

AMERICAN COLLEGE OF RADIOLOGY IMAGING NETWORK

ACRIN 6682

PHASE II TRIAL OF ^{64}Cu -ATSM PET/CT IN CERVICAL CANCER

Agent Name: ^{64}Cu -ATSM / IND Number: 62675

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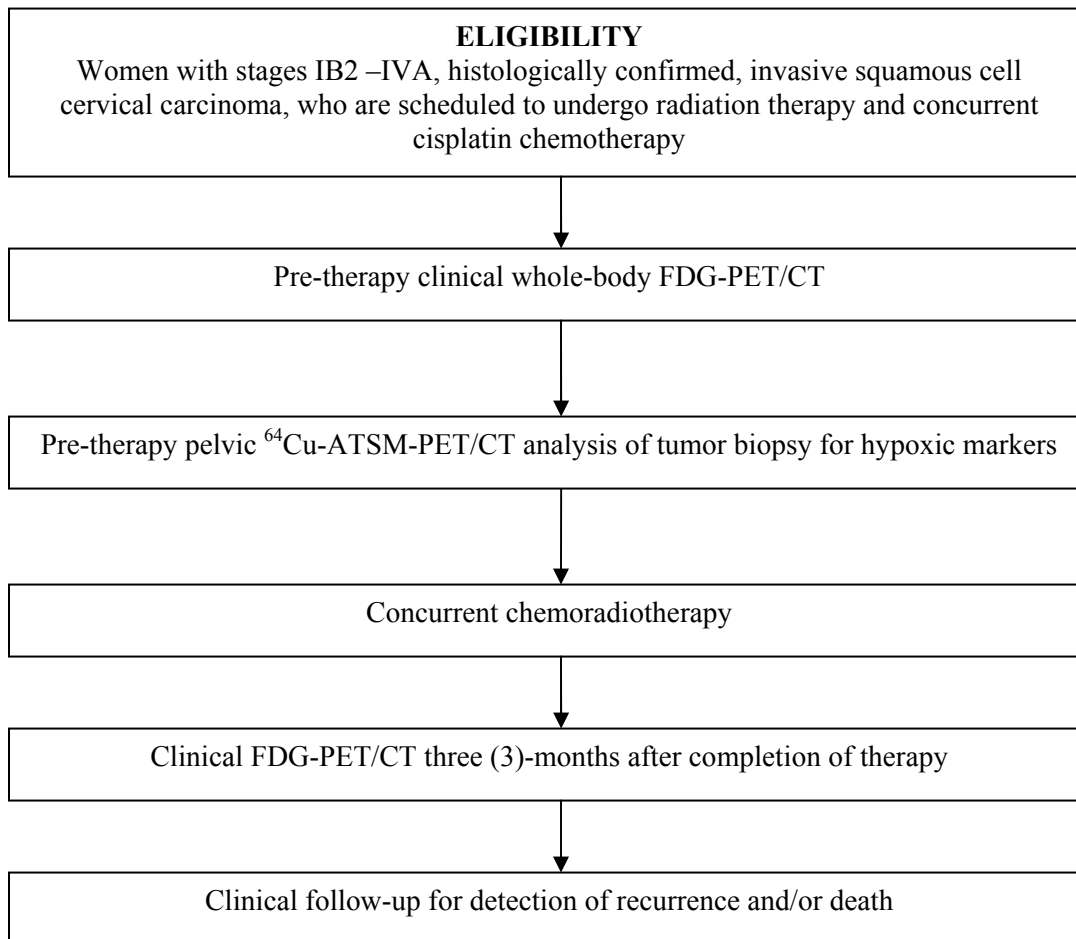
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ACRIN 6682

PHASE II TRIAL OF ⁶⁴Cu-ATSM PET/CT IN CERVICAL CANCER

SCHEMA



***Patients eligible and registered to the ANZGOG 0902/GOG-0274 OUTBACK Trial are eligible to participate on the ACRIN 6682 protocol and can be co-enrolled on both studies. Please see section 8.0 for specific information related to patient activity and co-enrollment schedule.**

SPECIFIC HYPOTHESES

1. ⁶⁴Cu-ATSM-PET/CT distinguishes patients with poorer survival rate from those with better survival rate prior to initiation of therapy.
2. ⁶⁴Cu-ATSM-PET/CT provides unique prognostic information different from that revealed by known prognostic factors in an invasive squamous cell cervical cancer.

SAMPLE SIZE

Total of 100 female participants will be enrolled into the study at a minimum of three (3) institutions.

1.0 ABSTRACT

This protocol for human research study is conducted according to United States and international standards of Good Clinical Practice Guidelines (International Conference on Harmonisation (ICH) Guidelines), applicable government regulations (i.e. Code of Federal Regulations), and the American College of Radiology Imaging Network (ACRIN) research policies and procedures.

In the United States, cervical cancer is the third most common gynecologic malignancy, with an estimated 11,150 new cases and 3,670 deaths in 2007. Approximately one-third of patients with advanced cervical cancer develop disease recurrence after concurrent chemoradiotherapy, and the majority of these recurrences occur within the first 2 years after completion of therapy.

Tumor hypoxia is an important prognostic factor in cervical cancer and predicts for decreased overall and disease-free survival. In part, this is because hypoxic tumors are resistant to radiation and chemotherapy. One possible way to improve the effectiveness of chemoradiotherapy is to improve tumor oxygenation. Various strategies designed to overcome tumor hypoxia and its effects on tumor behavior have had limited success, in part because a noninvasive clinical tool for determining tumor oxygenation has not been available. Previously, the only established method for assessing the oxygenation status of tumors *in vivo* used invasive oxygen electrodes.

Several single-center studies have shown that the novel tracer, ^{60}Cu -labeled diacetyl-bis(N^4 -methylthiosemicarbazone) (^{60}Cu -ATSM), accumulates avidly in hypoxic tissues. Clinical studies using positron emission tomography (PET) with ^{60}Cu -ATSM have demonstrated an inverse relationship between tumor uptake of this tracer and response to therapy in patients with lung and rectal carcinomas, and outcome in cervical and rectal carcinomas. The short half-life of ^{60}Cu precludes its widespread use. Another longer-lived copper radionuclide, ^{64}Cu , which could be distributed commercially, also can be used to label ATSM, and a recent small comparison study shows similar uptake of ATSM labeled with these two radionuclides. Accordingly, validation of the results of previous studies with ^{60}Cu -ATSM in a multicenter setting with ^{64}Cu -ATSM is warranted as a prelude to further development of this promising radiopharmaceutical.

Aim: The trial aims to show that the ^{64}Cu -ATSM uptake assessed by PET/CT is a biomarker predictive of the behavior of cervical cancer and patient outcome.

Design: Women with newly-diagnosed, locally-advanced squamous cell cervical carcinoma (stages IB2-IVA) will undergo PET/CT with ^{64}Cu -ATSM before initiation of standard of care treatment with concurrent cisplatin and radiation therapy (external beam and brachytherapy) per National Comprehensive Cancer Network (NCCN) guidelines. Patients will be followed for up to 3 years after completion of the study visits to assess progression of disease and overall survival. The trial will examine whether high pretreatment ^{64}Cu -ATSM uptake in the primary cervical tumor is associated with lower progression-free survival. It will also assess whether high ^{64}Cu -ATSM uptake is associated with (and predictive of) lower overall survival, earlier local recurrence, and a higher rate of development of distant metastatic disease. Tumor ^{64}Cu -ATSM uptake also will be correlated with tumor volume and several other markers of tumor hypoxia.

Hypotheses: The two hypotheses underlying this trial are that (i) ^{64}Cu -ATSM-PET/CT distinguishes patients with poorer survival from those with better survival prior to initiation of therapy and (ii) ^{64}Cu -ATSM-PET/CT provides unique prognostic information different from that revealed by known prognostic factors in an invasive squamous cell cervical cancer.

Endpoints: The primary endpoints of this study are the relationship between ^{64}Cu -ATSM uptake in the primary cervical tumor and progression-free survival after chemoradiotherapy. Secondary endpoints include the relationship between ^{64}Cu -ATSM uptake and overall survival; rates of local recurrence and development of distant metastasis; frequency of complete metabolic response; accuracy in predicting outcomes (e.g., survival, recurrence, and distant metastasis); tumor volume and the frequency of lymph node metastasis at diagnosis; markers of tumor hypoxia assessed by immunohistochemistry on biopsy tissue from the primary tumor.

2.0 BACKGROUND AND SIGNIFICANCE

2.1 Tumor Hypoxia

Since the 1930s, hypoxia (oxygen concentration of ≤ 1000 ppm) has been recognized as an important determinant of tumor biology in solid tumors. Hypoxia has been found in a wide range of tumors, including cervical cancer (1). Several studies have demonstrated that the pretreatment oxygenation status of tumors can predict overall survival, disease-free survival, and/or local tumor control in patients with cervical cancer (1).

2.2 Significance of Tumor Hypoxia

Numerous animal studies have confirmed that hypoxia contributes to radiation resistance (2-4). Evidence suggests that tumor hypoxia contributes to resistance to standard radiation therapy, some chemotherapy, and chemoradiation, and subsequently, to poorer clinical outcome (5). Studies of tumors transplanted into rodents have shown that the proportion of cells in the hypoxic fraction of such tumors increases with tumor size and may range from $< 10\%$ to as much as 50% of the total viable cell population (6). The level of oxygen delivery can alter these tumors' responses to radiation (6-9). Disrupting oxygen delivery increases radiation resistance, while improving it increases tumor radiosensitivity (10-21). As of 1995, overview analyses of more than 10,000 patients in 83 randomized trials showed that improvement in tumor oxygenation significantly improves locoregional disease control with an odds ratio of 1.21 (95% confidence interval, 1.12-1.30) and overall survival with an odds ratio of 1.13 (95% confidence interval, 1.05-1.21) (22). Clinical studies continue to show that hypoxia in human tumors contributes to therapy failure (23-28).

Hypoxia also appears to contribute to resistance to some chemotherapeutic agents (29-31). The exact mechanism is not known; but several mechanisms have been proposed: differential uptake and metabolism of drugs by hypoxic versus fully oxygenated cells (31, 32), reduced proliferation of hypoxic tumor cells (33, 34) and slower progression of hypoxic cells through the cell cycle (35-37). The effectiveness of chemotherapy can be improved by either enhancing oxygen delivery to a tumor or using hypoxia to selectively activate drugs (38-40).

2.3 Measurement of Tumor Hypoxia

Direct and indirect evidence demonstrate that human tumors contain hypoxic cells, and these cells are considered to affect tumor behavior and response to therapy. Measurement of hypoxia is possible with invasive methods including polarographic needle electrodes, exogenous markers of hypoxia (such as 2-nitroimidazole pimonidazole), and endogenous markers (such as carbonic anhydrase-IX), as well as noninvasive imaging techniques. Only imaging is noninvasive; all others require direct access to the tumor or analysis of tumor biopsy material. Some markers, for example pimonidazole, require injection of the drug prior to biopsy. The invasive nature of these methods makes it difficult to assess hypoxia routinely in a clinical setting.

2.4 In Vivo Assessment of Tumor Hypoxia in Cervical Cancer with Invasive Oxygen Electrodes

Polarographic oxygen electrodes (Eppendorf GmbH, Hamburg, Germany) permit direct determination of oxygen tension in human tumors. Early clinical studies using polarographic oxygen electrodes demonstrated that hypoxic tumors, including cervical tumors, respond poorly to radiation therapy (41-43). Use of smaller and more accurate electrodes demonstrated significant intra- and inter-tumor variability in oxygen levels in patients with cervical cancer (44-47). Tumor hypoxia detected by this method is predictive of locoregional response to radiotherapy and survival in cervical cancer, and significantly increases the risk of nodal and distant metastases (48). In patients with advanced cervical cancer, it has been shown that a tumor's oxygen status, as measured by invasive oxygen electrodes, was the single most important prognostic factor, independent of various patient demographics (such as age and menopausal status) and pretreatment tumor characteristics (such as clinical tumor stage and size, histological type, and differentiation) (49, 50). Unfortunately, the oxygen electrode method is invasive, technically demanding, and useful only for studying tumors accessible to the electrodes. Moreover, sampling errors can be significant because tumors are not homogeneously oxygenated.

2.5 Tissue Markers of Tumor Hypoxia

Several *in vitro* assays have been used to assess tumor hypoxia in human tumors. These methods evaluate endogenous molecular markers of tissue hypoxia. The hypoxia-inducible factor-1 (HIF-1) transcription is considered to be a master regulator of more than 70 genes involved in tumor metabolism (such as surface transmembrane carbonic anhydrases, glucose transporters, glycolytic enzymes), angiogenesis (such as the

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vascular endothelial growth factor [VEGF]), apoptosis (such as the apoptosis-inhibitor protein *bcl-2*), and erythropoiesis (such as the erythropoietin) (1). Hypoxia also induces transcription of other factors such as activator protein-1 (AP-1), chaperone proteins such as heat shock protein (Hsp) and osteopontin (OPN).

Carbonic anhydrase-IX (CA-IX) expression is dependent on hypoxia (51-56). Focal overexpression of CA-IX has been found in > 90% of several types of cancers including cervical cancer (57). CA-IX is a transmembrane protein that catalyzes the reversible hydration of carbon dioxide to carbonic acid. An acidic extracellular pH has been found to be a fundamental property of the malignant phenotype. CA-IX is overexpressed in von Hippel-Lindau (VHL) defective tumors because the VHL tumor suppressor gene regulates transcription of CA-IX gene. There is evidence that the CA-IX gene is markedly induced under hypoxic conditions in tumors (58). Loncaster et al. demonstrated that CA-IX expression is predictive of survival in cervical cancer after adjustment for age, stage, and tumor grade (58). Lee et al. recently demonstrated that CA-IX expression in primary cervical cancer was significantly associated with lymph node metastasis ($p = 0.03$) and poorer disease-free survival (relative risk, 6.1; 95% confidence interval, 1.3-28.3; $p = 0.02$, multivariate analysis) (59). Kim et al. studied tumor tissues obtained from 59 patients with cervical cancer before and after radiotherapy (to doses of either 10 or 20 Gy) (60). They found that CA-IX expression was a significant factor associated with metastasis-free survival ($p = 0.008$; hazard ratio, 34.8). Tumor CA-IX expression was not altered following either 10 or 20 Gy of radiotherapy. Thus, CA-IX expression may be an important indicator for identifying patients who require more aggressive systemic therapy.

Glucose transporter protein-1 (GLUT-1) mediates cellular glucose uptake and is regulated by hypoxia and inhibition of oxidative phosphorylation through two *cis*-acting elements in GLUT promoter (61). HIF-1-mediated overexpression of glucose transporters has been demonstrated *in vitro* (62). GLUT-1 is one of 14 structurally related, membrane-bound, facilitative glucose transporter proteins that are involved in glucose transportation across the cell membrane. Overexpression of GLUT-1 was found in breast, cervix, and rectal cancers, and is correlated with tumor aggressiveness (61). In carcinomas of the uterine cervix, a significant correlation between GLUT-1 and the exogenous hypoxia marker pimonidazole, and between expression of GLUT-1 and CA-IX, was found (63). This further supports a relationship between GLUT-1 and hypoxia. Airley et al. found that absence of GLUT-1 significantly increased the likelihood of metastasis-free survival ($p = 0.022$) in patients with cervical cancer (64). However, its correlation with tumor oxygenation is still disputed in several studies (61).

VEGF is one of the critical survival genes whose expression is up-regulated under hypoxic conditions (55, 65). VEGF is involved in the growth and survival of endothelial cells, vascular permeability, and angiogenesis (61). VEGF expression is mostly regulated by oxygen. Under hypoxia, HIF-1 up-regulates VEGF expression, and this activates tumor angiogenesis (61). Tanaka et al. demonstrated that squamous cell carcinoma of the uterine cervix showed hypoxia, which correlated with abundant vascularity, and VEGF expressed in the hyperplastic epithelium appears to promote angiogenesis in squamous cell carcinoma of the uterine cervix (66). VEGF expression increases from normal epithelium to squamous cell carcinoma in cervical cancer, suggesting that VEGF expression is involved in the promotion of angiogenesis in cervical cancer (67).

OPN is a glycoposphoprotein that has a variety of physiological functions. OPN is expressed in many tumors and has been described to be a prognostic indicator of tumor progression and survival in a number of solid neoplasms and linked to a metastatic phenotype (68, 69). OPN contributes to progression and invasion in cancers of the breast, lung, liver, prostate, stomach, and colon (70). Sakaguchi et al. demonstrated strong staining for OPN in 62% of cancer cells or stromal cells of metastatic lymph node lesions in patients with cervical cancer (70). The OPN level was significantly ($p < 0.05$) increased in these metastatic lymph node lesions of cervical cancers. The prognosis of the patients with significant increase of OPN in cervical cancers was extremely poor in comparison to the patients with no increase of OPN. This indicates that OPN may contribute to lymph node metastasis and its advancement, and that the OPN level in metastatic lesions may be a prognostic indicator in cervical cancers.

2.6 Noninvasive Measurement of Tumor Hypoxia by Functional Imaging

Investigations over the past 15 years have led to quantitative, non-invasive methods for radionuclide imaging of hypoxia, particularly by PET (71, 72). Much of the work in hypoxia imaging by PET has used labeled **nitroimidazoles**, a class of compounds whose metabolism and tissue retention is dependent upon the state of tissue oxygenation. After entering a viable cell, nitroimidazoles are reduced to RNO₂ radical, regardless of

intracellular oxygen concentration. In the presence of tissue oxygen, the radical is immediately reoxidized to superoxide, and the original uncharged misonidazole leaves the cell. If intracellular oxygen levels are low, however, the RNO₂ radical is further reduced to a more reactive form, which binds covalently to intracellular macromolecules and remains within the cell. The nitroreduction that occurs in hypoxic cells is believed to be enzyme mediated (73-75), and nitroimidazole compounds have a high affinity for xanthine oxidase, a nitroreductase (76). The most extensively studied radiolabeled nitroimidazole for *in vivo* imaging is [¹⁸F]fluoromisonidazole (FMISO), which is lipophilic and therefore diffuses readily through cell membranes. Cell culture studies and *in vivo* studies using a variety of transplanted rodent animal tumors have shown that intracellular retention of FMISO is dependent on oxygen concentration, and that the rate of FMISO binding can be up to 28 times higher under hypoxic conditions than under normoxic conditions (73, 75, 77-79).

Based on *in vivo* FMISO biodistribution studies in animals and humans, tissue hypoxia has been defined as an FMISO tissue-to-blood ratio ≥ 1.2 by 2 hrs after radiotracer administration (79). Elevated ratios (≥ 1.4) have been observed in tumors, and were used to estimate the “fractional hypoxic volume” (FHV) (79). Rasey et al. used FMISO to study 37 cancer patients before therapy (80). They observed hypoxia in 36 of the 37 tumors, and FHVs ranged from 0% to 94.7%. The distribution of hypoxia was heterogeneous, and the extent of hypoxia varied markedly between tumors in the same site or of the same histology. In a recent study, Rajendran et al. have shown that the results of pre-therapy FMISO PET were predictive of survival in patients with head and neck cancers (81).

To date, there is no established method for identifying patients who will benefit from hypoxic-directed therapy. However, Rischin et al. recently demonstrated that FMISO-PET is useful in directing hypoxia-specific treatment in patients with head and neck cancer; the uptake of FMISO predicted the greater effectiveness of tirapazamine therapy (82). In this study, only patients with increased FMISO uptake benefited from the addition of tirapazamine to radiotherapy. This is very important considering the side effects of tirapazamine. Iodinated tracers such as ¹²³I-iodoazomycin arabinoside (IAZA) also have been used to assess hypoxia by single-photon imaging methods in preclinical and pilot clinical studies (83). Several other potentially hypoxia-measuring radiopharmaceuticals labeled with ¹⁸F or ^{99m}Tc have been developed, and are currently being evaluated in animal models and patients with solid tumors (84-88).

