB Cyclotron PET Production Facility and dispensed from UAB Nuclear Pharmacy. It will be administered to the patient in the PET imaging suite at the UAB Advanced Imaging Facility, in the basement of WTI. The injection will be infused nominally over one minute and followed by a saline flush.

Study Procedures and Schedule of Events

Patients who are identified as potential candidates for the FMISO PET study will be approached to explain the nature of the study and to get their informed consent to be enrolled in the protocol. Imaging will be performed on inpatient or outpatient basis.

All patients entering the study will have had diagnostic CT or MRI scans of the abdomen as part of their cancer staging evaluation.

Imaging Acquisition Procedure for FMISO PET involves the following steps:

1. Baseline vital signs including resting blood pressure, pulse rate, and breathing rate will be measured and recorded within 60 minutes prior to the start of imaging.
2. Women of childbearing potential will receive a urine test prior to the procedure to rule out pregnancy
3. Placement of 1 intravenous catheter for injection of the radiopharmaceutical.

4. Careful patient positioning with the liver centered within the PET/CT scanner field-of-view. The patient will be made as comfortable as possible on the imaging table.
5. Attenuation correction will be obtained using the low dose CT scan on the PET/CT scanner.
6. Intravenous injection of FMISO ($\leq$ 15 μg (range 7 mCi-10 mCi) not to exceed 10 mCi, followed by saline flush.
7. The patient will be imaged in a dynamic fashion for 30 minutes and then again for a 30-minute static scan after a 60-minute wait period
8. Data analysis: The regional tissue:blood ratio values will be determined and hypoxic volume of the primary tumor and metastases will be calculated.
9. The study team will conduct a 24hr follow up call after FMISO administration to elicit and record any adverse event.

5.4 Duration of Follow Up.

Being an imaging only trial, we will not be seeing the patients in nuclear medicine after the last imaging is completed. We will be able to obtain clinical follow-up information from medical records generated at clinic visits and update our data base at 6 month intervals, for two years. Final analysis will take place at the completion of patient accrual.

5.5. Data Collection. Treatment selection is based on current clinical practices.

6. ADVERSE EVENTS REPORTING

Note: No adverse events have been attributed to Positron-Emission Tomography (PET) imaging/diagnostic administration of FMISO at the levels described in the Investigators Brochure. Therefore, no adverse events are expected as a result of the intravenous (IV) administration of FMISO for typical PET imaging applications such as tumor hypoxia.

Note: As with many IV administered agents, FMISO could cause an allergic reaction that could potentially pose a threat to life (anaphylaxis). This has not been observed in limited human exposure to date. Reasonable precautions should be taken, consistent with normal radiologic and clinical facility practice. The patient should be monitored until the PET procedure is completed, and trained personnel and emergency equipment should be available per facility standards.

Qualifying Adverse Events (AEs), including Serious Adverse Events (SAEs), as defined herein, will be reported via the FDA Adverse Event Expedited Reporting System (AERS). For the FMISO IND we will report adverse events based on the FDA final rule for IND safety reporting requirements under 21 CFR part 312 published on September 29, 2010 and implemented on March 28, 2011. This investigational study is not a BA or BE study so 21 CFR part 320 is not applicable. Adverse events will also be reported to the UAB IRB according to their requirements.

6.a. General Definitions (from 21 CFR 312.32 (a))

Adverse Event (AE): An **Adverse Event** is an untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related. For this study, the drug is FMISO and adverse events would include any events experienced by a study participant during the Adverse Event reporting period defined in **Table 3.** whether or not it was considered to be related to the FMISO. At the conclusion of the imaging study, the imaging technologist will observe the patient and also inquire if they are back to their usual state of health. If a negative answer is received, then the physician will be called to investigate this report as a possible adverse reaction.

Adverse Reaction:

An **Adverse Reaction** is any adverse event caused by a drug. In this study, the drug is FMISO.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the IND drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An **adverse event or suspected adverse reaction** is considered "**unexpected**" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

An **adverse event or suspected adverse reaction is considered "serious**" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include: allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

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

Investigational Agent: An investigational agent is any agent held under an Investigational New Drug (IND) application. For purposes of this study, FMISO is the investigational agent.

