A Feasibility Study Using Fluorine-18-Labeled Fluoro-Misonidazole Positron Emission Tomography to Detect Hypoxia in Colorectal Cancer Patients

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please note: All listed above must have complete the mandatory Human Subjects Education and Certification and Good Clinical Practice Certification Programs.

Participating Institutions – *If multicenter study coordinated by MSK:*	**PI's Name**	**Site's Role**
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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a 2-stage feasibility study of the non-invasive detection of tumor hypoxia using fluorine-18-labeled fluoro-misonidazole (^{18}F-FMISO), a PET tracer of hypoxia. The primary objective is to determine the feasibility of a non-invasive method of detecting hypoxia, ^{18}F-FMISO-PET imaging, in colorectal cancer patients, with feasibility defined as the ability to identify regions of interest (ROI) on the ^{18}F-FMISO scan that distinguish hypoxic versus normoxic regions in colorectal tumors.

Please note that all patients entered on this protocol will undergo the standard of care for their disease site. The information obtained from the use of ^{18}F-FMISO PET scans will NOT be used to guide patients' treatment on this protocol.

This study is being performed under the FDA's Radioactive Drug Research Committee (RDRC) program.

Schema:

1.1 Evaluation and consent.

1.2 A CT scout and attenuation scan will be performed on a combination PET/CT scanner to identify and localize the tumor at the center of the 15 cm PET scanner field of view. The patient will be injected while in the scan position with the ^{18}F-FMISO hypoxia-specific radiotracer. Simultaneous with the injection, a dynamic PET will be initiated, to collect radiotracer pharmacokinetic information.

1.3 The dynamic PET image frames will be used to analyze the hypoxia-specific radiotracer uptake and assist in determining the distribution of tumor hypoxia.

1.4 Digital autoradiography will be performed on the resected specimen in patients who undergo surgery on the same day as the ^{18}F-FMISO PET.

2.0 OBJECTIVE AND SCIENTIFIC AIMS

Our primary objective is to determine the feasibility of a non-invasive method of detecting hypoxia, using ^{18}F-FMISO-PET imaging in colorectal cancer patients. Feasibility is defined as the ability to identify regions of interest (ROI) on the ^{18}F-FMISO-PET images that distinguish between hypoxic and normoxic regions within colorectal tumors.
Secondary objectives include determining the volume of hypoxic tumor ROIs as a proportion of the entire tumor volume by this non-invasive imaging technique. ROIs are defined as those voxels, within the tumor volume defined on FDG PET/CT, for which the ^{18}F-FMISO radioactivity concentration is greater than 1.2 times that

measured in blood. Selection of this threshold is based on the largest body of ^{18}F-FMISO studies in humans (20, 21).

We will also obtain pharmacokinetic data on the biodistribution of ^{18}F-FMISO in patients with colorectal cancer to determine the optimal interval between drug administration and PET imaging.

Digital autoradiography, when performed, will allow us to determine the correspondence between the micro-distribution of ^{18}F-FMISO and the tumor histology.

3.0 BACKGROUND AND RATIONALE

3.1 Hypoxia

Hypoxia is a characteristic feature of malignant solid tumors that has been well established (1,2). Unlike healthy tissues, many tumors contain a fraction of hypoxic cells, which immunohistochemical studies suggest consist of areas of up to several hundred microns in diameter that are poorly perfused (3). It is well known that hypoxia renders tumor cells up to three times more resistant to ionizing radiation than aerobic cells (1-3). In addition to increased radioresistance, hypoxia is associated with a more aggressive phenotype and increased metastatic potential (4,5). It has also been shown in several studies to be an important determinant of loco-regional control of head and neck tumors (6,7).

A large amount of direct and indirect evidence also supports the presence of a significant hypoxic fraction in human rectal cancer. Hemoglobin saturation of red blood cells within microvessels averaged 43% in rectal cancer biopsies vs. 80% in the corresponding biopsies of normal rectal mucosa (8). Direct *in vivo* pO_2 measurement with polarographic electrodes in human rectal cancer have revealed a mean pO_2 of 20-30 mm Hg (8-11), with 23-45% of all measured values below 10 mm Hg (10,11). Also noted in these studies is the marked heterogeneity of oxygenation in rectal tumors. This heterogeneity may explain the wide extent of rectal cancer response to preoperative combined modality therapy (12).

There is mounting evidence that hypoxia is not only a potentially important prognostic factor in patients with rectal cancer, but in colon cancer patients as well. Studies utilizing immunohistochemical techniques have identified certain proteins, up-regulated during hypoxic conditions, correlate with increased rates of lymph node and distant metastasis (30), tumor recurrence and overall survival (31,32). This data suggests that hypoxic tumors may behave more aggressively than normoxic tumors.