An alternate approach to hypoxia imaging has relied upon the use of radiometal chelates. This method offers the potential for preparation of the radiopharmaceutical by “kit” labeling of a stable precursor with a positron-emitting radionuclide such as ⁶⁰Cu or ⁶⁴Cu. This is analogous to the way most ^{99m}Tc compounds are produced in clinical nuclear medicine practice, and offers the possibility of a hypoxia-imaging radiopharmaceutical with widespread availability. One such radiometal chelate is copper(II)-diacetyl-bis(N⁴-methylthiosemicarbazone) (Cu-ATSM), developed by Fujibayashi and colleagues, that appears to be a promising agent for imaging hypoxic tissue (89-93). Because of its attractiveness as a readily available hypoxia imaging agent and promising single-center results, it will be further evaluated in this trial as a marker for tumor hypoxia.

2.7 Copper(II)-diacetyl-bis(N⁴-methylthiosemicarbazone): Cu-ATSM

Copper(II)-diacetyl-bis(N⁴-methylthiosemicarbazone), Cu-ATSM, labeled with a positron emitting isotope of copper (⁶⁰Cu, ⁶¹Cu, ⁶²Cu, or ⁶⁴Cu) has been shown, *in vitro* and *in vivo*, to be selective for hypoxic tissue. *In silico* studies have explored the mechanism of its hypoxia selectivity, and clinical studies with this agent have shown non-invasive imaging data that are predictive of cancer patients’ response to conventional therapy. The evolution of Cu-ATSM, and the *in vitro*, *in vivo*, and clinical studies with this tracer has been recently reviewed (94).

The use of copper radionuclides presents many advantages over other better-established metal radionuclides such as ^{99m}Tc. The positron-emitting isotopes of copper (⁶⁰Cu, ⁶¹Cu, ⁶²Cu, and ⁶⁴Cu) are particularly versatile given their range of decay schemes [⁶⁰Cu ($t_{1/2} = 0.40$ h, $\beta^+ = 93\%$, EC = 7%); ⁶¹Cu ($t_{1/2} = 3.32$ h, $\beta^+ = 62\%$, EC = 38%); ⁶²Cu ($t_{1/2} = 0.16$ h, $\beta^+ = 98\%$, EC = 2%); and ⁶⁴Cu ($t_{1/2} = 12.7$ h, $\beta^+ = 17.4\%$, EC = 43%)]. Copper-64 is the most commonly used copper isotope, and its production and use has now been reported in the United States, Europe, and Japan. With a half-life of 12.7 hrs, it is ideally suited for clinical PET studies that can be conducted over a 48-hour period. This longer half-life also allows for the distribution of this radionuclide beyond the site of production. This model has been successful with ¹⁸F, with distribution from regional production centers to imaging centers that may not have direct access to a cyclotron; however, with a considerably longer half-life, even wider distribution will be possible for ⁶⁴Cu. Copper-64 has a relatively low maximum β^+ -energy of 0.66 MeV. This is similar to the corresponding value for ¹⁸F, and PET images with ⁶⁴Cu are accordingly of very high quality.

2.7.1 Preclinical Oncologic Studies

The evolution of Cu-ATSM, and the *in vitro*, *in vivo*, and clinical studies with this tracer has been recently reviewed (94). In 1999, the *in vitro* kinetics of ^{64}Cu -ATSM in EMT6 cells were compared to ^{18}F -FMISO, the misonidazole drug described earlier (95). Uptake of ^{64}Cu -ATSM, ^{64}Cu -PTSM, and ^{18}F -FMISO into EMT6 cells was investigated at dissolved oxygen concentrations of 0 (anoxia), 1×10^3 , 5×10^3 , 5×10^4 and 2×10^5 (normoxia) ppm. This study showed a sigmoidal inflection (i.e., the threshold of selectivity) between 5×10^3 (3.8 mm Hg) and 1×10^3 ppm (0.8 mm Hg) of dissolved O_2 which is centered around pO_2 levels of tumor hypoxia (2–3 mm Hg). ^{18}F -FMISO also showed oxygen concentration-dependent uptake, but with lower percentages than ^{64}Cu -ATSM; ^{64}Cu -PTSM showed 83% to 85% uptake into the cells after 1 hr, independent of O_2 concentration. Compared to ^{18}F -FMISO, Cu-ATSM exhibited more efficient uptake and superior washout kinetics in hypoxic and normoxic cells offering the possibility of a superior means of detecting tumor hypoxia by PET imaging.

In 1999, a comparative biodistribution of ^{64}Cu -ATSM with ^{64}Cu -PTSM in BALB/c mice bearing EMT6 tumors was reported (95). The biodistribution data of ^{64}Cu -ATSM and ^{64}Cu -PTSM showed optimal tumor uptake after 10 min post injection, suggesting a rapid trapping mechanism for Cu-ATSM in solid tumors. *Ex vivo* autoradiography of tumor slices following co-injection of ^{60}Cu -PTSM and ^{64}Cu -ATSM into the same animal showed ^{60}Cu -PTSM uniform throughout the EMT6 tumor but heterogeneous uptake of ^{64}Cu -ATSM, indicative of trapping of ^{64}Cu -ATSM into the ‘hypoxic’ regions of the tumors. Using oxygen needle electrode measurements of the solid tumor, PET, and electronic autoradiography, a strong relationship between low tumor pO_2 and high Cu-ATSM accumulation was observed in 9L gliosarcoma tumors in rats (96). By chemical manipulation of tumor pO_2 , a significant increase in Cu-ATSM was observed in hypoxic-induced tumors. This was the first study confirming that Cu-ATSM uptake in cancerous tissues *in vivo* was dependent upon the tissue pO_2 .

One of the most important validation studies of Cu-ATSM as an agent for delineating hypoxia was reported by Yuan et al. in 2006 (97). The authors compared the autoradiographic distributions of ^{64}Cu -ATSM with a well-established hypoxia marker drug in R3230 mammary adenocarcinomas, fibrosarcomas (FSA), and 9L gliomas. There was close correlation of ^{64}Cu -ATSM uptake and hypoxia in R3230Ac and 9L tumors, but not in FSA tumors. The same relationship was observed with 2 other hypoxia markers—pimonidazole and CA-IX—in the FSA tumors. This study strongly confirmed that, on a histological level, ^{64}Cu -ATSM is a valid PET hypoxia marker in most tumor types.

The retention mechanism of Cu-ATSM has been hypothesized and explored by a number of groups (89-93, 98-103). Two major mechanisms have been proposed. A proposed mechanism of Cu-ATSM retention was first reported by Fujibayashi et al., where it was suggested that Cu(II)-ATSM reduction only occurred in hypoxic cells and that the resultant Cu(I) was irreversibly trapped (104). Additional studies on the mechanism of Cu-ATSM retention were reported by Dearling et al. (91) and Maurer et al. (93). These reports suggested that reduction of Cu(II)-ATSM took place in both normoxic and hypoxic cells resulting in unstable Cu(I)-ATSM. This unstable species would slowly dissociate, and if completely dissociated (in hypoxic cells), it would be irreversibly trapped, but in the presence of oxygen (normoxic cells) the Cu(I)-ATSM would be reoxidized to Cu(II)-ATSM and diffuse back out.

In summary, pre-clinical studies of Cu-ATSM, labeled with a positron-emitting radionuclide of copper (^{60}Cu , ^{61}Cu , ^{62}Cu , or ^{64}Cu), have shown the agent to be selective for hypoxic cancers and ischemic myocardial tissue. Mechanistic studies examining the hypoxia selectivity of Cu-ATSM have presented a number of mechanisms that explain the activity of this agent. From these studies and the clinical data (see below) it has been shown that Cu-ATSM is a very effective PET agent for clinically delineating many hypoxic human malignancies, but, as with all radiopharmaceuticals, it is not a universal agent. Animal studies have shown that care should be taken in particular regard to Cu-ATSM in the imaging of prostate tumors.

2.7.2 Clinical Studies of Cu-ATSM

To date, investigators at Washington University have used ^{60}Cu -ATSM to evaluate slightly over 100 cancer patients (20 with non-small cell lung cancer [NSCLC], 18 with head and neck cancer, 41 with cervical cancer, 13 with breast cancer, 6 with brain tumors, and 21 with rectal cancer). Images were

evaluated qualitatively and quantitatively and the investigators found heterogeneous uptake in these cancers.

2.7.2.1 Quantitative Analysis

Initial quantitative analysis was performed at Washington University in 10 patients with lung cancer and 2 patients with head and neck cancer who also underwent radial artery catheterization to allow rapid blood sampling during dynamic PET imaging. Blood samples were evaluated after octanol fractionation and chromatographically. These studies showed that ^{60}Cu -ATSM moves rapidly into tissues in the first 5 min and that ^{60}Cu -ATSM blood levels are remarkably stable from 10 to 60 min. While there is undoubtedly ongoing metabolism/clearance of ^{60}Cu -ATSM at this time, a pseudoequilibrium state is apparently maintained by slow ^{60}Cu -ATSM movement back out of normoxic tissues into blood.

2.7.2.2 Kinetic Analysis

A three-compartment model was used to characterize the tracer kinetics in tumors and muscle, in an attempt to estimate the rate at which the tracer was irreversibly trapped within the tumor. Initial attempts at modeling included three-compartment analysis similar to that used in the FDG model, Patlak graphical analysis, and simple tissue slope analysis. Three-compartment parameter estimation did not yield unique stable parameters (for K1, K2 and K3), while both the Patlak and tissue slope approaches were stable and gave very similar results. Muscle and blood activities were similar—nearly flat over the time of the scan. The tumor slope index (% change/min, calculated as: the tumor slope divided by average muscle activity) was found to be the most suitable parameter for quantitative analysis of tumor uptake of ^{60}Cu -ATSM (105, 106).

The slope method can be calculated on a pixel-by-pixel basis, permitting the creation of a parametric image that should better reflect ^{60}Cu -ATSM trapping and remove non-specific influences such as high transport and non-specific binding in well-perfused tumor tissue. Furthermore, the area of peak tumor slope can be easily identified. Peak slope index (%/min) values have been calculated for tumors of 13 patients with lung cancer and 27 patients with cervical cancers whose ^{60}Cu -ATSM uptake was measurable by PET (mean and standard deviation of tumor slope index $2.3 \pm 1.3\%$ /min and $5.0 \pm 4.7\%$ /min, respectively).

2.7.2.3 Semiquantitative Analysis

In addition, overall tumor uptake of ^{60}Cu -ATSM has been assessed semiquantitatively by determining the tumor-to-muscle (T/M) uptake ratio. These two quantitative methods (T/M ratio and peak slope index) have been compared in patients with NSCLC and cervical cancers and found to reveal similar information. This is not surprising considering the constant tissue uptake of ^{60}Cu -ATSM 10 min after injection. Because of these observations, the simpler T/M ratio method for quantitative analysis of the data will be used in this trial.

In patients with NSCLC, rectal cancer, and cervical cancer, ^{60}Cu -ATSM uptake was compared with response to therapy and/or survival. In NSCLC, an arbitrarily selected T/M threshold of 3.0 was found to be an accurate cutoff value for distinguishing responders from nonresponders; all responders had $T/M < 3.0$ and all nonresponders had $T/M \geq 3.0$. Overall tumor uptake of ^{60}Cu -ATSM also was assessed using the standardized uptake value (SUV). Although SUV has been found to be very useful in FDG-PET studies, the SUVs of ^{60}Cu -ATSM measurements were extremely variable, did not correspond with other measures (e.g., T/M ratio), or response to therapy; thus, the SUVs were not useful for assessing tumor uptake of ^{60}Cu -ATSM (107). In a recent study of patients with rectal cancer undergoing neoadjuvant therapy, a median T/M ratio of 2.6 discriminated those with worse prognosis from those with better prognosis. Both overall and progression-free survivals were worse with hypoxic tumors ($T/M > 2.6$) than with non-hypoxic tumors ($T/M \leq 2.6$) (both $p < 0.05$). In addition, 2 of the 3 tumors with *no change* in size had $T/M > 2.6$ (positive predictive value, 66%) while only 2 of the 14 tumors with decreased size had $T/M > 2.6$ (negative predictive value, 86%). Three of the 4 tumors *not* down staged had $T/M > 2.6$ (positive predictive value, 75%), while only 1 out of the 13 down-staged tumors had $T/M >$

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2.6 (negative predictive value, 92%). The mean T/M ratio for down-staged tumors (2.2) was significantly lower than that of non-down-staged tumors (3.3) ($p = 0.03$) (108).

Several studies have shown that the magnitude of tumor FDG uptake, as measured by the SUV, in patients with lung cancer, breast cancer, and several other tumors has a significant predictive value that is independent of established prognostic factors, such as cell type, tumor size, and stage (109, 110). There is also evidence that hypoxia increases FDG uptake *in vitro* (100, 111). Therefore, to assess whether ^{60}Cu -ATSM-PET provides unique information, we assessed the relationship between tumor uptake of this tracer with that of FDG, as measured by the maximum SUV (SUV_{max}) within the tumor volume of interest (VOI). In 14 patients with NSCLC, there was no significant difference in tumor SUV_{max} of FDG between nonresponders (10.9 ± 4.1) and responders (12.7 ± 10.4) ($p = 0.7$). In addition, there was no significant correlation between tumor uptake of ^{60}Cu -ATSM and FDG ($r = 0.04$; $p = 0.9$) (107). In patients with rectal cancer, tumor FDG uptake did not correlate with ^{60}Cu -ATSM uptake ($r = 0.4$; $p = 0.9$) and there was no significant difference in mean tumor FDG uptake between patients with hypoxic tumors and those with normoxic tumors ($p = 0.3$) (108). These studies demonstrate that hypoxia imaging with Cu-ATSM adds distinct and incremental information to FDG-PET imaging, similar to reported findings for FDG- versus FMISO-PET (112).

2.8 ^{60}Cu -ATSM-PET in Patients with Cervical Cancer

The Washington University investigators have studied 38 patients with cervical cancer by ^{60}Cu -ATSM-PET. All patients had advanced cervical cancer (stage IB1 in 3, stage IB2 in 3, stage IIA in 1, stage IIB in 18, stage IIIA in 1, stage IIIB in 11, stage IVA in 1) with lesions > 2.0 cm. Clinical FDG-PET demonstrated markedly increased FDG uptake in the cervical cancer of all of these patients. The amount of tumor uptake of ^{60}Cu -ATSM was variable. The tumor of one patient had no discernable ^{60}Cu -ATSM uptake (considered T/M = 1 for analysis). The tumors of the remaining 37 patients showed measurable ^{60}Cu -ATSM uptake on PET (mean and standard deviation, T/M of 4.0 ± 2.5 , $n = 38$; tumor peak slope index of $5.0 \pm 4.7\%/min$, $n = 27$).

At the last follow-up (mean and \pm standard deviation, 28 ± 25 months), 27 patients were alive (24 with no evidence of cervical cancer and 3 with a recurrence of cervical cancer) and 11 had died (10 due to recurrent cervical cancer and 1 due to intercurrent disease). A log rank test was used to determine the cut-off uptake value that was strongly predictive of prognosis. Tumor uptake of ^{60}Cu -ATSM was inversely related to progression-free survival and cause-specific survival (log-rank, $p = 0.006$ and $p = 0.04$, respectively). We found that a T/M threshold of 3.5 best discriminated those likely or unlikely to develop recurrence; the 3-year progression-free survival of patients with normoxic tumors ($\text{T/M} \leq 3.5$) was 71% and that of patients with hypoxic tumors ($\text{T/M} > 3.5$) was 28% ($p = 0.01$). No significant difference in total radiation dose or overall treatment time was noted between patients with T/M ratios above or below 3.5. No significant difference was found in the frequency of lymph node involvement between patients with T/M above (9/16; 56%) and below 3.5 (9/22; 41%) ($p = 0.6$). Also, no significant correlation was found between disease stage and tumor uptake of ^{60}Cu -ATSM ($p = 0.46$). To assess whether ^{60}Cu -ATSM-PET provides unique information, the correlation between cervical tumor uptake of this tracer and the SUV_{max} for FDG was evaluated. No significant correlation was found between tumor SUV_{max} for FDG and tumor uptake of ^{60}Cu -ATSM ($R^2 = 0.006$, $p = 0.63$). The mean tumor FDG uptake in hypoxic tumors was 11.7 ± 4.2 and was 11.5 ± 8.4 in normoxic tumors ($p = 0.9$ by unpaired t-test) (113).

2.9 Comparison of ^{60}Cu -ATSM and ^{64}Cu -ATSM

The initial Washington University studies were performed using ^{60}Cu -labeled ATSM, which was suitable for local production; however, for radiopharmaceutical distribution to multiple centers, longer-lived ^{64}Cu is desirable. The longer half-life of ^{64}Cu (12.7 hours) would allow shipment from a single production site to other US imaging facilities, thus making hypoxia imaging more widely available. In addition, the lower positron energy for ^{64}Cu than for ^{60}Cu reduces image blurring and leads to better spatial resolution (4.7 mm vs. 6.3 mm).

To document that ^{64}Cu -ATSM images have adequate image quality and that the semiquantitative measure of tumor uptake is similar between ^{60}Cu -ATSM and ^{64}Cu -ATSM, the Washington University investigators compared the two agents in 10 patients with advanced cervical cancer. The tumors of all of the patients showed ^{60}Cu -ATSM and ^{64}Cu -ATSM uptake on PET (T/M of 5.9 ± 1.6 and 7.4 ± 1.9 , respectively). A good correlation was observed between the uptake of ^{60}Cu -ATSM and ^{64}Cu -ATSM ($r = 0.95$, $p < 0.0001$). The image quality was comparable; although, generally, the images with ^{64}Cu -ATSM had a better target-to-background ratio and, in most cases,

tumors were seen more clearly than with ⁶⁰Cu-ATSM. In addition, the spatial distribution of tumor uptake (or hypoxia) seen with ⁶⁰Cu-ATSM and ⁶⁴Cu-ATSM performed on two different days (range, 1 to 8 days) was similar. These studies support the feasibility of ⁶⁴Cu-ATSM imaging and show the results are quantitatively equivalent to ⁶⁰Cu-ATSM, for which there is a larger body of experience (114).

Given that ⁶⁰Cu decays by positron decay with the concurrent emission of numerous cascade gamma photons, the fortuitous cascade coincidences were removed by convolution of the cascade gamma ray kernel. Using this technique, the fully corrected projection data (corrected for normalization, attenuation, and scatter) were further corrected by subtraction of the cascade background. The images were then reconstructed from those corrected data with the same reconstruction algorithm. All imaging with ⁶⁰Cu was performed in 2D mode since the collimator septa minimize contamination by cascade coincidences. In addition, the patient data were quantitatively analyzed, and T/M ratios were measured on the images obtained with ⁶⁴Cu-ATSM and ⁶⁰Cu-ATSM processed with cascade subtraction. This analysis demonstrated that similar T/M ratios are obtained with both radionuclides and that application of the cascade subtraction correction improves the correlation between ⁶⁴Cu-ATSM and ⁶⁰Cu-ATSM measurements. The T/M for ⁶⁰Cu-ATSM processed with cascade subtraction was 7.3 ± 1.8—closely similar to that reported above for ⁶⁴Cu-ATSM (7.4 ± 1.9). The slope of the regression line was 1.002 with the corrected ⁶⁰Cu-ATSM values versus 1.238 without the cascade subtraction correction. The corresponding correlation coefficients were 0.88 and 0.95, respectively (114). These data support the equivalence of results using ⁶⁴Cu-ATSM and ⁶⁰Cu-ATSM.

2.10 Relationship of Cu-ATSM Uptake and Tumor Hypoxia Markers in Cervical Cancer

In a pilot study, immunohistochemistry was performed on pre-therapy tumor biopsies from 15 patients (6 hypoxic and 9 normoxic tumors based on ⁶⁰Cu-ATSM uptake). Some of the markers were over-expressed more often in the hypoxic tumors in this small group of patients (Table 1) (115). These preliminary results are encouraging. Now it is warranted to study the correlation between ⁶⁴Cu-ATSM uptake and various hypoxic markers in a larger number of patients. These correlative studies will be further explored in this trial.