6.b. AE Reporting Requirements

The investigators on this protocol will report any suspected adverse events that occur after FMISO administration and within the specified follow-up period to Dr. O'Malley and they will work together to determine whether there was an adverse event or adverse reaction and the severity of the adverse event or reaction.

All AEs will be followed by the investigators until resolution, stabilization, scientifically and clinically satisfactory explanation as to attribution and etiology is achieved, or until subject is lost to follow up.

6.c. CAEPR / ASAE for FMISO

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. The Agent Specific Adverse Event List (ASAEL) would include the expected adverse events associated with the use of FMISO. At this time, there have been no reported AEs associated with the use of FMISO and so no ASAE are expected as a result of IV injection of FMISO in our study population with the specifications for our drug product. We will continue to update our CAEPR and ASAE lists as this study progresses, including by reviewing the literature and our in-house data safety monitoring. If any are found, we will begin an ASAE list. Any information on reported AEs for FMISO will be provided by the sponsor to all of the investigators on this protocol.

6.d. Potential but Unexpected Adverse Events for FMISO

No adverse reactions have been reported for FMISO based on a review of published literature, publicly available reference sources, and adverse drug reporting systems.

Other general risks for PET/CT imaging include:
The injection site may become infected.
The dose might be extravasated into tissues surrounding the vein catheter leading to localized pain / discomfort.

Radiation risks: FMISO injection contributes to lifetime radiation accumulation. The smallest dosage for imaging and safe handling are used for these protocols. The organ and total body doses associated with FMISO imaging are comparable to those associated with other widely used clinical nuclear medicine procedures and are presented in Section **2.2g** of this protocol. **Table 1.** summarizes the radiation dosimetry for FMISO.

6.e. Review of Safety Information.

As required by 21 CFR 312.32(b), the physician investigators will promptly review all information relevant to the safety of the drug. The physician investigators will also be providing much of this information to the local IRB as well for data safety and review monitoring. The review will include determining whether there is a safety event over time and the causality. As there are no expected events, all events that will be monitored will be unexpected. Reporting will be as described in **Table 3.**

Characterization of the severity of an Adverse Event: Adverse events will be graded as below.

Grade: Grade denotes the **severity** of the AE. An AE is graded using the following categories:

- **Mild**
- **Moderate**
- **Severe**
- **Life-threatening or disabling**
- **Fatal**

NOTE: Severity is graded on the Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria for Adverse Events (CTCAE) based scale for each adverse event. For example, an abnormal hemoglobin value is graded for severity from 1 to 5 [death] based upon where that value falls on the CTCAE scale of abnormal hemoglobin values. "Severity" is NOT the same as "Seriousness." All appropriate clinical areas should have access to a copy of the most current CTCAE and a copy of the CTCAE can be downloaded from (http://ctep.cancer.gov).

Attribution of cause: The physician investigators will determine whether an adverse event was related to a medical treatment or procedure. Definitions taken from our work with CTEP and NIH give the following definitions for "Attribution" that we will adopt for this IND study: Attribution is a clinical determination, by the investigator, as to whether an AE is related to a medical treatment or procedure. Attribution categories are:

- **Definite:** The AE is **clearly related** to a treatment or procedure
- **Probable:** The AE is **likely related** to a treatment or procedure
- **Possible:** The AE **may be related** to a treatment or procedure
- **Unlikely:** The AE is **likely unrelated** to a treatment or procedure
- **Unrelated:** The AE is **clearly not related** to a treatment or procedure
- **NOTE:** Attribution is part of the assessment of an adverse event. Determining that an event is 'unlikely related' or 'unrelated' to a study agent or procedure does NOT make the event unreportable, or disqualify the event as an AE. As defined above, an AE is reportable as specified herein if it occurred: **"during the Adverse Event reporting period defined in the protocol**, or by applicable guidance, regulation, or policy."

6.f. Adverse Event Reporting

Expedited AE reporting for this study will be done through the Cancer Consortium IRB and FDA and as required by FDA MedWatch. These requirements are briefly outlined in the table below. **Table 3. Reporting Requirements**.