As hypoxia is a known cause of radioresistance (1-3)(particularly important with regard to tumors that are treated with radiation therapy, such as locally advanced or recurrent rectal cancers and head & neck cancers), a number of methods aimed at improving tumor oxygenation are currently under investigation (ie. hyperbaric oxygen therapy (33), carbogen breathing (34) or administration of vasoactive agents (35)). Currently, the most extensively studied method includes administration of radiosensitizing agents (eg. Tirapazamine) concurrently with radiotherapy (36).

Tirapazamine, a hypoxic cytotoxin, preferentially targets hypoxic cells and induces DNA double strand breaks, leading to cell death. As demonstrated in head and neck cancer, cancer of the uterine cervix and non small cell lung cancer, tirapazamine holds promise as both a radiosensitizer and has synergistic chemotherapeutic effect when combined with cisplatin. This drug has been studied here at MSKCC in patients with cervical and non-small cell lung cancer (IRB Protocols 94-082, 94-114, 96-062), and is currently under investigation as an adjunct in patients undergoing chemoradiotherapy for cervical cancer (IRB Protocol 07-128).

If we are able to demonstrate that colon cancers contain a significant hypoxic fraction, we can justify the study of tirapazamine (or similar compounds) in patients with colorectal cancer. Currently, oxaliplatin is both highly active and widely used in the treatment of colon cancer patients. If active these patients, tirapazamine, in conjunction with oxaliplatin, may prove more efficacious than current chemotherapeutic regimens.

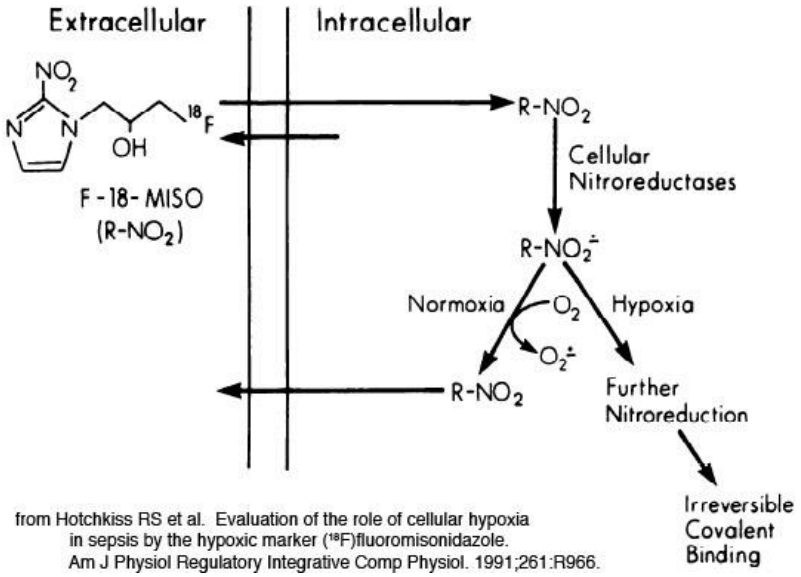
PET is a noninvasive imaging modality with the potential to identify tumor hypoxia through the use of hypoxia-targeting radiotracers. If these hypoxic regions are identified, they (a) may translate into a new prognostic indicator of therapeutic outcome and (b) be specifically targeted with additional radiation and perhaps translate into further improvement in local control.

3.2 ^{18}F-FMISO PET

^{18}F-FMISO PET is a recently developed imaging modality useful in detecting and quantifying hypoxic regions within tumors. This tool has been studied both *in vitro* and *in vivo* across a wide range of human tumor types. While it is well established that polarographic needle electrode assessment of tissue oxygen tension is considered the "gold standard" for detecting and measuring degree of hypoxia, we continue to investigate new, less invasive techniques. A number of studies comparing ^{18}F-FMISO PET imaging to needle electrode measurements in advanced head and neck tumors and soft tissue sarcomas have validated its use in studying hypoxia (13, 29)

Radiolabeled nitroimidazoles (including ^{18}F-FMISO) have emerged as useful non-invasive markers of hypoxia. These molecules, in their native form, are lipophilic and freely diffusible across cell membranes. Once intracellular, they undergo an

enzymatic reduction reaction. In the presence of oxygen, this reaction is rapidly reversed and the molecule is free to diffuse out of the cell, obtaining transmembrane equilibrium. In a hypoxic environment, the reduced molecule covalently binds to intracellular macromolecules, effectively trapping it within the cell (20, 28).



from Hotchkiss RS et al. Evaluation of the role of cellular hypoxia in sepsis by the hypoxic marker (^{18}F)fluoromisonidazole. Am J Physiol Regulatory Integrative Comp Physiol. 1991;261:R966.