Table 1: Hypoxic tumors in a pilot study

Marker	Hypoxic (Cu-ATSM > 3.5, n=6)	Normoxic (Cu-ATSM < 3.5, n=9)	Chi Square p Value	Univariate Survival*
VEGF	5/6	4/9	0.132	0.76
EGFR	5/6	3/9	0.057	0.31
COX-2	4/6	2/9	0.085	0.04
Apoptosis	5/6	1/9	0.005	0.0001
CA-IX	6/6	4/9	0.025	0.86
Lymph Nodes	6/6	3/9	0.0098	0.13

* Mantel-Cox p value for progression-free survival

2.11 FDG-PET, Predictor of Response and Prognosis in Cervical Cancer

FDG-PET (and PET/CT) are widely used for the initial staging of locally advanced cervical cancer, most notably because they lead to improved detection of metastatic disease to pelvic and para-aortic nodes, as well as distant metastasis (116). In addition, several features on pre-therapy FDG-PET have been shown to be strongly correlated with prognosis. These features include primary tumor SUV_{max}, metabolic tumor volume, tumor heterogeneity, and the presence of abnormal FDG uptake in pelvic nodes and para-aortic nodes (117-123). Accordingly, one secondary aim of this trial will be to confirm these earlier single-center observations in a multicenter study.

In the last decade, numerous studies have evaluated the use of FDG-PET for monitoring tumor response to chemotherapy and radiotherapy (124). These studies have shown that treatment-induced changes in tumor FDG uptake and residual FDG uptake after completion of therapy are significantly correlated with patient survival (124). In 152 patients with cervical cancer, we have found that FDG-PET performed approximately 3 months after chemoradiotherapy was highly predictive of prognosis (125). Patients with a normal FDG-PET after therapy were characterized by an excellent prognosis, with a 5-year survival rate of 90%. In contrast, 5-year survival was only 45% in patients with persistent FDG uptake at the site of the primary tumor. If the post-therapeutic PET scan demonstrated new metastatic lesions, the prognosis of the patients was poor (5-year survival, 15%). We recently validated these finding—that the results of a 3-month post-therapy FDG-PET are predictive of long-term

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survival and clinical outcome—in 92 patients with cervical carcinoma who were treated with external irradiation, brachytherapy, and concurrent chemotherapy (126). These patients underwent post-therapy FDG-PET 2 to 4 months (mean, 3 months) after completion of therapy. Similarly, we found that the degree of response based on FDG-PET was predictive of progression-free and overall survival. FDG-PET showed a complete metabolic response in 65 patients (71%), a partial metabolic response in 15 (16%), and progressive disease in 12 (13%). Their 3-year progression-free survivals were 78%, 35%, and 0%, respectively ($p < 0.0001$). The 3-year cause-specific survivals were 100%, 51%, and 0%, respectively ($p < 0.0001$). Multivariate analysis demonstrated that the post-therapy metabolic response was more predictive of survival outcome than all known pre-treatment prognostic factors ($p < 0.0001$). In addition, multivariate analysis demonstrated that the post-therapy metabolic response was more predictive of survival outcome than all known pre-treatment prognostic factors ($p < 0.0001$). Thus, 3-month post-therapy FDG uptake is a metabolic biomarker of tumor response and is a robust surrogate for prolonged follow-up to determine long-term survival outcome in cervical cancer. It will be performed in this trial to allow for an interim analysis of the prognostic accuracy of ^{64}Cu -ATSM PET findings.

2.12 Significance of ACRIN 6682 Trial and Cu-ATSM

Cervical cancer is the most common gynecologic malignancy worldwide. Most patients with invasive squamous cell cervical cancer present with advanced-stage disease and are treated with irradiation and concurrent cisplatin-based chemotherapy. Tumor hypoxia in cervical cancer has been recognized to be an important biologic prognostic factor that affects responsiveness to therapy and patient outcome.

Recently, invasive measurements of tumor oxygenation have identified subsets of patients with hypoxic tumors. Accordingly, more generally applicable methods need to be developed for identifying hypoxic tumors, since interventions designed to overcome hypoxia or mitigate its effects are likely to be effective in such tumors. Preclinical studies demonstrated the selective and rapid uptake of Cu-ATSM in hypoxic tumor tissue. These studies also showed that the distribution of Cu-ATSM is similar to that of histology markers pimonidazole and EF5 and the PET tracer FMISO.

The accumulated clinical data using ^{60}Cu -ATSM in cervical cancer suggest that: (1) advanced cervical cancer can be successfully imaged with ^{60}Cu -ATSM-PET; (2) the uptake of ^{60}Cu -ATSM in cervical cancers is heterogeneous, in keeping with the expected heterogeneity of tumor oxygenation; and (3) the T/M uptake ratio appears valuable for characterizing tumor hypoxia since higher values associate with poor response to therapy and worse outcome. It is anticipated that Cu-ATSM-PET/CT not only will predict tumor behavior, but also will prove to be a reliable tool for monitoring the effectiveness of various strategies known to overcome hypoxia, ultimately leading to more effective treatments for this cancer.

Based on these single-center results, it is now warranted to study PET/CT with ^{64}Cu -ATSM in a multicenter trial in order to define better the role of this tracer in predicting the behavior of cervical cancer. This will be achievable with use of longer-lived ^{64}Cu -ATSM, which can be easily distributed to any center in the US.

3.0 STUDY OBJECTIVES

The primary aim of this study is to define the role of pre-therapy ^{64}Cu -labeled diacetyl-bis(N^4 -methylthiosemicarbazone) (^{64}Cu -ATSM) in predicting prognosis and determining the behavior of an invasive squamous cell cervical cancer.

3.1 Primary Endpoint

3.1.1 To determine if higher ^{64}Cu -ATSM uptake is associated with lower progression-free survival.

3.2 Secondary Endpoints

3.2.1 To determine if higher ^{64}Cu -ATSM uptake is associated with lower overall survival.

3.2.2 To determine if higher ^{64}Cu -ATSM uptake is associated with earlier primary cervical tumor recurrence and a higher rate of development of distant metastatic disease.

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- 3.2.3 To determine if higher ^{64}Cu -ATSM uptake is associated with a lower frequency of complete metabolic response on FDG-PET/CT performed 3 months after completion of radiation and chemotherapy.
- 3.2.4 To estimate the accuracy ^{64}Cu -ATSM uptake as a predictor of:
 - 3.2.4.1 Progression-free survival;
 - 3.2.4.2 Overall survival;
 - 3.2.4.3 Primary tumor recurrence;
 - 3.2.4.4 Future development of distant metastatic disease.
- 3.2.5 To evaluate the performance of ^{64}Cu -ATSM uptake as a predictor of lymph node metastasis at study entry.
- 3.2.6 To evaluate whether ^{64}Cu -ATSM uptake correlates with tumor volume at study entry.
- 3.2.7 To examine the relationship between tumor uptake of ^{64}Cu -ATSM and other markers of tumor hypoxia, including VEGF, GLUT-1, CA-IX, and OPN.
- 3.2.8 To compare the predictive ability of pre-therapy ^{64}Cu -ATSM-PET to that of post-therapy FDG-PET/CT.
- 3.2.9 To assess whether pre-therapy FDG-PET/CT findings are predictive of progression-free survival.

4.0 STUDY DRUG INFORMATION

4.1 Description of ^{64}Cu -labeled diacetyl-bis(N^4 -methylthiosemicarbazone) (IND # 62675)

- 4.1.1 IND Holder Name: Farrokh Dehdashti, MD
- 4.1.2 Chemical Name: ^{64}Cu -labeled diacetyl-bis(N^4 -methylthiosemicarbazone)
- 4.1.2 Other Name: ^{64}Cu -ATSM
- 4.1.2 Molecular Formula: $\text{CuC}_8\text{H}_{14}\text{S}_2\text{N}_6$
- 4.1.3 Description: ^{64}Cu -ATSM is a positron-emitting radiopharmaceutical for use in conjunction with PET/CT imaging. The radioactive half-life of ^{64}Cu -ATSM is 12.7 hours.
- 4.1.4 Solution Preparation: ^{64}Cu -ATSM will be synthesized at each participating institution (or at a commercial radiopharmacy that has agreed to perform the compounding for that participating institution) using GMP-produced, pyrogen-free H_2ATSM kits. The lyophilized kit contains 15 mg H_2ATSM and an injection-grade sugar. This kit is reconstituted with 10 mL of sterile pyrogen-free acetate buffer solution containing 10% injection-grade propylene glycol supplied in a separate glass vial with septum access by the manufacturer of the kit. ^{64}Cu -ATSM is prepared by aseptically combining the contents of the reconstituted kit with $^{64}\text{CuCl}_2$ solution (supplied in 0.1 M HCl) followed by vigorous mixing of the vial at room temperature for 30 seconds. The final ^{64}Cu -ATSM is a pyrogen-free, clear solution with pH ~6.4. This solution will be filtered using a 0.22 μm sterile pyrogen-free filter prior to injection.
- 4.1.5 Mechanism of Action: Cu(II) -ATSM is biochemically reduced to unstable Cu(I) -ATSM and retained in hypoxic cells. In the presence of oxygen (normoxic cells), the Cu(II) -ATSM diffuses back out of the cells. The retention mechanism of Cu -ATSM has been explored by a number of groups (89-92, 98-103).
- 4.1.6 How Supplied: ^{64}Cu -ATSM will be made at each participating institution (or commercial radiopharmacy) as an isotonic, sterile, pyrogen-free, clear, and colorless/pale yellow solution.
- 4.1.7 Dosage and Route of Administration: 18 to 25 mCi of ^{64}Cu -ATSM will be administered intravenously as a bolus.
- 4.1.8 Storage: $^{64}\text{CuCl}_2$ solution should be stored upright in an appropriate lead or tungsten alloy-shielded container at room temperature if required.
- 4.1.9 Stability: Refer to the guidelines from the provider, but typically ^{64}Cu -ATSM should be used within 3 hours of the end of synthesis.

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- 4.1.10 *Precaution:*** ^{64}Cu -ATSM should be administered by nuclear medicine personnel trained to handle radioactive material.
- 4.1.11 *Synthesis, Apyrogenicity, and Purity:*** ^{64}Cu -ATSM will be synthesized using the method described in the Investigator's Brochure (IB) and in consultation with the radiochemistry personnel at Washington University School of Medicine. The final product will be tested before release for pyrogens and radiochemical purity as described in the IB.

4.2 Supplier of ^{64}Cu -ATSM

$^{64}\text{CuCl}_2$ solution will be supplied by Washington University School of Medicine. H_2ATSM kits will be manufactured by Proportional Technologies, Inc (Houston, TX), and supplied to participating sites by Washington University School of Medicine.

- 4.2.1 *Drug Ingredient Ordering:*** The investigator or the authorized investigator designee may request the $^{64}\text{CuCl}_2$ solution by completing a Drug Request form and faxing it to 314-362-9940. Any questions regarding the order or the agent can be directed to Tom Voller (vollert@wustl.edu; phone 314-362-8433). Suzanne Lapi, Ph.D (lapis@mir.wustl.edu) should be copied on all email requests and questions. The production date for $^{64}\text{CuCl}_2$ solution will be set between the site and Washington University in St. Louis (WUSTL) personnel, and the $^{64}\text{CuCl}_2$ solution along with the appropriate number of kits will be sent to the site. The radioactive materials will be shipped via AirNet or FedEx directly to the ACRIN 6682 participating institution (or the commercial radiopharmacy that has agreed to perform the compounding for that participating institution). Shipments will be tracked by both WUSTL and the receiving site.
- 4.2.2 *Drug Returns:*** H_2ATSM kits (non radioactive materials) are shipped to the site in advance. However, the radionuclide, $^{64}\text{CuCl}_2$ solution will be made and shipped to the participating institution (or commercial radiopharmacy) the day before the participant is to be injected. The participating institution (or commercial radiopharmacy) will be in receipt of the $^{64}\text{CuCl}_2$ solution the day of study imaging. If for any reason the study imaging is unable to be completed, the participating institution (or commercial radiopharmacy) will allow the radioactivity of the $^{64}\text{CuCl}_2$ solution to decay and then discard it appropriately per site's policies and procedures. A copy of the policy should be available upon request. Unused kits at the end of the trial will be returned to Washington University School of Medicine, St. Louis, Missouri.
- 4.2.3 *Drug Accountability:*** The investigator or the investigator-designee must maintain a detailed record of receipt, disposition, and return/destruction dates of $^{64}\text{CuCl}_2$ solution and H_2ATSM kits received from Washington University School of Medicine, using either the Drug Accountability Record form available on the ACRIN web site (www.acrin.org/6682_protocol.aspx) or by calling the ACRIN 6682 project manager or the institution's standard drug accountability document.

4.3 Toxicology of ^{64}Cu -ATSM

Adverse events (AEs) will be evaluated at the study-related imaging session, especially during the administration of ^{64}Cu -ATSM and PET/CT scan. AEs are defined as any signs of illness or symptoms that have appeared or worsened since the administration of ^{64}Cu -ATSM. Participants will be queried for potential AEs from the investigational radiopharmaceutical, ^{64}Cu -ATSM. Other AEs will be specifically monitored during the infusion, including localized discomfort at the intravenous (IV) catheter injection site, pain, and any other symptoms related to the investigational trial component—the ^{64}Cu -ATSM PET/CT scan (see Section 12.0 for AE reporting requirements).

Study-specific AEs will be reviewed by the medical monitor and various oversight committees and individuals on an on-going, regularly scheduled basis.

5.0 STUDY OVERVIEW

In this phase II prospective trial, the overall goal is to develop a clinically relevant PET-based method for delineating and measuring tumor hypoxia in cervical cancers. A total of 100 women with biopsy-proven, newly-diagnosed invasive squamous cell carcinoma of the cervix (stages IB2 to IVA, based on the FIGO staging system) who plan to receive standard of care treatment with concurrent cisplatin and radiation therapy (external beam and

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brachytherapy) per NCCN guidelines, or eligible women randomized to either Arm A or B of the OUTBACK Trial will be enrolled.

At least three (3) ACRIN-qualified institutions will participate and accrue to this study. Additionally, ACRIN will collaborate with Gynecologic Oncology Group (GOG) and the leadership for the Australian New Zealand Gynaecological Oncology Group (ANZGOG) on the (ANZGOG)-0902 OUTBACK trial (<http://clinicaltrials.gov/ct2/show/NCT01414608>). Eligible participants randomized on this trial to either the Control Group (Arm A) or the Intervention Group (Arm B) are eligible for recruitment to ACRIN 6682.

Eligible participants will be actively involved in the trial for approximately one (1) year. The chemoradiotherapy during this time period is approximately 52 calendar days or 1.5 months. However, the chemoradiotherapy treatment time period will vary for OUTBACK participants and their randomization assignments. The participant will be clinically followed for up to 3 years to assess progression of disease and survival for this trial. However, the participant will continue with clinical follow-up up to 5 years per standard of care, or at the discretion of the participant's treating physician.

6.0 PARTICIPANT SELECTION/ELIGIBILITY CRITERIA

Eligible participants for this trial are women with biopsy-proven, newly-diagnosed invasive squamous cell carcinoma of the cervix (stages IB2 to IVA, based on the FIGO staging system) who plan to receive standard of care treatment with concurrent cisplatin and radiation therapy (external beam and brachytherapy) per NCCN guidelines, or eligible women randomized to either Arm A or B of the OUTBACK Trial.

6.1 Inclusion Criteria

- 6.1.1** Patient with histologically proven invasive squamous cell cervical cancer - primary stages IB2 – IVA;
- 6.1.2** Patient with Karnofsky performance status of ≥ 70 ;
- 6.1.3** Patient meets one of the following criteria:
 - 6.1.3.1** If FDG-PET/CT was performed within 4 weeks of enrollment on an ACRIN-qualified scanner and showed only pelvic nodal (or no nodal) disease, patient is eligible and will undergo standard of care treatment with concurrent cisplatin and radiation therapy (external beam and brachytherapy) per NCCN guidelines;
 - 6.1.3.2** If FDG-PET/CT was performed within 4 weeks of enrollment on an ACRIN-qualified scanner and showed para-aortic nodal metastasis, patient is eligible and will undergo standard of care treatment with cisplatin and radiation therapy (external beam and brachytherapy) per NCCN guidelines, as well as radiotherapy to para-aortic nodes;
- NOTE:** If the FDG-PET/CT must be performed at baseline due to not meeting the above criteria, patients of child-bearing potential must have a negative urine or serum pregnancy test result within 7 days prior to FDG-PET/CT imaging per institution's standard of care.
- 6.1.4** Patient must be an adult female 18 years of age or older;
- 6.1.5** Patient of childbearing potential must agree to use medically appropriate contraception if sexually active:
 - 6.1.5.1** Women who have had a tubal ligation at least 12 months ago or a hysterectomy will be considered not "of child-bearing potential".
 - 6.1.5.2** Postmenopausal women must have been amenorrheic for at least 12 consecutive months to be considered not "of child-bearing potential."
- 6.1.6** Patient must be able to lie flat for the duration of the PET/CT scan;
- 6.1.7** Patient must be able to give study-specific, IRB-approved informed consent, including authorization for release of personal health information.

6.2 Exclusion Criteria

- 6.2.1** Patients with stage IVB disease will be excluded by whole-body FDG-PET/CT:
 - 6.2.1.1** If FDG-PET/CT show distant metastasis or supraclavicular metastasis and this is confirmed, patient is not eligible to go on study.

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- 6.2.2 Patients with recurrent invasive carcinoma of the uterine cervix regardless of previous treatment;
- 6.2.3 Patients who have known metastases to lungs, supraclavicular lymph nodes, or other organs outside of the pelvis or abdominal lymph nodes at the time of the original clinical diagnosis;
- 6.2.4 Patients who had a prior pelvic or abdominal lymphadenectomy performed for any reason;
- 6.2.5 Patients who have received prior pelvic radiation therapy for any reason;
- 6.2.6 Patients who are pregnant or breastfeeding;
- 6.2.7 Patients with septicemia or severe infection;
- 6.2.8 Patients with uncontrolled or poorly controlled diabetes;
- 6.2.9 Patients with circumstances that will not permit completion of the imaging studies or required clinical follow-up;
- 6.2.10 Patients with other invasive malignancies, with the exception of non-melanoma skin cancer, who had (or have) any evidence of the other cancer within the last 5 years or whose previous cancer treatment contraindicates this protocol therapy.

6.3 Recruitment and Screening

The investigative team at each participating site must include a gynecologic oncologist and/or a radiation oncologist, and a radiologist and/or nuclear medicine physician. Potential study participants will be seen by a gynecologic oncologist and/or a radiation oncologist as part of their standard care for their cervical cancer. At the time of the patient's visit, the standard-of-care treatment will be discussed along with possible participation in the ACRIN 6682 trial. If the patient agrees to participate, the consent will be obtained by the site principal investigator or investigator-designee.

Investigators selected to participate in the trial are required to complete an ACRIN Protocol Specific Application (PSA) found on the ACRIN web site (www.acrin.org/6682_protocol.aspx). The PSA requires the following information:

1. Documentation of the number of patients treated in the previous 2 years who would meet protocol eligibility;
2. Documentation of the site's recruitment potential;
3. Detailed description of how the patients will be identified, informed about the study, and consented into the trial.

ACRIN will work with the protocol team and site investigators to determine materials that would be helpful for participant recruitment. Site investigators will be responsible for obtaining IRB approval of recruitment materials provided by ACRIN.

ACRIN will develop a trial communications plan that will describe the production of materials to aid participant recruitment. All materials used for participant recruitment will be reviewed and approved by each institution's IRB.

6.4 Inclusion of Women and Minorities

The study population for this trial only includes women. The ACRIN participating institutions will not exclude potential participants from participating in this or any study solely based on ethnic origin or socioeconomic status. Every attempt will be made to enter all eligible patients into this protocol and therefore address the study objectives in a patient population representative of the entire cervical cancer population treated by participating institutions.