	Unexpected	**Expected**

	Adverse Reaction **(known or suspected attributable to the use of FMISO**		**AE not attributable to FMISO**	**AE, AR**
	Serious including life-threatening (or death)	Nonserious	Life-Threatening or serious or not serious	None are expected for FMISO
Reporting Time Requirement to the FDA	**Report to FDA ASAP and within 7 days of discovery of event (section 6.ii.8.7)**	Annual Continuation Review submission	Annual Continuation Review submission	Not applicable to FMISO
Reporting Form for the FDA	IND Safety report of potentially serious risk	Annual Reports / Case reports	Annual Reports / Case reports	Not applicable to FMISO
Reporting Time Requirement to the local IRB	**Report to IRB ASAP within 10 days of discovery of event** (suspected is defined as 50% probability attributable to FMISO study) this also includes any increased risks with the study even without an AE	At continuation review time	At continuation review time	Not applicable to FMISO
Reporting form for the IRB	Expedited Reporting Form for Unanticipated Problems or Noncompliance and Adverse Event Reporting Form	Form for Unanticipated Problems or Noncompliance, Case reports on continuation form, Data Safety Monitoring Reports	Form for Unanticipated Problems or Noncompliance, Case reports on continuation form, Data Safety Monitoring Reports	Not applicable to FMISO

6.g. Expedited Adverse Reaction Reporting Guidelines

Life-threatening (or fatal) adverse reactions must be reported within 7 days to the FDA. The FDA should be notified as soon as the adverse reaction is discovered by telephone or fax or email. The instructions and forms are available at http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm The report should be sent ASAP by mail and followed with a follow-up report. Individual IND safety reports to FDA are submitted on the Medwatch FDA Form 3500A as an "IND Safety Report". The form should be sent to The Director, Office of Generic Drugs in the Center for Drug Evaluation and Research at FDA. The address and phone numbers are available at:
http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm119100.htm.

All life threatening adverse reactions reports are submitted to the FDA, THE UAB IRB and to all investigators. A copy of the report is kept on file.

7. STATISTICAL CONSIDERATIONS

7.1 Study Design/Endpoints

This is a Phase II study of a PET imaging agent, [^{18}F]FMISO, in patients with HCC who will be receiving standard care with TACE, and with radiation. The imaging study will not impact the patient's treatment. The objective of this study is to evaluate the potential of [^{18}F]FMISO as a non-invasive indicator of tissue hypoxia in HCC patients to provide tumor imaging data.

The primary endpoint is changes in [^{18}F]FMISO parameters (HV and T:B_{max}) between baseline and follow-up scans. The regional tissue: blood values will be determined and hypoxic volume of the primary tumor and metastases will be calculated. The number of pixels in the tumor volume with a T/B ratio $\geq$1.2, indicating significant hypoxia, will be determined and converted to milliliter units to measure the HV. In each tumor region, we will also locate the pixel with the maximum T/B ratio and include this variable, T/Bmax, in our analysis. HV evaluates the volume of tumor that has crossed the threshold for hypoxia and T/Bmax depicts the magnitude of hypoxia.

The exploratory endpoints include overall survival (OS), disease free survival (DFS) and response to XRT by RECIST, mRECIST or EASL (because enhancement changes are known to better reflect treatment response than size changes) criteria.

7.2 Sample Size/Accrual Rate

This is a Phase II study in patients with Hepatocellular Carcinoma (HCC). We propose a sample size of 20 patients for this initial clinical trial because of patient recruitment based on low HCC incident from UAB tumor register data. If the results are promising but inconclusive, they will be used to define a larger multi-center trial. We expect to have a full enrollment in 2 years. From the previous study (Rajendran 2006), a significant hypoxia was identified in 58 patients (79%) in head and neck cancer. The mean

FMISO T/Bmax was 1.6 (range 1.0–3.5) and the mean HV was 40.2 mL (range 0–1092). We expect at least 60% hypoxia/uptake (e.g. TB ratio changes from baseline >1.2) will be identified in HCC patients in this study, thus with a size of 20 patients we will be able to have an accurate estimation of patients who will have untaken of [^{18}F] FMISO with~ 10% error, and produces a two-sided 95% confidence interval with a width equal to 0.448 for each FMISO PET scan. The size will be also able to evaluate pre-post changes of 1.5 effect size for T/Bmax based on the above published data using one group paired t-test with >80% power at 5% alpha level. Due to the nature of exploratory to generate hypothesis data for future study, the intra correlation between FMISO PET scans within the same subject will not considerate in the sample size consideration this time.