At our institution, in a rat prostate cancer tumor model, as well as in two colorectal cancer xenograft models, ^{18}F-FMISO microPET imaging has been used to detect tumor hypoxia. These results were corroborated by measuring pO_2 directly using the OxyLite probe system (14). Preliminary results support the feasibility of imaging hypoxic subvolumes in some tumors, and we propose its use in studying colorectal tumors.

3.3 PET Studies

PET studies using hypoxic markers have also been performed in patients both at other centers as well as at MSKCC. For example, Rasey, et al. has demonstrated the efficacy of PET imaging with ^{18}F-FMISO in quantifying hypoxia in both head and neck and lung cancer patients (13). Hypoxia was observed in 97% of the tumors studied with ^{18}F-FMISO PET imaging, and median fractional hypoxic volumes of 48% for lung cancer and 9% for head and neck cancer were detected. The extent of hypoxia varied markedly between tumors of the same site or of the same histology. Hypoxia was also distributed non-uniformly between regions within a given tumor.

Eschmann, et al. suggests that the efficacy of radiotherapy can be predicted on the basis of the kinetic behavior of ^{18}F-FMISO in tumors of head and neck and non-small cell lung cancer (15). Dr. Nancy Lee, in the Dept. of Radiation Oncology at MSKCC, has successfully completed a study of 20 head and neck cancer patients using ^{18}F-FMISO PET, in which 18/20 showed ^{18}F-FMISO tumor uptake (16). In

this protocol, we propose to investigate ^{18}F-FMISO PET imaging in patients with colorectal cancer.

3.4 Trials

A phase I trial of concurrent tirapazamine, a hypoxic cell sensitizer, cisplatin, and radiotherapy in the treatment of advanced head and neck cancer has been completed at the Peter MacCallum Cancer Institute in Australia (17). All patients underwent ^{18}F-FMISO PET to demonstrate tumor hypoxia. All patients also had baseline FDG PET scans and the ^{18}F-FMISO and FDG scans were co-registered. ^{18}F-FMISO PET scans in this study were obtained two hours after radiotracer administration. All PET imaging was performed on a dedicated PET scanner with the data processed using measured attenuation correction and iterative reconstruction. Fourteen out of the fifteen patients had detectable hypoxia on baseline ^{18}F-FMISO scan with focal abnormality corresponding to a region of increased FDG uptake in either the primary lesion or the nodal mass. In all cases, the intensity of ^{18}F-FMISO uptake was less than the corresponding FDG abnormality. On the co-registered PET images, necrotic regions, as evidenced by central photopenia on FDG PET, demonstrated ^{18}F-FMISO distribution only at the inner border of FDG uptake. In those regions without necrosis on FDG PET, only the central part of the metabolically active lesion had ^{18}F-FMISO retention. The pattern of ^{18}F-FMISO uptake was consistent with the expected pattern of hypoxia in tumor tissue being adjacent to areas of tumor necrosis or in the center of non-necrotic lesions. The rapid normalization (i.e. reduction in tumor uptake) of ^{18}F-FMISO PET scans suggests successful treatment of the hypoxic component.

In another phase I trial, also done at the Peter MacCallum Cancer Institute in Australia (18), patients with inoperable rectal cancer or symptomatic primary rectal cancer with metastasis were given oxaliplatin on day 1 of weeks 1, 3 and 5 of radiotherapy. Dose level 1 was oxaliplatin 70 mg m^{-2} with 5-FU 200 mg m^{-2} day^{-1} continuous infusion 96 h week^{-1}. On dose level 2, the oxaliplatin dose was increased to 85 mg m^{-2}. On dose level 3, the duration of the 5-FU was increased to 168 h week^{-1}. Pelvic radiotherapy was 45 Gray (Gy) in 25 fractions over 5 weeks with a boost of 5.4 Gy. FDG- and ^{18}F-FMISO-PET were used to assess metabolic tumor response and hypoxia. In total, 16 patients were accrued. Dose-limiting toxicities occurred in one patient at level 2 (grade 3 chest infection), and two patients at level 3 (grade 3 diarrhea). Dose level 2 was declared the recommended dose level. FDG-PET imaging showed metabolic responses in 11 of the 12 primary tumors assessed. Four of six tumors had detectable hypoxia on ^{18}F-FMISO scans.

A prospective study from Germany (22) on 103 patients with locally advanced cancer of the uterine cervix demonstrated that tumor oxygenation, measured with a standardized polarographic method, is a strong pre-therapeutic prognostic factor. Patients with hypoxic tumors had a significantly worse disease-free and

overall survival following surgery alone, mainly due to locoregional failures with or without distant metastases.