Women of all ethnic groups are eligible for this trial. In conformance with the National Institutes of Health (NIH) Revitalization Act of 1993 with regard to inclusion of women and minorities in clinical research, the projected gender and minority accruals are shown below:

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Gender and Minority Accrual Estimates

Ethnic Category	Sex			
	Females	Males	Unknown	Total
Hispanic or Latino	8	N/A	N/A	8
Not Hispanic or Latino	92	N/A	N/A	92
Ethnic Category: Total of all patients	100	N/A	N/A	100
Racial Category				
American Indian or Alaskan Native	1	N/A	N/A	1
Asian	2	N/A	N/A	2
Black or African American	18	N/A	N/A	18
Native Hawaiian or other Pacific Islander	0	N/A	N/A	0
White	79	N/A	N/A	79
Racial Category: Total of all patients	100	N/A	N/A	100

7.0 SITE SELECTION

7.1 Institution Participation and Requirements

At least three (3) ACRIN-qualified institutions will participate and accrue for this study. Additionally, ACRIN will collaborate with GOG and the leadership for the ANZGOG on the (ANZGOG)-0902 OUTBACK trial (<http://clinicaltrials.gov/ct2/show/NCT01414608>). Eligible participants randomized on this trial to either the Control group (Arm A) or the Intervention Group (Arm B) are also eligible for recruitment to ACRIN 6682.

The potential sites for this study are ACRIN participating institutions that meet qualifications for participating in this study. Each institution must complete a PSA (Appendix IV) and have the PET/CT scanner approved prior to the institution participating in the study (Appendix V). Detailed information for PET/CT Qualification Procedures, the application to become qualified, and the PSA can be accessed at www.acrin.org/6682_protocol.aspx.

7.1.1 PET/CT Qualification

To participate in this study, the site must be able to conform to all of the criteria described in the PSA and the ACRIN PET/CT Qualifying Application, which are available on the ACRIN web site (www.acrin.org/6682_protocol.aspx). This process includes submission of test images to ACRIN. The test images will be reviewed by one or more of the study investigators for compliance. Only after approval by ACRIN can an institution enroll participants on this study. Centers that have received PET/CT approval for other ACRIN studies may be eligible for expedited qualification.

7.2 FDA Form 1572, IRB Approval Letter, and Informed Consent

All institutions must have study-specific IRB approval for the protocol and site-specific informed consent form, along with a completed FDA Form 1572. The informed consent form is included in this protocol as Appendix I. The investigator and the investigator-designated research staff must follow OHRP-approved consent procedures (Title 45, Part 46 Code of Federal Regulations), as well as those set by the local IRB at the institution. Prior to registering the first participant, the completed FDA Form 1572, a copy of the IRB approval letter, and a copy of the IRB-approved, institutional study-specific informed consent form must be on file at ACRIN Headquarters (fax: 215-717-0936, ATTN: ACRIN Protocol Development and Regulatory Compliance Department) prior to enrolling the first study participant.

7.3 Accrual Goals and Monitoring

The ACRIN Biostatistics and Data Management Center (BDMC) will monitor participant accrual. Total target accrual for this study is 100 participants. During the first year, accrual will be reviewed monthly with the

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intention of discovering and resolving any recruitment barriers. In particular, starting approximately one month after a site is approved to begin participant enrollment, the site's actual accrual will be compared to the average monthly accrual potential described in their PSA. With the participation of GOG sites in the (ANZGOG)-0902 OUTBACK trial, it is anticipated that accrual will be a minimum of four patients per month.

The ACRIN Steering Committee regularly reviews the overall trial accrual and may request information about a trial's accrual performance to better understand general accrual barriers or issues. In addition, accrual and safety information will be reviewed by a designated ACRIN Data and Safety Monitoring Committee (DSMC) at regularly scheduled meetings.

8.0 STUDY PROCEDURES

Participants will be evaluated clinically by history and physical examination, routine laboratory and radiological studies, and tissue biopsy. Participant will begin study visits approximately 4 weeks and 3 months after completion of chemoradiotherapy, and then have follow-up visits that will occur every 3 months for the first 2 years and every 6 months during the 3rd year to assess for progression of disease under the supervision of the treating radiation and/or gynecologic oncologist as part of the trial. For participants enrolled from the Intervention Group (Arm B) of the Outback Trial, the post-therapy FDG-PET/CT must be performed at least 3 months after completion of chemoradiotherapy AND at least 2 weeks after completion of adjuvant chemotherapy. All participants will continue with clinical follow-up for up to 5 years per standard of care, or at the discretion of the participant's treating physician.

8.1 FDG-PET/CT Studies

For study participation, a prior FDG-PET/CT scan that was obtained on ACRIN-qualified equipment may be used to determine eligibility and as the baseline FDG-PET/CT if the study was performed up to four (4) weeks prior to enrollment. If the FDG-PET/CT study has been judged adequate for this trial, it will not be necessary to repeat the FDG-PET/CT scan at Baseline Visit. All subsequent PET/CT scans must be performed on the same ACRIN-qualified PET/CT scanner. Glucose testing must be conducted prior to each FDG-PET/CT. Site may conduct the glucose testing per institution's standard of care.

8.2 Treatment

For study participation, all eligible participants must be scheduled and treated with definitive irradiation (both external beam radiation and intracavitary brachytherapy) and concurrent cisplatin chemotherapy in accordance with current standards of care (www.nccn.org/professionals/physician_gls/PDF/cervical.pdf). For this study, potential participants must be scheduled to receive concurrent chemotherapy which consists of 6 weekly cycles of cisplatin, in order to be eligible for participation in the study. Participants randomized to the Intervention Group (Arm B) of the Outback Trial and enrolled into ACRIN 6682 will also be treated with 4 cycles of 3 weekly adjuvant chemotherapy using carboplatin and paclitaxel. The treatment duration for these patients takes approximately 52 calendar days or 2.5 months.

8.3 Post-treatment Evaluation

Participants will be evaluated for disease recurrence both locally in the pelvis and at distant sites. Follow-up will consist of medical history, physical examination, radiologic examinations, and tissue biopsies of the accessible sites of suspected tumor recurrence, as clinically indicated by the treating physician to prove or exclude recurrent disease. Participants whose disease sites are not accessible to biopsy will be followed closely both clinically and by appropriate radiologic examination. Participants will be evaluated for symptoms (bleeding, sciatic or obturator pain, leg edema and/or hydronephrosis) and/or clinical findings (evidence of cervical/vaginal recurrence or persistent parametrial nodular induration) suspicious for pelvic recurrence.

In general, patients with distant metastatic disease also have pelvic recurrence. However, a subgroup of patients has recurrence only at distant sites and typically presents with symptoms (pain often osseous or in the gluteal region, respiratory symptoms, and/or hydronephrosis) and/or clinical findings (palpable lymph nodes, in particular supraclavicular and groin lymph nodes) suspicious for recurrence at distant sites. As part of this study to assess response to therapy, all participants will undergo FDG-PET/CT at 3 months after completion of chemoradiotherapy. FDG-PET/CT study will be used for detection of clinically occult disease. Because residual/recurrent cervical cancer is a rapidly progressive disease, clinical evidence of disease will become

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evident in a short period (a few weeks to months). FDG-PET/CT imaging will be performed and evaluated in a similar clinical fashion as the pre-therapy scan by on-site radiologists and/or nuclear medicine physicians.

Based on the results of FDG-PET/CT, assessed qualitatively, at 3 months, participants will be classified as having metabolic complete response (no residual abnormal FDG uptake, i.e., FDG uptake in all previously detected sites of disease is equivalent to background); metabolic partial response (residual disease—there is persistent disease apparent on clinical examination and/or increased FDG uptake in one or more previously detected sites of disease); or progressive metabolic disease (new sites of abnormally increased FDG uptake are identified). Persistent disease or new sites of disease should be confirmed by biopsy (at which time it will be considered a progression event). New sites of abnormal FDG uptake also should be confirmed by biopsy of an accessible site of disease. If disease is not accessible for biopsy, additional imaging and/or close clinical follow up will be used to document disease. A progression event in such cases is defined as having occurred at the time of either biopsy confirmation or clinically unequivocal progression. Participants who develop disease recurrence after initial response to therapy—evident on clinical or radiological examinations (and confirmed by biopsy where practical)—will be categorized as having disease progression.

For the purposes of this trial, disease progression is thus defined as (1) metabolic partial response (confirmed) at 3 months post-therapy, (2) progressive metabolic disease (confirmed) at 3 months post-therapy, or (3) recurrence of disease (confirmed) after an initial complete response.

8.4 ⁶⁴Cu-ATSM Safety Assessment

Given the documented safety profile of ⁶⁴Cu-ATSM in more than 110 participants with no clinically detectable AE related to the radiotracer, there should be no significant AE reported. However, because of the investigational status of the radiotracer, ⁶⁴Cu-ATSM safety assessment will be conducted before injection of ⁶⁴Cu-ATSM, 15 minutes post injection (before the patient goes into the scanner), and at approximately 75 minutes post injection (after completion of the scan, before the participant leaves the PET facility). If there are significant changes in vital signs accompanied by signs or symptoms suggesting an adverse reaction to the drug, the patient will be monitored until the site investigator or investigator-designee judges that the patient may safely leave the PET facility. If necessary, appropriate treatment will be provided and documented. In addition, concomitant medication taken by the participant prior to 2 weeks and/or during the time of the AE must be collected and documented.

All AEs that occur within 24 hours of ⁶⁴Cu-ATSM administration must be collected and reported per the AE reporting requirements in the Adverse Event Reporting Section 12.0. A follow-up with the participant must be performed after 24-hour post injection of the investigational radiotracer to ensure complete documentation and assessment. The follow-up contact may be done either in person or with a telephone call. It is imperative that all protocol-specific AEs are reported to ACRIN in a timely manner, particularly for serious AEs, to ensure timely reporting by the Investigational New Drug (IND) holder to the FDA.

Any participant who has a serious AE during or after administration of ⁶⁴Cu-ATSM, such that imaging cannot be completed safely, in the judgment of the site investigator, will be withdrawn from the study. Site will need to immediately notify ACRIN regarding any study-specific AEs and report the discontinuation of the participant from the study to obtain further instructions. Refer to Adverse Event Reporting Section 12.0.

8.5 Baseline Visit for Eligibility & Registration

- Obtain signed consent on an IRB-approved informed consent form prior to performing any study-related procedures;
- Obtain medical history, including allergy history;
- Obtain concomitant medication;
- Perform a physical examination;
- Obtain a whole-blood sample, if the participant consents, and send to Washington University for future correlative studies. A kit and instructions for shipment of the blood sample will be provided. NOTE: If a repeat pre-therapy FDG-PET/CT is necessary, blood draw can coincide with the glucose testing.
- Review imaging studies (and reports) obtained per institution's standard of care to exclude stage IVB disease;

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- Review study (and report) for clinical FDG-PET/CT to assess that the study was performed within 4 weeks of enrollment on an ACRIN-qualified scanner.
 - If necessary due to ineligibility of the previous scan, schedule and perform clinical FDG-PET/CT (see Section 10.0)
 - If a repeat FDG-PET/CT scan is performed to determine eligibility, a standard-of-care pregnancy test will be required to be completed within 7 days of the scan.
- Determine if patient meets all eligibility requirements according to Section 5.0. If a repeat FDG-PET/CT must be performed, determination of eligibility (completion of Visit 0) will be completed upon review of the repeat FDG-PET/CT;
- Register the participant using the web-based registration application once the potential participant has completed all baseline visit procedures and have been determined to be eligible for participation (see Section 9.0);
- Send cervical biopsy that was obtained for the diagnosis of invasive cervical squamous cell carcinoma to Washington University Pathology Laboratory (see Section 11.0).

An eligible participant of childbearing potential must have a negative pregnancy test with either a high-sensitivity urine test (capable of detecting beta-human chorionic gonadotropin at a concentration ≤ 25 mIU/mL) or a qualitative or quantitative serum pregnancy test within 7 days prior Visit 1 and having the ^{64}Cu -ATSM PET/CT.

8.6 Visit 1: ^{64}Cu -ATSM PET/CT Within 14 Days After Baseline Visit

Visit 1 will occur within 14 days after the Baseline Visit. Participants will undergo PET/CT scan as described in Section 10.0.

- Obtain vital signs prior to injection of ^{64}Cu -ATSM;
- Inject 18-25 mCi ^{64}Cu -ATSM, 30 to 40 minutes prior to imaging;
- Obtain vital signs 15 minutes after injection of ^{64}Cu -ATSM;
- Perform the ^{64}Cu -ATSM-PET/CT for 30 minutes;
- Obtain vital signs 75 minutes after injection of ^{64}Cu -ATSM;
- Assess for any new and/or changes in concomitant medication;
- Assess for any AEs after completion of the ^{64}Cu -ATSM-PET/CT.

NOTE: To assess any AEs that occurred **within 24 hours after ^{64}Cu -ATSM injection** and are related to the investigational radiotracer, site should conduct a follow-up contact **after 24 hours post ^{64}Cu -ATSM injection** to ensure AEs are properly reported and documented. This can be done either in person or via telephone contact. Refer to section 8.4 for further details.

8.7 Visit 2: Within 4 Weeks After ^{64}Cu -ATSM-PET/CT

- All participants are expected to initiate chemoradiotherapy within 4 weeks after PET/CT;
- Assess for any AEs related to the investigational radiotracers.

NOTE: Initiation date of chemoradiotherapy will be recorded on the participant's study chart and appropriate case report form. Any delays in treatment must also be documented and an explanation must be provided. The date of completion of chemoradiotherapy must be documented.

8.8 Visit 3: 4 Weeks After Completion of Chemoradiotherapy

- Routine clinical follow up, including physical examination and laboratory tests.

8.9 Visit 4: 3 Months (± 4 Weeks) After Completion of Chemoradiotherapy

- Participants will return approximately 3 months (± 4 weeks) after completion of chemoradiotherapy for:

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- Obtain a whole-blood sample, if the participant has consented previously, and send to Washington University for future correlative studies. NOTE: The blood draw can coincide with the glucose testing for follow-up FDG-PET/CT;
- Obtain glucose testing prior to FDG administration;
- Perform FDG-PET/CT for assessment of response to therapy;
- Review the updates on medical history and the results of the physical examination and laboratory tests.

NOTE: If the FDG-PET/CT indicates a suspicious lesion, additional imaging and/or clinical follow up will be conducted to assess progression of disease.

8.10 Visit 4B – FOR Intervention Group (Arm B) Participants ONLY: At Least 3 Months After Completion of Chemoradiotherapy AND at Least 2 Weeks After Completion of Adjuvant Chemotherapy

In addition to Visit 4, Intervention Group (Arm B) participant will return for Visit 4B. The following procedures must be performed 2 weeks after completion of adjuvant chemotherapy, but must be completed within 6 weeks after completion of adjuvant chemotherapy.

- Obtain a whole-blood sample, if the participant has consented previously, and send to Washington University for future correlative studies. NOTE: The blood draw can coincide with the glucose testing for follow-up FDG-PET/CT;
- Obtain glucose testing prior to FDG administration;
- Perform follow-up FDG-PET/CT for assessment of response to therapy;
- Review updates on medical history and the results of the physical examination and laboratory tests.

8.11 Participant's Clinical Follow-up Visits – Per Institution's Standard of Care

All participants will be treated and followed clinically per the institution's standard of care or as recommended by the participant's treating physician after Study Visit 4 and the completion of their chemoradiotherapy. The participant will be clinically followed for up to 3 years from the end of the study visits to assess progression of disease and overall survival. However, the participant will continue with clinical follow-up for up to 5 years per standard of care, or at the discretion of the participant's treating physician.

8.11.1 Follow-up Visits: Every 3 Months for the First 2 Years

- Routine clinical follow up, including updates to medical history, physical examination, laboratory test results, and imaging, if clinically indicated to assess progression of disease.
- Contact the participant's treating physician to obtain information pertaining to disease progression and vital status.

8.11.2 Follow-up Visits: Every 6 Months for the 3rd Year

- Routine clinical follow up, including updates to medical history, physical examination, laboratory test results, and imaging, if clinically indicated to assess progression of disease.
- Contact the participant's treating physician to obtain information pertaining to disease progression and vital status.

8.12 Off-Study Criteria

If a participant completes the ⁶⁴Cu-ATSM imaging study and initiates chemotherapy or radiation treatment, her follow-up should continue per protocol. Participants will go off-study and will be replaced to complete target accrual if the ⁶⁴Cu-ATSM imaging study was not completed or if at least one form of therapy was not initiated.

8.12 Study Procedures Timetable

PROCEDURES	Baseline Visit: Eligibility and Registration	VISIT 1: 14 Days After Baseline Visit—Day of ⁶⁴Cu-ATSM-PET/CT	VISIT 2: Within the 4 Weeks After ⁶⁴Cu-ATSM-PET/CT	VISIT 3: 4 Weeks After Completion of Standard of Care Chemoradiotherapy	VISIT 4: 3 Months (± 4 Weeks) After Completion of Standard of Care Chemoradiotherapy	VISIT 4B: FOR ARM B ONLY – 2 weeks after, but within 6 weeks after, Adjuvant Chemo-therapy	FOLLOW-UP VISITS: Every 3 Months for the First 2 Years/Then Every 6 Months for the 3rd Year
Informed consent	X						
Eligibility/registration	X						
Medical history, including allergy history	X						
Concomitant medication	X	X					
Physical examination	X			X	X	X	X
Clinical lab tests (per standard of care as clinically indicated)				X	X	X	X
Pregnancy test (urine or serum) within 7 days prior to ⁶⁴ Cu-ATSM-PET/CT		X ¹					
Review diagnostic imaging studies obtained for staging	X						X ²
Whole-blood sample (if participant consents)	X				X	X	
Glucose testing	X ³				X ³	X ³	
FDG-PET/CT	X ⁴				X	X	
⁶⁴ Cu-ATSM injection ~30 minutes prior to PET/CT		X					
Vital signs (Prior to ⁶⁴ Cu-ATSM injection then 15 and 75 minutes after injection)		X					
⁶⁴ Cu-ATSM-PET/CT		X					
Initiation of standard of care chemoradiotherapy per NCCN Guidelines			X				
Cervical biopsy sent for hypoxic markers analysis	X						
Adverse event assessment		X	X				

¹ Negative pregnancy test for participants who are of childbearing potential must be confirmed within 7 days prior Visit 1 and the ⁶⁴Cu-ATSM PET/CT. If the negative test is greater than within 7 days, pregnancy test must be repeated with a negative result.

² Per investigator's discretion.

³Glucose testing must be conducted prior to each FDG-PET/CT. Site may conduct the glucose testing per institution's standard of care.

⁴ Baseline FDG-PET/CT will not be repeated if the FDG-PET/CT was completed within 4 weeks of enrollment on an ACRIN-qualified scanner. If necessary to repeat scan, pregnancy test must be repeated with a negative result within 7 days of FDG-PET/CT scan.

9.0 DATA MANAGEMENT / ONLINE REGISTRATION SYSTEM

9.1 Using the Online Registration System

Once the investigator-designated research staff (i.e. the Research Associate [RA]) has completed the eligibility checklist (Appendix II) and the participant has been found to be eligible to participate in the trial, the participant will be consented. Upon obtaining a signed informed consent form, the information of the study participant will be registered by logging onto the ACRIN web site (www.acrin.org), which is available 24 hours a day, 7 days a week.

9.2 Unsuccessful Registrations

- 9.2.1** ACRIN and protocol-specific requirements for Institution participation are maintained within the Administrative database. The protocol specific attributes are then interfaced with the web application for on-line verification of site participation acceptance. If the institution has not met all the regulatory requirements based on the required attributions within the database, a screen that includes a brief explanation of the failure to gain access to the registration screens is projected. If during the completion of the eligibility questions a participant is deemed ineligible based on a response, a message box appears to instruct the research staff to contact the Data Management Center (DMC).
- 9.2.2** In the unlikely event that the ACRIN web registration site is not accessible, participating sites may still register a participant by faxing the completed eligibility checklist to the DMC at ACRIN (215-717-0936, ATTN: PARTICIPANT REGISTRATION). ACRIN staff will either fax or email a response to the registering site with the confirmation of registration and participant case number as soon as possible.

9.3 General

- 9.3.1** The ACRIN web address is www.acrin.org.
- 9.3.2** Data collection and management will be performed by the Biostatistics and Data Management Center (BDMC) of ACRIN under the direction of Dr. Constantine Gatsonis. The Biostatistics Center (BC) is located at Center for Statistical Sciences at Brown University in Providence, RI, and the DMC is located at ACRIN in Philadelphia, PA.
- 9.3.3** Participant enrollment and data collection occurs through a series of programmed screens accessed through the ACRIN web site to register/randomize participants, collect participant data, and maintain calendars of data submissions for each participant. By using the World Wide Web, ACRIN has made participant registration, data entry, and updated calendar information available to clinical sites 24 hours a day, 7 days a week. Each successful case registration is confirmed through an e-mail receipt containing registration/randomization confirmation and a case-specific calendar identifying timelines for data and image submission. If the confirmation e-mail is not received, the enrolling person should contact the DMC before attempting a re-registration.