7.3 Statistical analysis plan

The primary endpoint analysis of changes in [^{18}F]FMISO parameters (HV and T:B_{max}) between baseline and 2 follow-up scans will be using paired t-test. Proportion of patients with uptake of [^{18}F] FMISO will be reported as N and % and exact 95% confidence interval using the method of Clopper-Pearson. Descriptive analysis such as mean, standard decision at baseline and follow-up will be estimated with two–sided 95% confidence intervals for all [^{18}F]FMISO parameters from PET scans. Longitudinal analysis will be also applied to estimate the changes (slope) between baseline to the first treatment of TACE and sequential treatment of Stereotactic Radiotherapy (SBRT) with auto correlation covariance structure to control patients' intra-correlation. When repeat studies are done sequentially over the course of treatment, changes are expected to be substantial.

The exploratory endpoints analysis include using cox regression analysis to correlate [^{18}F]FMISO parameters with overall survival and progression free survival; and using logistics regression analysis to associate [^{18}F]FMISO parameters with clinical response (yes, no). Baseline [^{18}F]FMISO, FU [^{18}F]FMISO, and changes of [^{18}F]FMISO between baseline and FU will be used individually in order to identify if early image of [^{18}F]FMISO at baseline, and treatment with TACE alone (FU1) and with radiation (FU2) can predict survival or response. Survival is defined from the time of initial PET scan to the last follow up. Patient's demographics and other prognosis factors will be adjusted in the model. The set of prognostic variables considered in the analysis include standard clinical measures (age, tumor-node-metastasis status), as well as the PET measurements [HV, and FMISO tissue to blood ratio (T/Bmax)]. Due to the high correlation between HV and T/Bmax, and the resulting issue of multicollinearity, these variables will each considered separately with the other prognostic variables. Hazard ratio and Odds ratio will be estimated along with 95% CI. Log-rank tests will be used to compare Kaplan-Meier survival curves associated with patients above and below the median values for the variables of interest. Survival curves are presented with time measured in months. It is important to notice that patient survival will be evaluated every six month through medical record, therefore the Interval-censored survival analysis will be applied which simply extends the approach used for right-censored observations to left- and interval-censored observations (Sun 2006). The probability of a left-censored time is the probability that the true time is less than or equal to the censoring time. And, the probability of an interval-censored observation is the probability that the true time is less than or equal to the upper bound of the interval minus the probability that the true time is less than or equal to the lower bound of the interval. The SAS procedure NPMLE could be used. In addition, we will estimate the optimal cut point of [^{18}F] FMISO level using

ROC analysis based on the optimizations of sensitivity and specificity if there is an association between [^{18}F]FMISO level and outcomes. Due to small sample size, this analysis is exploratory in order to identify hypotheses for rigorous evaluation in future studies.

Safety analysis will be descriptive include frequency and proportion of patients have reported AE/SAE, by severity and attribution to study although we don't expect any AE/SAE occurs using the imaging agent based on previous published works.

All analysis will be carried out using statistical analysis software (SAS) v9.4.

7.4 Evaluation of response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following RECIST categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria (with the possible exception of those who received no study drug) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

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Appendix A

Class and Description	Comment
OPTN class 5	
Meets radiologic criteria for HCC	May qualify for automatic exception, depending on stage*
Class 5A:≥1cm and <2cm measured on late arterial or portal venous phase images	Increased contrast enhancement in late hepatic arterial phase AND washout during later phases of contrast enhancement AND peripheral rim enhancement (capsule or pseudocapsule).
Class 5A-g: same size as OPTN class 5A HCC	Increased contrast enhancement in late hepatic arterial phase AND growth by 50% or more documented on serial CT or MR images obtained ≤ 6 months apart
Class 5B: maximum diameter ≥2 cm and ≤ 5 cm	Increased contrast enhancement in late hepatic arterial phase AND either washout during later contrast phases OR peripheral rim enhancement (capsule or pseudocapsule) OR growth by 50% or more documented on serial CT or MR images obtained ≤6 months apart (OPTN class 5B-g)
Class 5T: prior regional treatment for HCC	Describes any residual lesion or perfusion defect at site of prior UNOS class 5 lesion
Class 5X: maximum diameter ≥5 cm	Increased contrast enhancement in late hepatic arterial phase AND either washout during later contrast phases OR peripheral rim enhancement (capsule or pseudocapsule)