3.5 Summary

Hypoxia is a characteristic feature of malignant solid tumors associated with poor prognosis and resistance to chemotherapy and radiation. It has also been shown (6) that the presence of hypoxia may reduce long-term survival post surgery. Hypoxia renders tumor cells up to three times more resistant to ionizing radiation than aerobic cells. The presence of hypoxic regions within tumors may be one factor leading to local failure after treatment with standard pre-operative radiotherapy doses. If these regions could be identified and verified using a non-invasive imaging technique prior to surgery, they could be specifically targeted using sophisticated planning techniques such as intensity modulated radiation therapy (IMRT) to deliver higher doses ionizing radiation with pre-operative radiotherapy. Future studies using IMRT to "dose paint" areas of hypoxia within tumors will build upon the results of this feasibility study. Ultimately, by the delivery of differential dose of radiation to the tumor, in combination with surgery, the local control rates of rectal cancer patients may further be improved.

4.0 OVERVIEW OF STUDY DESIGN AND INTERVENTION

4.1 Design

All patients will undergo the standard of care for their colorectal cancer and will have an FDG PET/CT scan performed within six weeks of a dynamic ^{18}F-FMISO PET/CT scan. We will accept FDG PET scans not performed at MSKCC if the outside study was performed as a dedicated PET/CT and the full data set (including all CT and PET images) is submitted digitally to us accompanied by a report clearly indicating the administered activity, uptake time and blood glucose level at the time of the study. The patient will receive no treatment between the two scans. Both scans will be performed before radiochemotherapy. The FDG PET scan will be used to delineate the tumor volume. The accompanying CT of both FDG and ^{18}F-FMISO will be co-registered, to allow selection of the ^{18}F-FMISO intra-tumoral hypoxic voxels.

Regions of interest (ROI) corresponding to hypoxic tumor sub-volumes will be defined as those voxels, within the tumor volume defined on FDG PET/CT, for which the ^{18}F-FMISO radioactivity concentration is greater than 1.2 times that measured in blood. The selection of this threshold is in concordance with the largest body of ^{18}F-FMISO studies in man (20, 21), which were successfully adopted in our own studies in head and neck cancers by Dr. Nancy Lee.

This is a feasibility trial using a 2-stage design. In the first stage, 15 patients will receive an ^{18}F-FMISO PET scan. If 4 or fewer patients have ROIs, the study will be stopped but if 5 or more patients have ROIs then an additional 15 patients at most will be scanned. We will therefore scan a maximum of 30 patients in order

to determine the hypoxia fraction in colorectal cancer using ^{18}F-FMISO. We anticipate the need to enroll approximately 50 patients to account for withdrawals of consent or inability of subjects to complete protocol requirement in order to achieve our target of 30 completed scans. Once this has been established, we propose a larger trial in order to correlate hypoxia fraction with treatment outcome.

Digital autoradiography will be performed on frozen tumor tissue sections obtained from the resected specimen in patients who undergo surgery on the same day as the ^{18}F-FMISO PET in order to determine the micro-distribution of ^{18}F-FMISO (see details in Section 4.2, "Intervention").

4.2 Intervention

^{18}F-FMISO PET scan

Dynamic PET scans will be performed on one of the GE Discovery PET/CT scanners in 2D mode. The patient will be set up in the radiotherapy treatment position with intra-venous lines for radiotracer injection and for venous blood sampling. A CT scan of a 15cm segment of the body (the field of view of the PET scanner) will be performed with the tumor at the field center. These images will be used for both attenuation correction and registration of the serial image set.

Immediately thereafter, a single 370 MBq (10 mCi) dose of ^{18}F-MISO will be injected as a bolus, and the dynamic scan initiated coincident with the injection. Data will be acquired in 1-minute time intervals continuously from 0-30 minutes. The patient will then be removed from the scanner. Since no further ^{18}F-FMISO will be injected in the patient, there will be no further radiation exposure from radiopharmaceutical. The 2nd PET scan (optional and performed at 90 minutes) and the 3rd PET scan (non-optional and performed between 120-240 minutes) will each consist of a 10-minute image of the tumor consisting of two consecutive 5-minute time frames. A 15-cm body segment low dose CT scan will be performed at each of these two additional image sessions for attenuation correction and image registration. The radiation dose of the first CT scan will be 0.9 mCi and the dose for the 2nd (optional) and 3rd (non-optional) scans will be 0.1 mCi each, for a total exposure of 1.0-1.1 mCi for all 3 CT scans. This total dose is approximately equivalent to one half (1/2) of the radiation exposure from a single diagnostic CT scan. To further improve patient image registration, the patient will have markers placed on the skin, inferior and superior to the lesion, to facilitate accurate laser set-up for the subsequent 2nd (optional) and 3rd (non-optional) scans.