9.4 Clinical Data Submission

- 9.4.1** Upon successful participant registration, a confirmation e-mail containing the registration and case specific calendar is sent to the research staff enrolling the participant via the web. In addition, the investigator-designated research staff may download the participant-specific data submission calendar, which lists all forms and designated reports required by protocol, along with the form-due dates at the DMC. These calendars will be updated as the study proceeds to reflect data that have been received, reply deadlines for queries about unclear data, deadlines for follow-up reports of AEs, or changes in the protocol that change the data being collected or the timeframe. Updated calendars for each participant can be obtained 24 hours a day from the ACRIN web site. The RA may use the calendar as a case management tool for data submission and follow-up scheduling.

- 9.4.2** The investigative site is required to submit data according to protocol as detailed on each participant’s calendar, as long as the case status is designated as open/alive or until the study is terminated. The case is closed when all data have been received, reviewed, and no outstanding data query exists for the case.
- 9.4.3** To submit data via the ACRIN web site, the appropriate investigator-designated research staff will log onto the ACRIN web site and supply the pre-assigned user name and password. Case report forms will be available on the web site through a series of links. Each web form is separated into modules; each module must be completed sequentially in order for the internal programming to be accurate. The user selects the link to the appropriate form and enters data directly into the web-based form. As information is entered into the web form application, various logic checks will be performed. These logic checks look for data that are missing, out of range, and/or in the wrong format (e.g., character data in a field requiring numeric responses). Such errors will be detected as soon as the user attempts to either submit the form or move to the next data element. They must be corrected before the form is transmitted to the DMC. The user will not be able to finalize form transmission to the DMC until all data entered pass these logic checks. Forms that are not completed in one sitting can still be submitted and completed at a later date. The form will remain available on the web until the “Complete Form Submission” button is depressed.
- 9.4.4** Once data entry of a form is complete, and the summary form reviewed for completeness and accuracy, the investigator or the research staff presses the “Complete Form Submission” button on the form summary screen and the data is transferred into the clinical database. No further direct revision of the submitted data is allowed after this point. E-mail confirmation of web data entry is automatically generated and sent to the site investigator or RA listing all of the data completed and just submitted. Should a problem occur during transmission and the e-mail confirmation of data submission is not received, the investigator or RA should contact the DMC for resolution of the submission.
- 9.4.5** If a temporary problem prevents access to the Internet, all sites are notified of the event and estimated down time through an ACRIN broadcast message. The investigative site should wait until access is restored to submit data. The site RA or investigator should notify the DMC of the problem, and the DMC will give an estimated time when access will be restored. If access will be unavailable for an extended period, sites must seek another Internet Service Provider (ISP). On a short-term basis, ACRIN can serve as an ISP.

9.5 Data Security

The registration and data collection system has a built-in security feature that encrypts all data for transmission in both directions, preventing unauthorized access to confidential participant information. Access to the system will be controlled by a sequence of identification codes and passwords.

9.6 Electronic Data Management

- 9.6.1** Data received from the web-based forms are electronically stamped with the date and time of receipt by the ACRIN server. The data are then entered into the database. A protocol-specific validation program is used to perform more extensive data checks for accuracy and completeness. Complementary validation programs are initiated at the Brown BC and the DMC. The logic checks performed on the data at this point are more comprehensive than those built into the web-based data entry screens. They include checking that answers are logical based on data entered earlier in the current form and the more-thorough checks. Data elements that fail validation are followed up by the DMC. The validation program generated by BC produces a log of errors, which is sent to the DMC for resolution. The program is frequently updated to incorporate exceptions to rules so that subsequent validity checks minimize the time the DMC needs to spend resolving problems. Additional data review will take place once the data are transferred to the BC. The BC will run thorough cross-form validations, frequency distributions to look for

unexpected patterns in data, and other summaries needed for study monitoring. Any errors found at the BC will be reported to the DMC for resolution. All BDMC communication with the participating sites is normally done through the DMC.

- 9.6.2** If checks at DMC or BC detect missing or problematic data, the DMC sends a Request for Information (Z1 query letter) to the site RA or investigator specifying the problem and requesting clarification. The DMC updates the participant's data submission calendar with the due date for the site RA or investigator's response.

9.7 Missing and Delinquent Data Submission

In addition to providing a data collection calendar for each case to the investigator, the DMC periodically prompts institutions for timely submission of data through the use of a Forms Due Report. Distributed at intervals via the electronic mail system directly to both the RA and the investigator at each site, this report lists data items (e.g. forms, reports, and images) that are delinquent and those that will be due before the next report date. In addition to prompting clinicians to submit overdue data, the Forms Due Report helps to reconcile the DMC's case file with that of the RA and/or investigator. Future Due Forms Report may be sent on an as needed basis in addition to past due reports. The site investigator or RA may use the Forms Due and Future Due Reports as a case management tool.

9.8 Data Quality Assurance

- 9.8.1** The BC at Brown University will maintain a study database at its site for monitoring data quality and for performing analyses. These data are drawn directly from the permanent database of the DMC. The transfer of data between the DMC and the BC has been validated through a series of checks consisting of roundtrip data verification in which data are sent back and forth to verify that the sent data are equivalent to the received data. These checks are repeated at random intervals during the course of a given study. Any discrepancies and other data quality issues will be referred to DMC for resolution, since only the DMC can correct the data file. No changes to the data will be made at the BC.
- 9.8.2** A goal of the monitoring of data is to assess compliance with the protocol and to look for unforeseen trends that may be indicative of procedural differences among clinical sites. If patterns are discovered in the data that appear to arise from causes specific to an institution, the BDMC will apprise ACRIN Headquarters and the site of the problem, and work with the site, along with ACRIN Protocol Development and Regulatory Compliance (PDRC) department, until the problem has been resolved. If the BDMC, along with the PDRC, cannot find a resolution to the problem, it will be brought to the ACRIN Quality Assurance (QA) Committee for further discussion and resolution.
- 9.8.3** In addition, the ACRIN QA Monitor will review case report forms and source documents at several different time points: after the first few participants have been enrolled and during the conduct of the trial, including when staff changes at the participating sites. In addition, the QA Monitor will review the initial and annual regulatory documents and any revised regulatory documents. This monitoring process ensures protocol and regulatory compliance and participants' welfare and safety, and provides resources to sites for clarification to the protocol and guidance in completion of the case report forms.

10.0 IMAGING PROTOCOL: PET/CT SCAN

10.1 Overview of PET and CT Data Acquisition

Acquisition protocols of the PET/CT studies are described in Appendix VI. Study participants will undergo a PET/CT study 30 minutes after injection of 18–25 mCi of ⁶⁴Cu-ATSM. The CT component of the PET/CT study will be performed for attenuation correction. Typical CT imaging parameters will be acquired using the vendor-recommended pre-set low-dose whole-body PET/CT imaging protocol.

10.2 Pre-therapy Evaluation

- 10.2.1** All potentially eligible patients with invasive squamous cell cervical cancer will be required to undergo a routine clinical evaluation including medical history and physical examination, radiological studies per institutional standard of care (typically diagnostic CT of the abdomen and pelvis to exclude stage IVB disease) and whole-body FDG-PET/CT prior to initiation of therapy. The volume of the primary tumor will be estimated by FDG-PET/CT employing a method previously described (119) to be performed by the ACIN Imaging Core Laboratory. All imaging done as part of clinical staging or restaging will be evaluated in a clinical fashion by on-site radiologists and nuclear medicine physicians.
- 10.2.2** FDG-PET/CT Identification of Advanced Disease: The presence or absence of lymph node metastasis and distant metastasis will be determined based on all available clinical data, including the pre-therapy FDG-PET/CT study. If the baseline FDG-PET/CT identifies stage IVB disease (including mediastinal or supraclavicular lymph node metastasis) that is amenable to biopsy, tissue confirmation (pathologically by tissue acquisition) must be obtained from the most technically accessible site. Such patients will not be eligible for participation into this study. Participants with suspicious FDG-PET/CT findings that are not amenable to biopsy will undergo additional imaging (CT, MRI, or bone scintigraphy) as clinically indicated and, if negative, they will be eligible to participate. Patients with pelvic and/or para-aortic or lymph node metastasis on FDG-PET/CT are eligible for participation into this study.

10.3 Post-therapy Evaluation

All patients will undergo a clinical FDG-PET/CT 3 months following completion of chemoradiotherapy in a clinical fashion similar to the pre-therapy FDG-PET/CT. For participants enrolled from the Intervention Group (Arm B) of the Outback Trial, the post-therapy FDG-PET/CT must be performed at least 3 months after completion of chemoradiotherapy AND at least 2 weeks, but within 6 weeks after completion of adjuvant chemotherapy.

10.4 Evaluation Criteria

- 10.4.1** All PET/CT images will be required to undergo centralized review to be organized by ACIN (see Section 10.5 for instructions on submitting images to ACIN). The central reading for ⁶⁴Cu-ATSM-PET/CT (for evaluation of tumor uptake of ⁶⁴Cu-ATSM) and FDG-PET/CT (for assessment of metabolic tumor volume) will be conducted at the ACIN Headquarters (Philadelphia, PA).
- 10.4.1.1** Images will be evaluated qualitatively for focal areas of abnormally increased ⁶⁴Cu-ATSM uptake in the primary tumor. T/M ratio will be determined in all cases, even if there is no tumor uptake. This will be performed by visually identifying ⁶⁴Cu-ATSM uptake in the region of the primary tumor seen on the PET/CT images. An FDG-PET/CT-guided circular region of interest of 1.0–1.5 cm in diameter will be drawn around the most intense region of the primary tumor to calculate the maximum uptake within the region. In addition, regions of interest will be drawn on bilateral gluteal muscle groups on at least 3 slices, and mean uptake will be calculated.
- 10.4.1.2** All patients will undergo clinical FDG-PET/CT prior to participation and 3 months after completion of chemoradiotherapy for assessment of response. These studies will be done according to the institution's clinical imaging protocol and will be evaluated in clinical fashion. Appropriate case report forms (CRFs) will be completed by the nuclear medicine physician at each site and will be sent to ACIN.

10.4.1.3 All participants are expected to start chemoradiotherapy within 4 weeks after completion of the ^{64}Cu -ATSM PET/CT. Any delay in initiation of therapy beyond 4 weeks must be clearly documented and an explanation provided. In addition, any subsequent delays in treatment must also be documented and an explanation provided. The date of completion of chemoradiotherapy must also be documented.

10.4.1.4 For participants enrolled from the Intervention Group (Arm B) of the Outback Trial, the post-therapy FDG-PET/CT must be performed at least 3 months after completion of chemoradiotherapy AND at least 2 weeks, but within 6 weeks after completion of adjuvant chemotherapy.

10.5 FDA Preliminary Public Health Notification for CT scans

As of July 14, 2008, FDA has released a preliminary public health notification to health professionals regarding possible malfunction of electronic medical devices caused by CT scanning. Please refer to the FDA website for the notification (www.fda.gov/MedicalDevices/Safety/AlertsandNotices/PublicHealthNotifications/ucm061994.htm) and to section 12.5.3 for AE reporting requirements.

10.6 Imaging Procedures

10.6.1 PET/CT Imaging

All participants will undergo a total of 3 PET/CT scans on this trial. Participants will have 2 clinical FDG-PET/CT studies: the first prior to therapy, and the second at 3 months after completion of chemoradiotherapy, or at least 3 months after completion of chemoradiotherapy AND at least 2 weeks, but within 6 weeks after completion of adjuvant chemotherapy for participants from the Intervention Group (Arm B) of the Outback Trial.

In addition, participants will have the investigational single-position ^{64}Cu -ATSM-PET/CT scan at Visit 1. The PET/CT Technical Assessment Form will be used to ensure protocol compliance. Detailed information for PET/CT qualification (Appendix V), its application, and the PET/CT Technical Assessment Form can be found on the ACRIN web site (www.acrin.org/6682_protocol.aspx).

There are no set criteria that require the institution to repeat the pre-therapy or post-therapy FDG-PET/CT studies, except that digital data from the pre-therapy FDG-PET/CT images should be available for measurement of the volume of the primary tumor. However, considering that PET/CT is standard of care for initial staging of cervical cancer, the study may be repeated, if it is judged by the original institution that the scan results are suboptimal and do not provide clinical information to: stage the patient, measure primary tumor volume prior to chemoradiotherapy, or assess response to therapy after completion of chemoradiotherapy.

10.6.2 ^{64}Cu -ATSM-PET/CT Imaging

^{64}Cu -ATSM Preparation: Please refer to Section 4.0 for detailed drug information.

^{64}Cu -ATSM-PET/CT Imaging: Participant preparation for scanning will include placement of an IV angiocatheter (typically, a 20- or 22-gauge angiocath) or a butterfly needle in a vein of the participant's arm for the injection. Inject 18–25 mCi of ^{64}Cu -ATSM as a bolus, and flush the catheter with 20–40 mL of normal saline solution. All participants must void immediately prior to imaging to ensure clearance of bladder activity. Participants will be positioned in the scanner, typically head first with arms up or on their chest/upper abdomen. A single-position PET/CT image at the level of the primary tumor will be performed. A low-dose CT scan of the pelvis will be used for positioning. Thirty to 40 minutes after the injection of 18–25 mCi of ^{64}Cu -ATSM, the CT component of the PET/CT study will be performed for attenuation correction. Typical CT imaging parameters will be acquired using the vendor recommended low-dose whole-body

PET/CT imaging protocol. Typical acquisition parameter for the low-dose CT scan for attenuation correction should be: kVp = 120; effective mAs = 30–80 (participant dependent); gantry rotation time ≤ 0.5 sec; maximum reconstructed width = 3–5 mm without overlap. The parameters should use the standard reconstruction algorithm, without any iodinated contrast. The PET emission data will be acquired as a 30-minute static image beginning immediately after completion of the CT acquisition.

10.6.2.1 ⁶⁴Cu-ATSM PET/CT Review at Imaging Institutions

⁶⁴Cu-ATSM-PET/CT images will be assessed for quality by trained PET/CT readers and also to ensure the primary tumor was included in the field of view. These images will be evaluated semiquantitatively at ACRIN Imaging Core Laboratory.

10.7 Image Quality Review

An ongoing review will be performed by the ACRIN Imaging Specialist to ensure protocol images meet the study-specific parameters. When the PET/CT scanner is upgraded or a new PET/CT scanner is installed, it will be necessary to have the scanner pre-qualified by the ACRIN Imaging Core Laboratory.

10.8 Core Laboratory Evaluation

The primary analyses for evaluation of study adherence will be based on data obtained from the semiquantitative analysis of the images at ACRIN Imaging Core Laboratory.

The reader will evaluate the images qualitatively for focal areas of abnormally increased uptake in the primary tumor and pelvic nodal region and semiquantitatively by determining T/M uptake ratio. However, only T/M ratio of the primary tumor will be determined. ⁶⁴Cu-ATSM-PET/CT images will be correlated with pre-therapy FDG-PET/CT images to assist in localization of the primary tumor. (See Appendix VI for details of image data analysis.)

10.9 Image Submission

10.9.1 For TRIAD Submission: The preferred image transfer method is via TRIAD, a software application that ACRIN provides for installation on a site's PC. One or several computers of choice within the institutional "firewall" and on the institutional network may be equipped with TRIAD software; Internet access is also required. The TRIAD application can then be configured as a DICOM destination on either scanner(s) and/or PACS system for direct network transfer of study related images into the TRIAD directory. When properly configured, the TRIAD software anonymizes, encrypts, and performs a lossless compression of the images before they are transferred to the ACRIN image archive in Philadelphia. Once equipment-readiness has been determined, imaging personnel from ACRIN will coordinate installation and training for the software. The Imaging Transmittal Worksheet (ITW) must accompany **all** submissions.

For more information, contact: TRIAD-support@phila.acr.org or call 215-940-8820.

10.9.2 For Submission Via Media: In the event that the transfer of image data is not available via TRIAD, images may also be sent on a CD/DVD-ROM to the ACRIN core lab for transfer to the image archive. All image data submitted to the ACRIN core lab must be in DICOM format.

The ITW must accompany **all** media submissions. PDF versions of the transmission worksheets are available for download at:

www.acrin.org/PROTOCOLSUMMARYTABLE/PROTOCOL6682/6682ImagingMaterials.aspx.

10.9.3 Images may be mailed to:

**American College of Radiology Imaging Network
ACR Imaging Core Laboratory
Attn: ACRIN 6682
1818 Market Street 16th floor
Philadelphia, PA 19103**

11.0 TISSUE AND BLOOD SPECIMEN COLLECTION

11.1 Tissue Analysis For Tumor Hypoxic Markers

To investigate the possible relationships between hypoxic markers and ^{64}Cu -ATSM uptake with progression-free survival and overall survival, the cervical biopsy obtained for diagnosis of invasive cervical squamous cell carcinoma will be analyzed for expression of hypoxic markers. No additional biopsy will be obtained. Several hypoxic markers at the level of protein expression including VEGF, GLUT-1, CA-IX, and OPN will be assessed using immunohistochemical staining.

For each participant, 20 additional 5- μm thick, unstained slides on chemoplus/plus slides will be made from the cervical biopsy obtained for initial diagnosis by the pathology department at the institution where the participant will be seen. For a limited-size of biopsy specimen, a minimum of 10 additional 5- μm thick, unstained slides on chemoplus/plus slides will be acceptable. For immunohistochemical stains of the above markers, these slides will then be sent to:

**Ian S. Hagemann, MD, PhD
Molecular Genetic Pathology
Department of Pathology and Immunology
Washington University in Saint Louis
660 South Euclid Ave
Campus Box 8118
Saint Louis, MO 63110**

The immunohistochemical analysis will be performed in a blinded fashion. Briefly, formalin-fixed, 5- μm paraffin-embedded sections will be deparaffinized in xylenes and rehydrated through graded alcohols. Antigen unmasking will be performed by heating slides immersed in Tris-HCl 0.05M, pH 8.5, containing EDTA 0.001M in a 'decloaking chamber' (Biocare Medical, Walnut Creek, CA) at 95°C for 5 min. The slides will then be allowed to cool in the buffer for 20 min. Endogenous peroxidase activity will be quenched with 0.6% H_2O_2 in de-ionized water for 20 min. To block nonspecific immunobinding sites, slides will be incubated in 1.5% bovine serum albumin (BSA) for 1 hr followed by sequential incubation with Avidin D and biotin blocking solutions for 15 min each (Vector Laboratories, Burlingame, CA).

Immunostaining will be performed by incubation with the appropriate primary antibodies (monoclonal or polyclonal) for the recommended time and dilution. A solution of non-immune immunoglobulin diluted to the same concentration will serve as a negative control. The slides will be washed with phosphate-buffered-saline (PBS) with 1.5% BSA and then incubated for 30 min with biotin-conjugated secondary IgG (1 $\mu\text{g}/\text{ml}$). Antibody binding sites will be visualized by avidin-biotin horseradish peroxidase complex solution (Vectastain Elite ABC kit, Vector Laboratories) and 3, 3'-diaminobenzidine (DAB). After rinsing in water, the slides will be counterstained with Gills hematoxylin (Sigma Chemicals, St. Louis, MO), dehydrated, and mounted. For staining of the cervical squamous carcinoma with each antibody, a positive control tissue slide (will stain with that antibody) and a negative tissue control slide (will not stain with that antibody) will also be included at the same time for quality control. Immunostains of the biopsy specimens will be scored based on the percentage of tumor

cells staining and the staining intensity. The percentage of tumor cells staining will be scored as follows: 0 = <1% tumor cells, 1 + = 1%–33% tumor cells, 2 + = 34%–66% tumor cells, and 3 + = >66% tumor cells. The staining intensity (if possible) will be given a score of 0 to 2 (0 = no staining, 1 = weak staining, 2 = moderate to strong staining). A composite score (range 0 to 6) will be generated for each biopsy as the product of percentage of tumor cells staining (range 0 to 3) and the staining intensity (range 0 to 2). VEGF, GLUT-1, CA-IX and OPN will be analyzed using similar immunoperoxidase techniques and scoring system. The immunostain results will be interpreted by Dr. Ian Hagemann, who will be blinded to the imaging results and will assign each biopsy with a composite score for each marker.

11.2 Blood Specimen Collection And Banking For Future Research

For each participant who consents to blood sample collection and banking for future research, a whole-blood specimen will be collected from participant at the baseline visit and at 3 months after completion of therapy. If the potential participant must have a repeat pre-therapy FDG-PET/CT scan because of the ineligibility of the previous scan, the blood draw can coincide with the blood glucose testing. Similarly, the blood draw can coincide with the glucose testing for follow-up FDG-PET/CT at 3 months. In collecting the blood sample, a total of 16 mL of the participant's blood will be collected (in two 10-mL yellow-top, acid citrate-dextrose, tubes).

A kit for blood collection with instructions and shipment information will be made available to the sites. The blood samples will be banked/stored at Washington University School of Medicine in St. Louis, Missouri for purposes of future correlative research. All information will be de-identified prior to shipment.

12.0 ADVERSE EVENTS REPORTING

12.1 Definition of Adverse Event

An **Adverse Event (AE)** is any untoward medical occurrence in a participant that does not necessarily have a causal relationship with the study procedure.

A **pre-existing condition** is one that is present at the start of the study. A pre-existing medical condition is defined as an AE if the frequency, intensity, or character of the medical condition worsens during the study period. At screening visit, any clinically significant findings/abnormalities should be recorded as a pre-existing condition. At the end of study, any new clinically significant findings/abnormalities that meet the definition of an AE must be documented as AEs.

12.2 Definition of Serious Adverse Event

A **Serious Adverse Event (SAE)** is defined as any untoward medical occurrence that:

- results in death;
- is life-threatening (at the time of the event);
- requires inpatient hospitalization or prolongation of an existing hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly/birth defect;
- is considered a medically-important event.

Medically-important events are those based upon appropriate medical judgment that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above.

12.3 Adverse Event Grading

Grade is used to denote the severity of the AE (refer to the CTCAE version 4.0):

- 1 – Mild
- 2 – Moderate
- 3 – Severe
- 4 – Life-threatening or disabling
- 5 – Fatal

12.4 Adverse Event Attribution

Attribution is used to determine whether an AE is related to a study treatment or procedure.

Attribution categories are:

- Definite – AE *is clearly related* to the study treatment or procedure.
- Probable – AE *is likely related* to the study treatment or procedure.
- Possible – AE *may be related* to the study treatment or procedure.
- Unlikely – AE *is doubtfully related* to the study treatment or procedure.
- Unrelated – AE *is clearly NOT related* to the study treatment or procedure.

12.5 Expected Adverse Events

12.5.1 Expected Adverse Events Associated with Blood Sample Collection – If Participant Consents

- Minor discomfort;
- Pain;
- Bruising;
- Bleeding;
- Infection.

12.5.2 Expected Adverse Events Associated with the Intravenous (IV) Catheter Placement for Injection of ⁶⁴Cu-ATSM:

- Minor discomfort;
- Pain at the injection site;
- Bruising;
- Bleeding;
- Phlebitis;
- Infection at the site of injection.

12.5.3 Expected Adverse Events and Potential Risks Associated with ⁶⁴Cu-ATSM (IND # 62675):

- Possible allergic reaction or allergic-like reaction.

NOTE: While none have been encountered to date, such a reaction could be serious or even cause death.

12.5.4 Expected Adverse Events Associated with PET/CT scan:

- Discomfort;
- Claustrophobia;
- Malfunction of implanted electronic medical devices, e.g., pacemakers, neurostimulators, insulin pumps (see note below).

NOTE: As of July 14, 2008, FDA released a preliminary public health notification of possible malfunction of electronic medical devices caused by CT scanning. Site should use CT scout views to determine if implanted or externally worn electronic medical devices are present and if so, their location relative to the programmed scan range. See section 10.5 for FDA warning and their recommendations.

12.5.5 Expected Adverse Events Associated with Radiation:

- The effective dose for ⁶⁴Cu-ATSM-PET/CT scanning, as performed in this trial, is approximately 4 rem (about --80% of the annual allowable exposure for occupational workers).

NOTE: The radiation dose from the ⁶⁴Cu-ATSM-PET/CT has not been shown to have any adverse effects.

12.5.6 Expected Adverse Events Associated with Standard of Care Practice

- Any AE that is a result of standard of care practice, e.g. chemoradiotherapy will be reported and managed per the institution's policies and procedures.

12.5.7 Expected Adverse Events Associated with ANZGOG 092/GOG-0274 OUTBACK Trial

- Any AE that is a result of the OUTBACK Trial will be reported and managed per the OUTBACK Trial by the GOG research team.

12.6 Source Documentation of Adverse Events

At each contact (site visit and/or telephone) with the study participant, the investigator or investigator-designee must seek information on AEs through discussion and, as appropriate, by examination. Information on all expected and unexpected AEs with the severity level of grades 1, 2, 3, 4, 5 that occur within 24 hours of ⁶⁴Cu-ATSM administration should be recorded immediately into the source document, e.g. ACRIN AE Log and/or progress notes of the study participant's chart, and retained at the site. In addition, concomitant medication taken by the participant prior to 2 weeks and/or during the time of the AE must be collected and documented.

Important: Recording of AEs on source document does not constitute reporting. Please ensure that AEs are reported to ACRIN per protocol-specific reporting requirements. ACRIN will collect and report all AEs that occur within 24 hours of ⁶⁴Cu-ATSM administration.

12.7 Reporting of Adverse Events

Prompt reporting of AEs is the responsibility of each investigator, clinical research associate, and/or nurse engaged in clinical research. Anyone uncertain about how an AE should be reported should contact the ACRIN headquarters at (215) 574-3150 and ask for the ACRIN AE Coordinator.

All unresolved AEs should be followed by the principal site investigator until the AE is resolved, otherwise explained, or the site has documented due diligence in attempting to procure the requisite medical records.

Any death or AE occurring at any time after a participant has discontinued or terminated study participation that may be **reasonably related** to the ⁶⁴Cu-ATSM-PET/CT trial should be reported.

Assignment of grades (severity level) and attribution for each AE is to be completed at the site by the site Principal Investigator.

12.8 Routine AE Reporting Process

Routine reporting is defined as documentation of all AEs (routine and serious) on the source documents and AE CRF, for submission to ACRIN for review and preparation of reports for review by the medical monitor, designated DSMC, and other regulatory oversight bodies, as well as, for the preparation of the final study report.

ACRIN will collect and report all AEs that occur within 24 hours of ⁶⁴Cu-ATSM administration. Sites must report all AEs (routine and serious) to ACRIN per the reporting requirements as noted below in Table A. Local Institutional Review Boards (IRBs) may stipulate additional AE reporting based upon their review of the protocol. These AEs must also be recorded in the AE CRF and reviewed by the site Principal Investigator (PI) in real time to determine grade and attribution of the event.

All AEs occurring during study participation require telephone reporting to the ACRIN AE Coordinator. Call (215) 574-3150 and ask for the ACRIN AE Coordinator; the coordinator will add the AE form to the calendar for web entry.

NOTE: The AE must also be documented in the participant's chart. Significant new information and/or follow-up information (e.g., test results . . .) on any ongoing AEs should be promptly reported to ACRIN. ACRIN AE helpline is available for any questions via phone at (215) 717-2763.

12.9 Expedited AE Reporting Process

IMPORTANT: ACRIN will collect and report all AEs that occur within 24 hours of ⁶⁴Cu-ATSM administration.

Expedited reporting is defined as the immediate notification to ACRIN at first knowledge of the serious AE (SAE). All SAEs require 24-Hour telephone notification; in addition to written report (refer to section 12.9.1 for 24-Hour Telephone Expedited Reporting instructions). Routine reporting requirements will also apply. All SAEs will be documented in the study participant's chart and AE CRFs. It is imperative that AEs, and particularly SAEs are reported to ACRIN as note in Table A to ensure timely reporting by the Investigational New Drug (IND) holder to the FDA (See section 12.9.2 for written Expedited Reporting instructions).

Sites must comply with study-specific reporting requirements, FDA regulations, and the local IRB reporting requirements (per local IRB policy). Per FDA reporting requirements, the IND holder at Washington University School of Medicine in St. Louis will report AEs as necessary.

NOTE: In addition to documentation listed above, the AE must also be documented in the participant's chart and an AE CRF in order to satisfy routine reporting requirements.

The following Table A summarizes expedited AE reporting requirements for the ⁶⁴Cu-ATSM_trial to ACRIN: AEs/SAEs that occur within 24 hours of ⁶⁴Cu-ATSM administration.

Table A: Expedited Reporting Requirements for ⁶⁴Cu-ATSM

Attribution	Grade 1	Grade 2	Grade 2	Grade 3	Grade 3	Grades 4 & 5	Grades 4 & 5
	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitalization; Unexpected without Hospitalization	Expected with Hospitalization; Expected without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required Routine reporting is required.	Not Required Routine reporting is required.	5 Calendar Days; Not Required Routine reporting is also required.	5 Calendar Days; Not Required Routine reporting is also required.	5 Calendar Days Routine reporting is also required.	5 Calendar Days Routine reporting is also required.
Possible Probable Definite	Not Required Routine reporting is required.	5 Calendar Days Routine reporting is also required.	Not Required Routine reporting is required.	5 Calendar Days; 5 Calendar Days Routine reporting is also required.	5 Calendar Days; Not Required Routine reporting is also required.	5 Calendar Days Routine reporting is also required.	5 Calendar Days Routine reporting is also required.

12.9.1 24-Hour Telephone Expedited Reporting to ACRIN for all SAEs

All SAEs that occur within 24 hours of ⁶⁴Cu-ATSM administration require telephone reporting within 24 hours of first knowledge of the event to the:

ACRIN SAE line at (215) 717-2763

Once the ACRIN AE Coordinator is notified of an SAE via 24 hour telephone report, the following individuals will be notified via email:

Study Chair

Farrokh Dehdashti, MD
Division of Nuclear Medicine
Mallinckrodt Institute of Radiology
Email: dhhdashtif@mir.wustl.edu

Co-Chair

Barry A. Siegel, MD
Division of Nuclear Medicine
Mallinckrodt Institute of Radiology
Email: siegelb@mir.wustl.edu

Medical Monitor

Janet Rader, MD
Division of Obstetrics and Gynecology
Medical College of Wisconsin
Email: jrader@mcw.edu

12.9.2 Completion of Serious Adverse Event Report

In addition to the 24-Hour Telephone Expedited Reporting Process, all SAEs that occur within 24 hours of ⁶⁴Cu-ATSM administration require the submission of an electronic serious adverse event (SAE) report within five (5) calendar days of first knowledge of the event.

ACRIN AE helpline is available for any questions via phone at (215) 717-2763.

The SAE report must be completed and faxed within five (5) calendar days to the following:

- ACRIN SAE Fax Number: (215) 940-8819;
- ACRIN contact to confirm receipt of the SAE report: (215) 717-2763

ACRIN AE Coordinator will forward the completed SAE report for review and assessment prior to FDA reporting:

Study Chair
Farrokh Dehdashti, MD

Co-Chair
Barry A. Siegel, MD

Medical Monitor
Janet Rader, MD

12.10 Local IRB Reporting

12.10.1 Adverse Event Reporting and Local IRB

AEs not requiring expedited reporting are reported to the local IRB in an annual report and/or continuing review report. All expedited AE reports should be sent to your local IRB per the local IRB policies and procedures. Please refer to your local IRB's policies regarding AEs/SAEs and safety reports.

12.10.2 Expedited Serious Adverse Event Reporting and Local IRB

All expedited reports should be sent to your local IRB per your local IRB policies and procedures.

13.0 ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice [International Conference of Harmonisation (ICH) guidelines], applicable government regulations, and ACRIN research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or IRB for a formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to ACRIN before implementation of the study. The investigator will provide ACRIN with the institution's federal wide assurance (FWA) number, along with the IRB approval letter and copy of the IRB approved informed consent form. The investigator will provide a copy(s) of IRB approval letter(s) for any amendment(s), and copy(s) of annual renewal(s).

All patients considering participation in this study will be provided an IRB-approved, site-specific informed consent form describing the study and providing sufficient information for potential participants to make informed decisions about their participation in this study (see Appendix I for a copy of the sample informed consent form). This informed consent form will be submitted along with the protocol for review and approval by the EC/IRB. The study participant MUST be consented with the EC/IRB approved informed consent form before the participant is subjected to any study procedures. The approved consent form MUST be signed and dated by the study participant or legally acceptable representative and the investigator-designated research staff obtaining the consent.

14.0 CONFLICT OF INTEREST

Any investigator and/or research staff member who has a conflict of interest with this study (such as patent ownership, royalties, or financial gain greater than the minimum allowable by their institution) must fully disclose the nature of the conflict of interest in accordance with ACRIN policies and applicable federal, state, and local laws and regulations.

15.0 PUBLICATIONS POLICY

Neither complete nor any part of the results of the study obtained under this protocol, nor any information provided to the investigator for the purposes of performing the study, will be published or passed on to any third party without the consent of ACRIN and the Study Chair. Any investigator involved in this study is obligated to provide ACRIN with complete test results and all clinical data obtained from those participating in this protocol. Investigators will follow ACRIN Publication Policy (available on the web at www.acrin.org/PublicationsPolicy.aspx).

16.0 INSTITUTIONAL MONITORING AND AUDITS

The investigator will permit study-related monitoring, auditing, and inspections of all study-related documents by the EC/IRB, government regulatory agencies, and ACRIN. The investigator will ensure the capability for inspection of all participating site's study-related facilities (e.g. imaging center, satellite sites). The investigator will allocate adequate time for these activities, allow access to all study-related documents and facilities, and provide adequate space to conduct audit visits.

16.1 Monitoring

Monitoring ensures protocol and regulatory compliance and to provide any clarification to the protocol and guidance to the completion of the case report forms (CRFs). Institutional monitoring will be implemented at several different time points: after first participant enrollment and during the conduct of the study. Instructions for monitoring preparation will be sent to the site prior to the implementation of monitoring. The instructions will specify regulatory documents and participant case records to be monitored. CRFs and source documents of selected study participants enrolled at each site will be reviewed. In addition, the initial regulatory documents and any revised regulatory documents will also be monitored.

16.2 Audits

Institutional audits will be completed within 18 months after first participant enrollment of each site's enrollment of its first ACRIN trial participant. Subsequent audits will be scheduled per the outcome of the initial audit. The audits will be conducted per procedures established by the Cancer Imaging Program (CIP) of the NCI. Instructions for preparation for the audit visit will be sent to the site prior to the scheduled audit visit. These instructions will specify which participant case records will be reviewed during the audit. On-site records will be verified against the submitted form, and the findings will be recorded on specially-prepared audit reports. Major discrepancies will be forwarded to the appropriate oversight body within ACRIN. IRB procedures, approvals,

and consent forms will also be reviewed at the time of the audit visit. The ACRIN Audit Manual is available online at www.acrin.org/pdrc.aspx.

To help sites prepare for monitoring and audits, and to assure that the investigator and the research staff maintain records appropriately, the ACRIN Headquarter staff will offer training to sites. This training will cover all aspects of data collection, including special instructions to obtain and file the various source documents needed to verify the accuracy of submitted data for this trial.

16.3 Source Documents

Source data are found in all information, original records of findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source documents represent the first recording of any observations made or data generated about a study participant while he or she is enrolled in a clinical trial. Source documents for each study participant substantiate the data that are submitted to ACRIN. Source documents must verify the eligibility criteria and data submitted on all CRFs.

Research records for each case should contain copies of the source documents for the data collected and reported to ACRIN. If data is abstracted from medical charts that are not filed at the investigative sites (e.g. hospital charts), copies of these records should be filed in the research chart. Every attempt must be made to obtain all records/charts that were used to abstract any study data for this protocol. This will prevent any discrepancies and the inability to verify the document and the data reported.

16.4 Case Report Forms (CRFs)

CRFs, both web-based and in paper form, are the primary data collection instruments for the study. All data requested on the CRFs must be recorded, and any missing data must be explained. If a space is left blank on paper CRFs because the procedure was not done or the question was not asked, “N/D” must be noted. If the item is not applicable to the individual case “N/A” must be noted. All entries on paper CRFs must be printed legibly in black ink on the paper CRFs. In the event of any entry errors, corrections must be made by drawing a **single straight line** through the incorrect entry, writing **the initials of the person making the correction, recording the date** when the correction is being made, and entering the correct data above the strike through. Do not use white out or an eraser. Please refer to ICH Good Clinical Practice Guidelines.

Data elements that are extracted from the medical record (such as participant history or official clinical interpretations of images, pathology, or surgery results) and recorded on the CRFs will be reviewed against the appropriate component of the medical record. Data elements gathered from signed participant questionnaires must be available for review. Required study image interpretation data that are more detailed in information than the image and not typically documented in the standard radiology report may be documented on the CRF; the CRF is therefore acceptable source documentation **if signed by the Investigator**. At the time of audit, the auditor will verify the occurrence of the imaging examination, the reader, and the date of the exam(s) from the medical record(s). Any use of approved CRFs as source documentation require a signature and date on the CRF with a reference to the information source (participant questionnaire, CT, MR, etc.). Any use of CRFs as source documentation when the protocol has designated the medical record documentation as the only acceptable source data will be considered a deficiency.

17.0 STATISTICAL CONSIDERATIONS

17.1 Study Design and Endpoints

This is a non-randomized study intended to investigate the efficacy of ⁶⁴Cu-ATSM uptake, as measured by the T/M ratio from pre-therapy ⁶⁴Cu-ATSM-PET/CT, in predicting progression-free and overall survival, and in identifying tumor hypoxia and subsequent risk of recurrence in patients with cervical cancer.

Women with newly diagnosed locally advanced squamous cell cervical carcinoma (stages IB2-IVA) will undergo PET/CT with ^{64}Cu -ATSM before initiation of standard of care chemoradiotherapy per NCCN guidelines. Participants will be followed for up to 3 years after completion of study visits to assess progression of disease and overall survival.

17.2 Specific Endpoints and Analysis Plans

17.2.1 Analysis for the Primary Endpoint

17.2.1.1 To determine if ^{64}Cu -ATSM uptake is associated with progression-free survival.

Cox regression modeling will be used for this analysis, with progression-free survival as the response variable and the T/M ratio entered as a continuous predictor. The model will also include variables encoding FIGO stage and the presence of pelvic and para-aortic lymph nodes on baseline FDG-PET/CT (127).

17.2.2 Analysis of Secondary Endpoints

17.2.2.1 To determine if higher ^{64}Cu -ATSM uptake is associated with lower overall survival.

The analysis for this aim will be similar to the analysis for the primary aim. Cox regression modeling will be used with overall survival as the response variable and the T/M ratio entered as a continuous predictor. The model will also include variables encoding FIGO stage, and the presence of pelvic and para-aortic lymph nodes on baseline FDG-PET/CT (127).

17.2.2.2 To determine if higher ^{64}Cu -ATSM uptake is associated with earlier primary cervical tumor recurrence and a higher rate of development of distant metastatic disease.

Cox regression will be used to examine the relation between the T/M ratio and time to primary cervical tumor recurrence. Logistic regression will be used to examine the relation between the T/M ratio and a binary response variable marking the development of distant metastatic disease.

17.2.2.3 To determine if higher ^{64}Cu -ATSM uptake is associated with a lower frequency of complete metabolic response on FDG-PET/CT performed 3 months after completion of chemoradiotherapy.

Logistic regression will be used to examine the relation between the T/M ratio and a binary response variable marking the presence of complete metabolic response of FDG-PET/CT performed at 3 months after completion of radiation and chemotherapy.

17.2.2.4 To estimate the accuracy ^{64}Cu -ATSM uptake as a predictor of: (a) progression-free survival, (b) overall survival, (c) primary tumor recurrence, and (d) future development of distant metastatic disease.