Note that the patient will receive the radiation dose from only one ^{18}F-FMISO injection and from 2-3 low-dose CT scans. For a detailed estimate of radiation absorbed doses to organs as a result of these scans, please refer to Table 1.

Blood pharmacokinetic sampling

For pharmacokinetic modeling, the dynamic data of the ^{18}F-MISO radiotracer in the tumor must be supplemented by clearance data from the blood. This data will be ascertained by ROI analysis on an artery defined on the PET images. A limited number of blood samples (up to a maximum of 3) will be drawn (if possible) for the purpose of calibrating the image ROI data. When blood samples are taken, they shall be acquired at pre-designated times in conjunction with the PET scan from a single I.V. line inserted into the patient prior to the procedure. One blood sample will be taken per imaging time point, for a total of 2-3 blood samples. Between 1 and 3 cc of blood will be removed at each time point (making the maximum volume of blood withdrawn during this study < 9 cc).

These samples will be weighed and counted on a sensitive well scintillation counter in the nuclear medicine clinic by skilled nuclear medicine laboratory personnel for the purpose of an accurate determination of the amount of radioactive tracer in the blood versus time. The blood sample data will allow tumor-to-blood ratios to be determined for each of the imaging time points and provide information on the evolution of radiotracer entrapment within the hypoxic tumor regions. The kinetics of tumor uptake and blood clearance will be used to determine the optimal time to perform the ^{18}F-FMISO scan. Once pharmacokinetic modeling is complete, blood sampling will be discontinued. Therefore, not all 30 patients who undergo ^{18}F-FMISO scans will necessarily have blood sampling during imaging.

Digital Autoradiography

Digital autoradiography will be performed on the resected specimen for those patients who undergo surgery on the same day as the ^{18}F-FMISO scan,. Two tissue samples will be analyzed. The samples will be cubes with approximate dimensions of 5mm x 5mm x 5mm. The first sample will be taken from an ^{18}F-FMISO-avid (“hot”) section of the resected tumor, and the second sample will be taken from an ^{18}F-FMISO-negative (“cold”) section of the resected tumor. In instances when the entire tumor is “cold” and the surgical specimen contains adequate tissue, the first sample will consist of representative tumor tissue and the second sample will consist of adjacent normal mucosa.

Tissue samples will be individually wrapped in heavy-duty Saran Wrap film and immersed into pre-chilled methylbutane (Fisher Scientific, Pittsburgh, PA) at

-80˚C for 10 minutes. The frozen tissue samples will then be embedded in Optimal Cutting Temperature (OCT) compound (VWR Scientific, Rochester, NY) on dry ice and transferred to a -80˚C freezer for 30 minutes. Sets of contiguous frozen 8µm-thick sections will be cut from each tissue sample using a Microm HM500 cryostat microtome (Microm, Waldorf, Germany) and collected on glass microscope slides. A minimum of three frozen tissue sections from each specimen will be placed in a film cassette against a Fujifilm BAS-MS2325 imaging plate (Fuji Photo Film Co, Tokyo, Japan). Latent images will be read out after a 24-48 hr exposure time using a Fujifilm BAS-1800II Bio-Imaging Analyzer (Fuji Photo Film Co) at 50µm pixel resolution. Digital autoradiography image intensity will be characterized by the machine readout parameter of photostimulable luminescence per square mm (PSL/mm2). Tracer uptake will be derived from region-of-interest analysis of the digital autoradiography images using dedicated software.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

F-18 FDG is the only commercially available radiopharmaceutical approved by the FDA for the intended use in study patients/subjects.

F-18 labeled FMISO is prepared and tested for quality assurance in this study within the Cyclotron/Radiochemistry Core Facility at MSKCC. The radiopharmaceutical is being utilized in this protocol with authorization of the MSKCC Radioactive Drug Research Committee (RDRC). ^{18}F-FMISO is prepared at an approximate specific activity of 1-2 Ci/µmole at end of bombardment time. For PET imaging of tumor uptake, 370 MBq (10.0 mCi) in 1-3 ml sterile saline will be administered IV. The maximum mass of fluoromisonidazole that will be administered is 1 microgram. The no observable effect level (NOEL) is at mass dose >1 milligram.

Radionuclide dosimetry

Biodistribution data on FMISO has been obtained for 60 patients at the University of Washington School of Medicine, and dosimetry was performed. Estimates of normal organ absorbed doses following FMISO administration were published by Graham et al. (22) and are summarized in the table on the following page.

Table 1
Radiation Absorbed Doses to Organs following ^{18}F-MISO administration and CT Scans

Target Organ	Median Dose (mGy/MBq)	Dose (cGy) per 370 MBq (10mCi) injection	Total Dose (cGy) per 370 MBq (10mCi) injection + CT Scans
Adrenals	0.0166	1.71	0.61

Brain	0.0086	0.32	0.32
Breasts	0.0123	0.46	0.46
Gall bladder wall	0.0148	1.65	0.55
Lower Large Intestine	0.0143	0.53	1.63
Small Intestine	0.0132	0.49	2.69
Stomach	0.0126	0.47	0.47
Upper Large Intestine	0.0140	0.52	0.52
Heart Wall	0.0185	0.68	0.68
Kidney	0.0157	0.58	1.68
Liver	0.0183	0.68	1.78
Lungs	0.0099	0.37	0.37
Muscle	0.0142	0.53	0.53
Ovaries	0.0176	0.65	1.75
Pancreas	0.0179	0.66	1.76
Red Marrow	0.0109	0.40	0.40
Bone Surface	0.0077	0.28	0.28
Skin	0.0048	0.18	1.28
Spleen	0.0163	0.60	1.70
Testes	0.0146	0.54	0.54
Thymus	0.0155	0.57	0.57
Thyroid	0.0151	0.56	0.56
Urinary Bladder Wall	0.0210	0.78	1.88
Uterus	0.0183	0.68	1.78
Eye Lens	0.0154	0.57	0.57
Total Body	0.0126	0.47	0.47

This study will entail a single injection of 370 MBq (10 mCi) of ^{18}F-MISO per patient. Absorbed dose estimates, derived from the University of Washington data, indicate that the urinary bladder wall is anticipated to experience the largest dose of any normal organ. The absorbed dose to the urinary bladder wall is estimated as 0.78 cGy (7.8 mSv) for the ^{18}F-FMISO administration. Patients will receive additional radiation dose due to the CT component of the study. This contribution is estimated to be 1.1 cGy (1 mSv) for the three PET-CT scans and will be applicable only to the exposed pelvic region of the patient. The dose to the eye from the ^{18}F-MISO administration is estimated to be 0.57 cGy (5.7 mSv). No additional eye dose will be delivered by CT scanning. The total body radiation dose from ^{18}F-MISO is estimated to be 0.47 cGy (4.7 mSv). These estimates are well below the values of 3 cGy and 5 cGy permitted by the FDA to radiation sensitive or normal organs respectively, for an experimental radiopharmaceutical. The total radiation dose for this investigation is thus within the prescribed guidelines set for RDRC approval. Additionally, this study will not significantly add to the total radiation dose experienced by the patient as a consequence of their treatment for rectal cancer.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

- Able to provide written informed consent
- Histologically confirmed diagnosis of colorectal adenocarcinoma
- 18 years of age or older
- Karnofsky performance status ≥ 70

6.2 Subject Exclusion Criteria

- Women who are pregnant (confirmed by serum b-HCG in women of reproductive age) or breast feeding

7.0 RECRUITMENT PLAN

All patients meeting the eligibility requirements will be considered for enrollme nt regardless of sex, race, or religion. A member of the colorectal research team will screen the clinics of the investigators named on the protocol to identify potentially eligible patients. These patients will be made aware of the protocol, its specific aims and objectives, and the potential risks and benefits the patient may incur. Patients will be required to read, agree to, and sign an IRB-approved informed consent form prior to registration on this trial. Patients will be consented by their treating physician.

During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/re search staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes

Because they will be asked to come in to the hospital for an additional day, patients will be given a $100 honorarium to cover gas, tolls, parking, meals and any incidental expenses associated with this additional day.

8.0 PRETREATMENT EVALUATION

All patients will undergo standard of care tests including an FDG PET scan.

9.0 TREATMENT/INTERVENTION PLAN

Within six weeks of the FDG PET scan, patients will undergo an ^{18}F-FMISO PET scan. Patients will receive no treatment between the two scans. The ^{18}F-FMISO scan will be conducted as follows:

- An I.V. line will be placed in each of the left and right forearms (i.e., a total of 2 I.V. lines). One line will be used exclusively for radiotracer injection and the other exclusively for blood sampling (see below).

- A scout view, followed by a low-dose CT of the tumor region will be obtained.

- The couch will automatically advance into the PET detector component of the scanner so that the tumor is in the center of the 15 cm PET field of view.

- The patient will be injected intravenously with 370 MBq (10 mCi) of ^{18}F-FMISO, simultaneously with the start of PET image acquisition.

- Dynamic images consisting of five 1-minute images and five 5-minutes images (duration 30-minutes) will be acquired within one 15 cm field of view.