Time-dependent ROC analysis will be used to evaluate the accuracy of ^{64}Cu -ATSM uptake as predictor of progression-free survival, overall survival, primary tumor recurrence, and future development of distant metastasis. In this analysis ROC curves for each outcome will be estimated at 1 year intervals (128, 129) In addition to providing an overall assessment of the predictive performance of ^{64}Cu -ATSM uptake, the estimated ROC curves will be used to determine optimal threshold values for each of the designated tasks and timepoints.

17.2.2.5 To evaluate the performance of ^{64}Cu -ATSM uptake as a predictor of lymph node metastasis at study entry.

The analysis for this aim will rely on logistic regression modeling in which the response will be a binary determination of nodal involvement made on the basis of FDG-PET/CT and the predictor variable will be the T/M ratio for ^{64}Cu -ATSM uptake.

- 17.2.2.6** *To evaluate whether ⁶⁴Cu-ATSM uptake correlates with tumor volume at study entry.*
- 17.2.2.7** *To examine the relationship between tumor uptake of ⁶⁴Cu-ATSM and other markers of tumor hypoxia, including VEGF, GLUT-1, CA-IX, and OPN.*

The last two secondary endpoints involve exploratory analyses of the relation between T/M ratio of ⁶⁴Cu-ATSM uptake and the results of various immunohistochemical tumor markers putatively related to tumor oxygenation. Scatter plots, summary statistics and linear or nonlinear regression will be used to describe the relationship between these variables.

- 17.2.2.8** *To compare the predictive ability of pre-therapy ⁶⁴Cu-ATSM-PET to that of post-therapy FDG-PET/CT.*
- 17.2.2.9** *To assess whether pre-therapy FDG-PET/CT findings are predictive of progression-free survival.*

The analytic strategy for the last two endpoints will be based on Cox regression modeling and will proceed similarly to the analysis of the primary aim. For example, the relation between pre-therapy and PFS will be assessed using a Cox regression model with PFS as the response variable and the T/M ratio entered as a continuous predictor.

17.3 Sample Size/Accrual Rate

A total of 100 participants will be enrolled into this study. Accrual will be monitored through out the enrollment phase of the trial to ensure adequate representation of participants from all participating institutions.

17.4 Sample Size and Power Considerations for the Primary Endpoint.

The sample size for this study was chosen to provide adequate power for the primary aim, which involves the evaluation of ⁶⁴Cu-ATSM uptake as a predictor of progression-free survival. We assumed that accrual will proceed at a uniform rate and will be accomplished in 18 months. We also assumed that each participant will be followed for a minimum of 18 months, resulting in a total of 36 months from the beginning of accrual to the end of study follow-up. Based on preliminary data from Washington University, we assumed that about 30% of all patients will have progressed by the end of the 3-year study period and that the coefficient of T/M ratio will be above 0.25 and most likely around 0.3. We also assume that the standard deviation of the T/M ratio will be 2 or higher and that the T/M ratio has negligible correlation to the other predictors in the model. The following table shows estimates of power to detect a Cox regression coefficient with the indicated magnitude. Computations were done using the PASS software (130). A two-sided test at level 0.05 was assumed for the comparison of progression-free survival curves. If the total sample size is 100 and 5% of the cases accrued do not provide usable data for the analysis, the sample size of cases with usable data will be 95 and the power to detect a regression coefficient of 0.30 with this sample size is 0.89.

Coefficient: Cox Regression Model	Sample size	Power
0.25	95	0.76
0.25	100	0.78
0.26	90	0.77
0.26	95	0.79
0.26	100	0.81
0.3	85	0.86
0.3	90	0.88
0.3	95	0.89
0.3	100	0.91

17.5 Interim Analysis

The timeframe for accomplishing accrual to the study (18 months) severely limits the ability to perform interim monitoring of the primary endpoint before accrual has been completed. As an alternative, the accuracy of ⁶⁴Cu-ATSM uptake to predict response status at 3 months can be used for purposes of monitoring for futility. Response will be determined by FDG-PET/CT performed at 3 months from initiation of therapy. The ROC curve for prediction of a response will be estimated on the basis of data from the first 50 cases and the null hypothesis H0: AUC<0.6 will be tested using a one-sided test at level 0.05 test. If the hypothesis is not rejected, accrual of additional study participants will stop. This analysis can be performed near the end of the first year of the study. The following table shows estimates of the power to reject the null hypothesis using the data from the first 50 participants. We assumed that approximately 70% of participants will be classified as responders by FDG-PET/CT at 3 months and that information on both the test result and response status will be available on all participants.

True AUC	0.75	0.8	0.85	0.9
Power to reject H0	54%	82%	97%	99%

17.6 Reporting Guidelines

Routine reports for this protocol will be included in the ACRIN Biostatistics Center Mid-Year and Year-End Updates and will be provided to oversight bodies, including DSMC, for review during each of its twice-yearly meeting. Routine reports will include:

- Accrual and participant characteristics;
- Timeliness and completeness, eligibility and protocol compliance, and outcome data;
- All reported AEs.

REFERENCES

1. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev* 2007; 26:225-239.
2. Beran M, Tribukait B. Haemopoietic aspects of the changes in the natural radiation resistance of mice after hypoxia. *Acta Radiol Ther Phys Biol* 1972; 11:225-239.
3. Elkind MM. Fundamental questions in the combined use of radiation and chemicals in the treatment of cancer. *Int J Radiat Oncol Biol Phys* 1979; 5:1711-1720.
4. Gambino JJ, Lindberg RG, Hayden P. A search for mechanisms of radiation resistance in pocket mice. *Radiat Res* 1965; 26:305-317.
5. Vaupel P, Harrison L. Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. *Oncologist* 2004; 9 Suppl 5:4-9.
6. Moulder JE, Rockwell S. Tumor hypoxia: its impact on cancer therapy. *Cancer Metastasis Rev* 1987; 5:313-341.
7. Moulder JE, Dutreix J, Rockwell S, Siemann DW. Applicability of animal tumor data to cancer therapy in humans. *Int J Radiat Oncol Biol Phys* 1988; 14:913-927.
8. Fowler JF. The second Klaas Breur memorial lecture. La Ronde--radiation sciences and medical radiology. *Radiother Oncol* 1983; 1:1-22.
9. Suit HD. Modification of radiation response. *Int J Radiat Oncol Biol Phys* 1984; 10:101-108.
10. Graeber TG, Osmanian C, Jacks T, et al. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* 1996; 379:88-91.
11. Graeber TG, Peterson JF, Tsai M, Monica K, Fornace AJ, Jr., Giaccia AJ. Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. *Mol Cell Biol* 1994; 14:6264-6277.
12. Giaccia AJ. Hypoxic Stress Proteins: Survival of the Fittest. *Semin Radiat Oncol* 1996; 6:46-58.
13. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. *Nature* 1992; 359:845-848.
14. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 1992; 359:843-845.
15. Brown LF, Berse B, Jackman RW, et al. Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. *Am J Pathol* 1993; 143:1255-1262.
16. Hlatky L, Tsiou C, Hahnfeldt P, Coleman CN. Mammary fibroblasts may influence breast tumor angiogenesis via hypoxia-induced vascular endothelial growth factor up-regulation and protein expression. *Cancer Res* 1994; 54:6083-6086.
17. Toi M, Hoshina S, Takayanagi T, Tominaga T. Association of vascular endothelial growth factor expression with tumor angiogenesis and with early relapse in primary breast cancer. *Jpn J Cancer Res* 1994; 85:1045-1049.
18. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res* 1995; 55:3964-3968.
19. Young SD, Marshall RS, Hill RP. Hypoxia induces DNA overreplication and enhances metastatic potential of murine tumor cells. *Proc Natl Acad Sci U S A* 1988; 85:9533-9537.
20. Rice GC, Hoy C, Schimke RT. Transient hypoxia enhances the frequency of dihydrofolate reductase gene amplification in Chinese hamster ovary cells. *Proc Natl Acad Sci U S A* 1986; 83:5978-5982.
21. Russo CA, Weber TK, Volpe CM, et al. An anoxia inducible endonuclease and enhanced DNA breakage as contributors to genomic instability in cancer. *Cancer Res* 1995; 55:1122-1128.
22. Overgaard J, Horsman MR. Modification of Hypoxia-Induced Radioresistance in Tumors by the Use of Oxygen and Sensitizers. *Semin Radiat Oncol* 1996; 6:10-21.

23. Brown JM, Giaccia AJ. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res* 1998; 58:1408-1416.
24. Brown JM. The hypoxic cell: a target for selective cancer therapy--eighteenth Bruce F. Cain Memorial Award lecture. *Cancer Res* 1999; 59:5863-5870.
25. Nordsmark M, Overgaard J. A confirmatory prognostic study on oxygenation status and loco-regional control in advanced head and neck squamous cell carcinoma treated by radiation therapy. *Radiother Oncol* 2000; 57:39-43.
26. Bussink J, Kaanders JH, van der Kogel AJ. Tumor hypoxia at the micro-regional level: clinical relevance and predictive value of exogenous and endogenous hypoxic cell markers. *Radiother Oncol* 2003; 67:3-15.
27. Koukourakis MI, Bentzen SM, Giatromanolaki A, et al. Endogenous markers of two separate hypoxia response pathways (hypoxia inducible factor 2 alpha and carbonic anhydrase 9) are associated with radiotherapy failure in head and neck cancer patients recruited in the CHART randomized trial. *J Clin Oncol* 2006; 24:727-735.
28. Theodoropoulos GE, Lazaris AC, Theodoropoulos VE, et al. Hypoxia, angiogenesis and apoptosis markers in locally advanced rectal cancer. *Int J Colorectal Dis* 2006; 21:248-257.
29. Kennedy KA, Teicher BA, Rockwell S, Sartorelli AC. The hypoxic tumor cell: a target for selective cancer chemotherapy. *Biochem Pharmacol* 1980; 29:1-8.
30. Rockwell S. Hypoxic cells as target for cancer chemotherapy. New York, NY: Raven Press, 1983.
31. Teicher BA, Lazo JS, Sartorelli AC. Classification of antineoplastic agents by their selective toxicities toward oxygenated and hypoxic tumor cells. *Cancer Res* 1981; 41:73-81.
32. Tannock I, Guttman P. Response of Chinese hamster ovary cells to anticancer drugs under aerobic and hypoxic conditions. *Br J Cancer* 1981; 43:245-248.
33. Twentyman PR. Comparative chemosensitivity of exponential- versus plateau-phase cells in both in vitro model systems. *Cancer Treat Rep* 1976; 60:1719-1722.
34. Valeriote F, van Putten L. Proliferation-dependent cytotoxicity of anticancer agents: a review. *Cancer Res* 1975; 35:2619-2630.
35. Bedford JS, Mitchell JB. The effect of hypoxia on the growth and radiation response of mammalian cells in culture. *Br J Radiol* 1974; 47:687-696.
36. Brock WA, Swartzendruber DE, Grdina DJ. Kinetic heterogeneity in density-separated murine fibrosarcoma subpopulations. *Cancer Res* 1982; 42:4999-5003.
37. Koch CJ, Kruuv J, Frey HE, Snyder RA. Plateau phase in growth induced by hypoxia. *Int J Radiat Biol Relat Stud Phys Chem Med* 1973; 23:67-74.
38. Stratford IJ, Adams GE, Bremner JC, et al. Manipulation and exploitation of the tumour environment for therapeutic benefit. *Int J Radiat Biol* 1994; 65:85-94.
39. Harrison L, Blackwell K. Hypoxia and anemia: factors in decreased sensitivity to radiation therapy and chemotherapy? *Oncologist* 2004; 9 Suppl 5:31-40.
40. Hoogsteen IJ, Marres HA, van der Kogel AJ, Kaanders JH. The Hypoxic Tumour Microenvironment, Patient Selection and Hypoxia-modifying Treatments. *Clin Oncol (R Coll Radiol)* 2007; 19:385-396.
41. Kolstad P. Intercapillary distance, oxygen tension and local recurrence in cervix cancer. *Scand J Clin Lab Invest Suppl* 1968; 106:145-157.
42. Pappova N, Siracka E, Vacek A, Durkovsky J. Oxygen tension and prediction of the radiation response. Polarographic study in human breast cancer. *Neoplasma* 1982; 29:669-674.
43. Gatenby RA, Kessler HB, Rosenblum JS, et al. Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. *Int J Radiat Oncol Biol Phys* 1988; 14:831-838.
44. Hockel M, Knoop C, Schlenger K, et al. Intratumoral pO₂ predicts survival in advanced cancer of the uterine cervix. *Radiother Oncol* 1993; 26:45-50.

45. Hockel M, Knoop C, Schlenger K, Vorndran B, Knapstein PG, Vaupel P. Intratumoral pO₂ histography as predictive assay in advanced cancer of the uterine cervix. *Adv Exp Med Biol* 1994; 345:445-450.
46. Hockel M, Schlenger K, Hockel S, Aral B, Schaffer U, Vaupel P. Tumor hypoxia in pelvic recurrences of cervical cancer. *Int J Cancer* 1998; 79:365-369.
47. Hockel M, Schlenger K, Knoop C, Vaupel P. Oxygenation of carcinomas of the uterine cervix: evaluation by computerized O₂ tension measurements. *Cancer Res* 1991; 51:6098-6102.
48. Pitson G, Fyles A, Milosevic M, Wylie J, Pintilie M, Hill R. Tumor size and oxygenation are independent predictors of nodal diseases in patients with cervix cancer. *Int J Radiat Oncol Biol Phys* 2001; 51:699-703.
49. Hockel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. *Cancer Res* 1996; 56:4509-4515.
50. Sundfor K, Lyng H, Trope CG, Rofstad EK. Treatment outcome in advanced squamous cell carcinoma of the uterine cervix: relationships to pretreatment tumor oxygenation and vascularization. *Radiother Oncol* 2000; 54:101-107.
51. Giatromanolaki A, Koukourakis MI, Sivridis E, et al. Expression of hypoxia-inducible carbonic anhydrase-9 relates to angiogenic pathways and independently to poor outcome in non-small cell lung cancer. *Cancer Res* 2001; 61:7992-7998.
52. Olive PL, Aquino-Parsons C, MacPhail SH, et al. Carbonic anhydrase 9 as an endogenous marker for hypoxic cells in cervical cancer. *Cancer Res* 2001; 61:8924-8929.
53. Raleigh JA, Chou SC, Bono EL, Thrall DE, Varia MA. Semiquantitative immunohistochemical analysis for hypoxia in human tumors. *Int J Radiat Oncol Biol Phys* 2001; 49:569-574.
54. Haustermans K, Hofland I, Van de Pavert L, et al. Diffusion limited hypoxia estimated by vascular image analysis: comparison with pimonidazole staining in human tumors. *Radiother Oncol* 2000; 55:325-333.
55. Gaffney DK, Haslam D, Tsodikov A, et al. Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) negatively affect overall survival in carcinoma of the cervix treated with radiotherapy. *Int J Radiat Oncol Biol Phys* 2003; 56:922-928.
56. Kim GE, Kim YB, Cho NH, et al. Synchronous coexpression of epidermal growth factor receptor and cyclooxygenase-2 in carcinomas of the uterine cervix: a potential predictor of poor survival. *Clin Cancer Res* 2004; 10:1366-1374.
57. Ivanov S, Liao SY, Ivanova A, et al. Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. *Am J Pathol* 2001; 158:905-919.
58. Loncaster JA, Harris AL, Davidson SE, et al. Carbonic anhydrase (CA IX) expression, a potential new intrinsic marker of hypoxia: correlations with tumor oxygen measurements and prognosis in locally advanced carcinoma of the cervix. *Cancer Res* 2001; 61:6394-6399.
59. Lee S, Shin HJ, Han IO, et al. Tumor carbonic anhydrase 9 expression is associated with the presence of lymph node metastases in uterine cervical cancer. *Cancer Sci* 2007; 98:329-333.
60. Kim JY, Shin HJ, Kim TH, et al. Tumor-associated carbonic anhydrases are linked to metastases in primary cervical cancer. *J Cancer Res Clin Oncol* 2006; 132:302-308.
61. Moon EJ, Brizel DM, Chi JT, Dewhirst MW. The potential role of intrinsic hypoxia markers as prognostic variables in cancer. *Antioxid Redox Signal* 2007; 9:1237-1294.
62. Iyer NV, Kotch LE, Agani F, et al. Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev* 1998; 12:149-162.
63. Airley RE, Loncaster J, Raleigh JA, et al. GLUT-1 and CAIX as intrinsic markers of hypoxia in carcinoma of the cervix: relationship to pimonidazole binding. *Int J Cancer* 2003; 104:85-91.
64. Airley R, Loncaster J, Davidson S, et al. Glucose transporter glut-1 expression correlates with tumor hypoxia and predicts metastasis-free survival in advanced carcinoma of the cervix. *Clin Cancer Res* 2001; 7:928-934.

65. Chiarotto JA, Hill RP. A quantitative analysis of the reduction in oxygen levels required to induce up-regulation of vascular endothelial growth factor (VEGF) mRNA in cervical cancer cell lines. *Br J Cancer* 1999; 80:1518-1524.
66. Tanaka H, Yasuda Y, Musha T, Ueda K, Nakamura Y. Hypoxia correlates with angiogenesis in cervical cancers. *Int J Clin Oncol* 2005; 10:35-39.
67. Branca M, Giorgi C, Ciotti M, et al. Down-regulated nucleoside diphosphate kinase nm23-H1 expression is unrelated to high-risk human papillomavirus but associated with progression of cervical intraepithelial neoplasia and unfavourable prognosis in cervical cancer. *J Clin Pathol* 2006; 59:1044-1051.
68. Bache M, Reddemann R, Said HM, et al. Immunohistochemical detection of osteopontin in advanced head-and-neck cancer: prognostic role and correlation with oxygen electrode measurements, hypoxia-inducible-factor-1alpha-related markers, and hemoglobin levels. *Int J Radiat Oncol Biol Phys* 2006; 66:1481-1487.
69. Weber GF. The metastasis gene osteopontin: a candidate target for cancer therapy. *Biochim Biophys Acta* 2001; 1552:61-85.
70. Sakaguchi H, Fujimoto J, Hong BL, Tamaya T. Clinical implications of osteopontin in metastatic lesions of uterine cervical cancers. *Cancer Lett* 2007; 247:98-102.
71. Padhani A. PET imaging of tumour hypoxia. *Cancer Imaging* 2006; 6:S117-121.
72. Rajendran JG, Krohn KA. Imaging hypoxia and angiogenesis in tumors. *Radiol Clin North Am* 2005; 43:169-187.
73. Rasey JS, Nelson NJ, Chin L, Evans ML, Grunbaum Z. Characteristics of the binding of labeled fluoromisonidazole in cells in vitro. *Radiat Res* 1990; 122:301-308.
74. Casciari JJ, Graham MM, Rasey JS. A modeling approach for quantifying tumor hypoxia with [F-18]fluoromisonidazole PET time-activity data. *Med Phys* 1995; 22:1127-1139.
75. Martin GV, Caldwell JH, Graham MM, et al. Noninvasive detection of hypoxic myocardium using fluorine-18-fluoromisonidazole and positron emission tomography. *J Nucl Med* 1992; 33:2202-2208.
76. Linder KE, Chan YW, Cyr JE, Malley MF, Nowotnik DP, Nunn AD. TcO(PnA.O-1-(2-nitroimidazole)) [BMS-181321], a new technetium-containing nitroimidazole complex for imaging hypoxia: synthesis, characterization, and xanthine oxidase-catalyzed reduction. *J Med Chem* 1994; 37:9-17.
77. Jerabek PA, Patrick TB, Kilbourn MR, Dischino DD, Welch MJ. Synthesis and biodistribution of 18F-labeled fluoronitroimidazoles: potential in vivo markers of hypoxic tissue. *Int J Rad Appl Instrum [A]* 1986; 37:599-605.
78. Grierson JR, Link JM, Mathis CA, Rasey JS, Krohn KA. A radiosynthesis of fluorine-18 fluoromisonidazole. *J Nucl Med* 1989; 30:343-350.
79. Koh WJ, Rasey JS, Evans ML, et al. Imaging of hypoxia in human tumors with [F-18]fluoromisonidazole. *Int J Radiat Oncol Biol Phys* 1992; 22:199-212.
80. Rasey JS, Koh WJ, Evans ML, et al. Quantifying regional hypoxia in human tumors with positron emission tomography of [18F]fluoromisonidazole: a pretherapy study of 37 patients. *Int J Radiat Oncol Biol Phys* 1996; 36:417-428.
81. Rajendran JG, Schwartz DL, O'Sullivan J, et al. Tumor hypoxia imaging with [F-18] fluoromisonidazole positron emission tomography in head and neck cancer. *Clin Cancer Res* 2006; 12:5435-5441.
82. Rischin D, Hicks RJ, Fisher R, et al. Prognostic significance of [18F]-misonidazole positron emission tomography-detected tumor hypoxia in patients with advanced head and neck cancer randomly assigned to chemoradiation with or without tirapazamine: a substudy of Trans-Tasman Radiation Oncology Group Study 98.02. *J Clin Oncol* 2006; 24:2098-2104.
83. Urtasun RC, Parliament MB, McEwan AJ, et al. Measurement of hypoxia in human tumours by non-invasive spect imaging of iodoazomycin arabinoside. *Br J Cancer Suppl* 1996; 27:S209-212.
84. Zhang X, Melo T, Ballinger JR, Rauth AM. Studies of 99mTc-BnAO (HL-91): a non-nitroaromatic compound for hypoxic cell detection. *Int J Radiat Oncol Biol Phys* 1998; 42:737-740.