- Patient will be taken out of the scanner.

- Patient will undergo a 2nd (optional) PET scan at 90 minutes and a 3rd (non-optional) PET scan at 120-240 minutes post-injection. These scans will consist of a 10-minute PET acquisition and a low-dose CT.

- When blood sampling is required, up to a maximum of 3 blood samples will be obtained using the second I.V. line immediately at selected times post-injection and during the PET scan procedure. Samples will be taken with each imaging time point. Between 1 and 3 cc of blood will be removed at each time point (making the maximum volume of blood withdrawn during this study < 9 cc).
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  - These samples will be weighed and counted in the Nuclear Medicine laboratory for the purpose of determining the blood clearance kinetics and the tumor input function.

- At the end of the entire study, the I.V. lines will be removed.

- The radiopharmacy will keep a log of all injected activity. All patient doses are permanently recorded in the Nuclear Pharmacy database. The injected ^{18}F-FMISO dose, injection time and scan time are also recorded in the PET image file header, and can be accessed from the PET images themselves.

- Digital autoradiography will be performed on up to two tissue samples from patients who undergo surgery on the same day as the ^{18}F-FMISO scan (see details in Section 4.2, "Intervention").

- The RSA assigned to the study will be responsible for identifying eligible patients from the colorectal service clinics. The RSA will work closely with the POAs and RSAs in the Department of Radiology to coordinate scheduling of patients' research procedures and will track patients who are on study to ensure they follow the treatment schedule outlined above.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

There are no follow-up requirements for this study. All patients will undergo standard of care for their colorectal cancer and will be evaluated accordingly.

11.0 TOXICITIES/SIDE EFFECTS

The safety of various radiopharmaceuticals has been reviewed. In a 5-year prospective study (1989 to 1994) of 18 institutions in the United States, the prevalence of adverse events following radiopharmaceutical administration was 0.0023% (23). The adverse experiences reported included rash, chest discomfort, light headedness, nausea, mild anaphylaxis, diaphoresis, vomiting and headache. In a prospective study of 22 PET centers from 1994 to 1997, there were no adverse events reported during 81,801 administered doses of PET pharmaceuticals (24). In a similar study of 17 nuclear medicine departments in Europe in 1996, the prevalence of adverse events was 11 events per 100,000 radiopharmaceutical administrations (25). There were no serious or life-threatening events, and no deaths reported in the literature.

A similar study to that proposed has been carried out at MSKCC featuring FMISO PET scanning in Head and Neck cancer patients (IRB # 04-070). No side effects, including allergic reactions and claustrophobia, have been noted (16). Study patients will also be exposed to radiation during the study, both from the CT component of the PET CT and from the ^{18}F-MISO itself. At the dose levels

outlined in section 5.0, there have been no reported radiation-related side effects in the literature.

In the unlikely event of an adverse reaction, this will be documented and reported by the Principal Investigator to the Institutional SAE Manager (307 East 63rd Street, 1st Floor) as well as to the RDRC.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

This is a pilot study which aims to obtain pharmacokinetic data on the biodistribution of FMISO in patients with colorectal cancer. It will also assess the feasibility of detecting hypoxia by non-invasive ^{18}F-FMISO PET imaging in colorectal carcinoma patients, and to do this will utilize a dynamic PET acquisition sequence that will provide kinetic tumor uptake data, which when combined with blood clearance kinetics through compartmental modeling may provide useful parametric maps of the variation of intra-tumor hypoxia. Whereas, in this study, we will not obtain direct gold-standard measurements of tumor hypoxia using a pO_2 microelectrode, we and others have confirmed by autoradiography that the ^{18}F-FMISO tracer distribution accurately correlates with both exogenous (pimonidazole) and endogenous (HIF-1α and carboanhydrase 9) immunohisto-chemical markers of hypoxia.

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient withdraws consent he/she will be removed from the study.

If at any time the patient develops unacceptable toxicity he/she will be removed from the study. Although we have never had any adverse effects from the FMISO administration and none have been reported elsewhere (see section 11.0), allergic reaction is always a possibility and any patient experiencing this would be sent immediately to the Urgent Care unit for treatment, reported to the Principal Investigator, and removed from the study.

If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis), the patient will be removed from the study.

Please note that all patients whether included in this study or not will receive the standard of care radiation treatments.

14.0 BIOSTATISTICS

This is a feasibility study of using ^{18}F-FMISO PET to detect tumor hypoxia in colorectal cancer. Prospective patients will be scanned using ^{18}F-FMISO PET to identify the hypoxic regions. Analysis of the PET data will be conducted by one

of the physicists in Nuclear Medicine, who will use a software package designed to analyze on a voxel by voxel basis the kinetics of FMISO uptake and clearance in the tumors.

The primary objective of the study is to determine the feasibility of the imaging with ^{18}F-FMISO in colorectal cancer patients. Feasibility is defined as identifying regions of interest (ROI) in the ^{18}F-FMISO PET scan. In an earlier protocol with FMISO in head and neck cancer, 18 of 20 patients had identifiable ROIs. Since published data (26, 27) reveal that upwards of 40% of all, non selected early and advanced rectal cancers are considered to be hypoxic, and since the patients we will be studying in this protocol have large bulky, locally advanced rectal cancers, we anticipate that perhaps as many as 70% of our rectal cancers will be hypoxic. Because of differences in histology between head and neck cancers (squamous carcinoma) and rectal cancers (adenocarcinoma) and possible differences in excretion, we consider identifying ROIs in 50% of patients to be desirable and 30% to be too low.

Two criteria will be used for identifying hypoxia voxels. The first approach is the traditional method reported in the literature by the University of Washington group which defines hypoxia as tumor voxels for which the concentration ratio of FMISO between tumor to blood FMISO is 1.2 or greater, at a single late imaging time point. The second approach utilizes the kinetic profile of FMISO uptake and clearance from each tumor voxel. With this approach, hypoxic voxels are defined as those for which the uptake is slow and continuously rising vis-a-vis highly perfused regions where the initial uptake is high but continuously declining. One reason for employing the second approach is that there is evidence that a single late imaging time point can be ambiguous and not uniquely define the tumor hypoxic regions without knowledge of the history of the signal intensity to that voxel.

As just mentioned, using both ^{18}F-FMISO radioactivity concentration and kinetic uptake profile data in the tumor to signify a positive scan, we propose to evaluate the feasibility using a 2-stage design. In the first stage, 15 patients will receive an ^{18}F-FMISO PET scan. If 4 or fewer patients have identifiable ROIs, then the study will be stopped. If 5 or more have ROIs then an additional 15 patients at most will be scanned. ^{18}F-FMISO imaging will be considered successful if ROIs are present in 13 or more of the 30 patients. However, even if the first 13 of 15 patients scanned have identifiable ROIs, we will continue to scan up to 15 additional patients in order to obtain a spectrum of hypoxic fraction versus treatment response. Pre-clinical studies are underway to determine the correspondence between the ROIs in the scan and hypoxia.

In addition to visualizing these regions we want to determine their volume as a proportion of the entire tumor volume. If the ROIs correspond to hypoxia then the larger these proportions, the more hypoxic the tumor and potentially worse the prognosis. This data will provide preliminary information that could be used for a

future trial with the ^{18}F-FMISO imaging as a prognostic marker. If the imaging is successful, at least 13 patients will provide information for this objective which will be valuable preliminary data.

Distribution of ^{18}F-FMISO based on the pharmacokinetic data will be assessed using descriptive statistics (mean, standard deviation, etc.) and graphic tools. For instance, we will use both histograms and box plots to depict the data.

The microdistribution of ^{18}F-FMISO will be analyzed quantitatively through digital autoradiography.

This design has 8% type I error and 80.4% power.

15.0 SUBJECT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming that the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record. All research material from this study will be handled with the same confidentiality as patient's other medical data.

16.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study

period and potential problems will be brought to the attention of the study team for discussion and action

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Clinical Research Administration. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation

17.0 PROTECTION OF HUMAN SUBJECTS

Risks of Study Participation: Potential risks as outlined in section 11.0 (Toxicities and Side Effects) will be discussed with patients as part of the informed consent process, and patients will be watched closely for signs of adverse events. Whether or not a patient decides to participate in this study, he or she will receive the current standard of care for their specific disease site.

Financial Costs to Patients: All diagnostic and therapeutic interventions except for the ^{18}F-FMISO-PET scans, research blood draws, and digital

autoradiography,are part of the current routine care of patients eligible for this study. A research grant will cover the cost of the ^{18}F-FMISO-PET scans, research blood draws, and digital autoradiography. There are no additional financial costs to the patient beyond the charges routinely incurred as part of standard medical care.

Patient Confidentiality: Patient/subject privacy and confidentiality will be maintained according to MSKCC guidelines and all data derived from this study will be kept in a secure database. All data and results will be anonymously reported with regard to individual subjects.

Voluntary nature of the study: Subjects will be made aware of the voluntary nature of the study as part of the informed consent process. They will be allowed to withdraw participation at any time without the risk of alteration in the quality of their medical care.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

17.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows: For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject's initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1

Any additional SAE reporting information required by the sponsor or drug supplier should be included in this section.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.
Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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