85. Evans SM, Kachur AV, Shiue CY, et al. Noninvasive detection of tumor hypoxia using the 2-nitroimidazole [¹⁸F]EF1. *J Nucl Med* 2000; 41:327-336.
86. Cook GJ, Houston S, Barrington SF, Fogelman I. Technetium-99m-labeled HL91 to identify tumor hypoxia: correlation with fluorine-18-FDG. *J Nucl Med* 1998; 39:99-103.
87. Piert M, Machulla HJ, Picchio M, et al. Hypoxia-specific tumor imaging with 18F-fluoroazomycin arabinoside. *J Nucl Med* 2005; 46:106-113.
88. Tatsumi M, Yutani K, Kusuoka H, Nishimura T. Technetium-99m HL91 uptake as a tumour hypoxia marker: relationship to tumour blood flow. *Eur J Nucl Med* 1999; 26:91-94.
89. Obata A, Yoshimi E, Waki A, et al. Retention mechanism of hypoxia selective nuclear imaging/radiotherapeutic agent cu-diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) in tumor cells. *Ann Nucl Med* 2001; 15:499-504.
90. Fujibayashi Y, Taniuchi H, Yonekura Y, Ohtani H, Konishi J, Yokoyama A. Copper-62-ATSM: a new hypoxia imaging agent with high membrane permeability and low redox potential. *J Nucl Med* 1997; 38:1155-1160.
91. Dearling JL, Lewis JS, Mullen GE, Welch MJ, Blower PJ. Copper bis(thiosemicarbazone) complexes as hypoxia imaging agents: structure-activity relationships. *J Biol Inorg Chem* 2002; 7:249-259.
92. Dearling JL, Lewis JS, Mullen GE, Rae MT, Zweit J, Blower PJ. Design of hypoxia-targeting radiopharmaceuticals: selective uptake of copper-64 complexes in hypoxic cells in vitro. *Eur J Nucl Med* 1998; 25:788-792.
93. Maurer RI, Blower PJ, Dilworth JR, Reynolds CA, Zheng Y, Mullen GE. Studies on the mechanism of hypoxic selectivity in copper bis(thiosemicarbazone) radiopharmaceuticals. *J Med Chem* 2002; 45:1420-1431.
94. Vāvere AL, Lewis JS. Cu-ATSM: A radiopharmaceutical for the PET imaging of hypoxia. *Dalton Transactions* 2007, In press.
95. Lewis JS, McCarthy DW, McCarthy TJ, Fujibayashi Y, Welch MJ. Evaluation of ⁶⁴Cu-ATSM in vitro and in vivo in a hypoxic tumor model. *J Nucl Med* 1999; 40:177-183.
96. Chao KS, Bosch WR, Mutic S, et al. A novel approach to overcome hypoxic tumor resistance: Cu-ATSM-guided intensity-modulated radiation therapy. *Int J Radiat Oncol Biol Phys* 2001; 49:1171-1182.
97. Yuan S, Yu Y, Chao KS, et al. Additional value of PET/CT over PET in assessment of locoregional lymph nodes in thoracic esophageal squamous cell cancer. *J Nucl Med* 2006; 47:1255-1259.
98. Blower PJ, Castle TC, Cowley AR, et al. Structural trends in copper(II) bis(thiosemicarbazone) radiopharmaceuticals. *Dalton Transactions* 2003:4416-4425.
99. Blower PJ, Dilworth JR, Maurer RI, Mullen GD, Reynolds CA, Zheng Y. Towards new transition metal-based hypoxic selective agents for therapy and imaging. *J Inorg Biochem* 2001; 85:15-22.
100. Burgman P, Odonoghue JA, Humm JL, Ling CC. Hypoxia-Induced increase in FDG uptake in MCF7 cells. *J Nucl Med* 2001; 42:170-175.
101. Cowley AR, Dilworth JR, Donnelly PS, Labisbal E, Sousa A. An unusual dimeric structure of a Cu(I) bis(thiosemicarbazone) complex: implications for the mechanism of hypoxic selectivity of the Cu(II) derivatives. *J Am Chem Soc* 2002; 124:5270-5271.
102. Cowley AR, Dilworth JR, Donnelly PS, White JM. Copper complexes of thiosemicarbazone-pyridylhydrazine (THYNIC) hybrid ligands: a new versatile potential bifunctional chelator for copper radiopharmaceuticals. *Inorg Chem* 2006; 45:496-498.
103. Toorongian SA, Mulholland GK, Jewett DM, Bachelor MA, Kilbourn MR. Routine production of 2-deoxy-2-[¹⁸F]fluoro-D-glucose by direct nucleophilic exchange on a quaternary 4-aminopyridinium resin. *Int J Rad Appl Instrum B* 1990; 17:273-279.
104. Fujibayashi Y, Taniuchi H, Yonekura Y, Ohtani H, Konishi J, Yokoyama A. Copper-62-ATSM: A new hypoxia imaging agent with high membrane permeability and low redox potential. *J. Nucl. Med.* 1997; 38:1155-1160.

105. Pu Y, Mintun MA, Dehdashti F, Lewis JS, Welch M. Potential value of parametric imaging of uptake rate of ⁶⁰Cu-ATSM in lung tumor to detect hypoxia. *Mol Imaging - Abstract 2002*; 1:205.
106. Mintun MA, Berger KL, Dehdashti F, Lewis JS, Chau C, Welch MJ. Kinetic analysis of the novel hypoxic imaging agent [⁶⁰Cu]ATSM in human neoplasms. *J Nucl Med* 2000; 41:58.
107. Dehdashti F, Mintun MA, Lewis JS, et al. In vivo assessment of tumor hypoxia in lung cancer with ⁶⁰Cu-ATSM. *Eur J Nucl Med Mol Imaging* 2003; 30:844-850.
108. Dietz DW, Dehdashti F, Grigsby PW, Malyapa RS, Myerson RJ, Picus J, Ritter J, Lewis JS, Welch MJ, Siegel BA. Tumor hypoxia detected by positron emission tomography with ⁶⁰Cu-ATSM as a predictor of response and survival in patients undergoing neoadjuvant chemoradiotherapy for rectal carcinoma: A pilot study. *Dis Colon Rectum* 2008; 51:1641-1648.
109. Oshida M, Uno K, Suzuki M, et al. Predicting the prognoses of breast carcinoma patients with positron emission tomography using 2-deoxy-2-fluoro[¹⁸F]-D-glucose. *Cancer* 1998; 82:2227-2234.
110. Vansteenkiste JF, Stroobants SG, Dupont PJ, et al. Prognostic importance of the standardized uptake value on (18)F-fluoro-2-deoxy-glucose-positron emission tomography scan in non-small-cell lung cancer: An analysis of 125 cases. Leuven Lung Cancer Group. *J Clin Oncol* 1999; 17:3201-3206.
111. Clavo AC, Brown RS, Wahl RL. Fluorodeoxyglucose uptake in human cancer cell lines is increased by hypoxia. *J Nucl Med* 1995; 36:1625-1632.
112. Rajendran JG, Mankoff DA, O'Sullivan F, et al. Hypoxia and glucose metabolism in malignant tumors: evaluation by [¹⁸F]fluoromisonidazole and [¹⁸F]fluorodeoxyglucose positron emission tomography imaging. *Clin Cancer Res* 2004; 10:2245-2252.
113. Dehdashti F, Grigsby PW, Mintun MA, Lewis JS, Siegel BA, Welch MJ. Assessing tumor hypoxia in cervical cancer by positron emission tomography with ⁶⁰Cu-ATSM: relationship to therapeutic response—a preliminary report. *Int J Radiat Oncol Biol Phys* 2003; 55:1233-1238.
114. Lewis JS, Laforest R, Dehdashti F, Grigsby P, Welch MJ, Siegel BA. An imaging comparison of ⁶⁴Cu-ATSM and ⁶⁰Cu-ATSM in cancer of the uterine cervix. *J Nucl Med*, in press.
115. Grigsby PW, Malyapa RS, Higashikubo R, et al. Comparison of Molecular Markers of Hypoxia and Imaging with (60)Cu-ATSM in Cancer of the Uterine Cervix. *Mol Imaging Biol* 2007; 9:278-283.
116. Kumar R, Chauhan A, Jana S, Dadparvar S. Positron emission tomography in gynecological malignancies. *Expert Rev Anticancer Ther* 2006; 6:1033-1044.
117. Sironi S, Buda A, Picchio M, et al. Lymph node metastasis in patients with clinical early-stage cervical cancer: detection with integrated FDG PET/CT. *Radiology* 2006; 238:272-279.
118. Xue F, Lin LL, Dehdashti F, Miller TR, Siegel BA, Grigsby P. F-18 fluorodeoxyglucose uptake in primary cervical cancer as an indicator of prognosis after radiation therapy. *Gynecol Oncol* 2005, In Press.
119. Miller TR, Grigsby PW. Measurement of tumor volume by PET to evaluate prognosis in patients with advanced cervical cancer treated by radiation therapy. *Int J Radiat Oncol Biol Phys* 2002; 53:353-359.
120. Miller TR, Pinkus E, Dehdashti F, Grigsby PW. Improved prognostic value of ¹⁸F-FDG PET using a simple visual analysis of tumor characteristics in patients with cervical cancer. *J Nucl Med* 2003; 44:192-197.
121. Kidd EA, Siegel BA, Dehdashti F, Grigsby PW. The standardized uptake value for F-18 fluorodeoxyglucose is a sensitive predictive biomarker for cervical cancer treatment response and survival. *Cancer* 2007; 110:1738-1744.
122. Grigsby PW, Siegel BA, Dehdashti F. Lymph node staging by positron emission tomography in patients with carcinoma of the cervix. *J Clin Oncol* 2001; 19:3745-3749.
123. Yen TC, See LC, Lai CH, et al. Standardized uptake value in para-aortic lymph nodes is a significant prognostic factor in patients with primary advanced squamous cervical cancer. *Eur J Nucl Med Mol Imaging* 2008; 35:493-501.
124. Weber WA, Wieder H. Monitoring chemotherapy and radiotherapy of solid tumors. *Eur J Nucl Med Mol Imaging* 2006; 33 Suppl 1:27-37.

125. Grigsby PW, Siegel BA, Dehdashti F, Rader J, Zoberi I. Posttherapy [18F] fluorodeoxyglucose positron emission tomography in carcinoma of the cervix: response and outcome. *J Clin Oncol* 2004; 22:2167-2171.
126. Schwartz JK, Siegel BA, Dehdashti F, Grigsby PW. Tumor response and survival predicted by posttherapy positron emission tomography in cervical carcinoma. *JAMA*, In press.
127. Kalbfleisch J, Prentice R. *The Statistical Analysis of Failure Time Data*. In. 2nd ed. New York: Wiley-Interscience: Hoboken, 2002; 31-51.
128. Heagerty PJ, Lumley T, Pepe MS. Time-dependent ROC curves for censored survival data and a diagnostic marker. *Biometrics* 2000; 56:337-344.
129. Heagerty PJ, Zheng Y. Survival model predictive accuracy and ROC curves. *Biometrics* 2005; 61:92-105.
130. Hintze J. NCSS, PASS, and GESS. NCSS Kaysville, Utah (www.ncss.com). 2006.

Appendix I

Informed Consent Form Template

ACRIN 6682

PHASE II TRIAL OF ^{64}Cu -ATSM PET/CT IN CERVICAL CANCER

[Note: ACRIN does not monitor compliance with the Health Insurance Portability and Accountability Act (HIPAA); that is the responsibility of the local institutions and their IRBs].

March 1, 2013 ICF version: The informed consent template has been revised to include language for potential study participants enrolled on the GOG OUTBACK Trial for participation on ACRIN 6682. If your institution is not participating in the OUTBACK trial, please disregard any revisions pertaining to OUTBACK Trial.

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part. Please take your time to make your decision about taking part. You may discuss your decision with your friends and family. You can also discuss it with your health care team. If you have any questions, you can ask your study doctor for more explanation.

You are being asked to take part in this study because 1) you have cervical cancer and you are scheduled to receive radiation and chemotherapy (also referred as chemoradiotherapy), or 2) you have consented to participate in the OUTBACK Trial.

This clinical trial involves imaging with combined positron emission tomography (PET) and computed tomography (CT, also called a CAT scan) scanners. One PET/CT scan in the study will use an investigational radioactive drug called copper-64 diacetyl-bis[N⁴-methylthiosemicarbazone], also known as ^{64}Cu -ATSM.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to determine if the investigational radioactive drug, ^{64}Cu -ATSM, and PET/CT scan results can help doctors find out if there are regions of poor oxygenation (hypoxia) in your tumor. Hypoxic regions of a tumor do not usually respond as well to treatment as other regions in the tumor that have a good oxygen supply. Hypoxic tumors can also be more aggressive than tumors that get lots of oxygen.

Finding areas of hypoxia in cervical cancer tumors is important for treatment of women with cervical cancer. Cancer cells that do not get a lot of oxygen tend to be more aggressive (they grow and spread faster) than cancer cells with normal or high levels of oxygen. Scientists believe cancers that get a little oxygen may be treated differently than cancers that get normal levels or a lot of oxygen. In the future, this hypoxia information could help doctors plan the best possible treatment for cervical cancer.

The goal of this clinical trial is to see if ^{64}Cu -ATSM-PET/CT scan can help doctors locate cancer tumor cells with low oxygen in your cervical cancer in order to predict and determine the behavior of cervical cancer so in the future the doctors can identify appropriate course of treatment. But, because this is a research study, you and your doctor will not know the results of the ^{64}Cu -ATSM-PET/CT scan and your treatment will not be affected by study results.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

About 100 people will take part in this study.

WHAT WILL HAPPEN IF I TAKE PART IN THIS RESEARCH STUDY?

Before you begin the study ...

To participate in this study, you will be asked to read and sign this consent form before you are enrolled to participate in this trial and any study procedures are performed. Your study doctor will determine whether or not you are eligible to participate by reviewing your medical history, medical records, and imaging scans. Your study doctor and/or research staff will obtain your medical history and medical records. If your prior PET/CT scans can not be used to determine your eligibility to participate in this trial, you will be asked to have one completed. If your scan indicates you are not eligible, you will not be able to participate.

If you are determined to be eligible and agreed to participate in this research study, you will be enrolled into the study. As part of this study, a portion of the cervical biopsy taken to determine that you have cervical cancer will be sent to the Washington University pathology laboratory for analysis. You will not need to have any additional biopsies to participate in the study.

During the study...

Standard medical procedures that are part of regular cancer care and would probably be done even if you do not join the study:

- Medical history, including allergies
- Current medication reporting
- Physical examination
- Pregnancy blood or urine test (if applicable, see details below)
- Pre-therapy PET/CT scan with fluorodeoxyglucose (FDG), an agent used for PET imaging
- Other imaging examination(s), if needed
- Chemotherapy (cancer fighting drugs) through a vein in your arm
- Radiation therapy

If you are of childbearing age and you decide to participate in the trial, you must have a negative urine or blood pregnancy test result within 7 days before Visit 1 (see Visit details in the Study Chart below). If you had a negative pregnancy test more than 7 days before the ⁶⁴Cu-ATSM-PET/CT scan at Visit 1, you will be asked to have another urine pregnancy test to make sure it is still negative.

If you have had an FDG-PET/CT scan within 4 weeks of this study on an ACRIN-qualified PET/CT scanner, you may not need to have another scan as the 'pre-therapy' PET/CT scan. If you have not had an FDG-PET/CT scan, or if the scan was completed more than 4 weeks before the study and/or it was not performed on a qualified scanner, you will be asked to have another FDG-PE/CT scan completed, which will be done at no charge to you or your insurance company. You will also have to have another urine pregnancy test to make sure it is negative if you are capable of becoming pregnant and are sexually active.

Medical procedures that are part of this study (that is, they are not standard of care):

- Blood sample collection and storage for future research before and after therapy (optional);
- Pre-therapy PET/CT scan with FDG, if not done as a standard clinical procedure (see above)
- PET/CT scan with ⁶⁴Cu-ATSM, investigational radioactive drug;
- Post-therapy PET/CT scan with FDG.

Blood sample collection – this is an optional procedure:

Your study doctors would like to collect and store your blood that may be later used to look for changes in blood associated with cervical cancer in future research. This is an optional procedure in this study, so you may choose to be in the study but not to have the blood sample collection. If you agree, the blood specimens about one (1) tablespoon will be collected, stored, and used in future research to learn more about cancer and other diseases. All your personal information will be removed from the sample before it is shared and stored.

The blood sample will be given only to the approved researchers and will not be sold. The research done on the blood will also be reviewed and approved by the researcher's institutional review board (IRB). The research done with your blood will probably not help you but it may help women who have cervical cancer in the future. Reports about the research done with your blood will not be given to you or your treating doctor. These reports will not be put into your medical records and it will not have an effect on your care.

I agree to participate in the blood sample collection and storage for future research portion of this study.

YES NO _____ **Participant's Initials**

Description of the PET/CT Scan and Other Procedures

The FDG-PET/CT scan will be repeated approximately 3 months after you finish treatment to confirm your cancer has responded to chemotherapy and radiation. If you were randomized to Arm B of the OUTBACK Trial, you will have an FDG-PET/CT scan approximately 3 months after completion of your initial chemotherapy radiation; this will be performed 2 weeks after completing your adjuvant chemotherapy. This FDG-PET/CT scan must be completed within 6 weeks after your adjuvant chemotherapy.

PET stands for "positron emission tomography," and CT stands for "computed tomography." PET/CT is a novel imaging technology that combines two imaging modalities (PET and CT images) into one image. This combination allows doctors to see the location and function of cells within the body.

About PET Scans

PET is a nuclear medicine imaging technique that uses radioactive drugs to produce a 3-D image of functional processes in the body.

About CT Scans

A CT scanner is a special kind of X-ray machine. Instead of sending a single X-ray through your body as with ordinary X-rays, CT sends several beams out simultaneously through the body from different angles. The computer processes the results, displaying them as a 2-D picture shown on a monitor.

About PET/CT Scan

The PET and CT scanners are similar in appearance. Many PET scanners also include a CT scanner (PET/CT scanners). The PET/CT scanner is a large machine with a hole in the middle. It looks like a donut with a table in the middle. The size of the opening is 27 to 30 inches wide. How much space you feel you have around you will depend on your body size and the scanner size. If you feel any anxiety over being in enclosed spaces, let your study doctor know. A mild sedative may be used to help you feel more comfortable during the exam.

PET/CT scanners allow images of both anatomy (CT) and function (PET) to be taken during the same examination. The PET/CT scan has the benefit of combining the PET scan information about cell function with the CT scan information about the size and shape of abnormal cells. Alone, each test has its limitations but when the results of the scans are fused together they provide the most complete information on cancer cell function and location.

Preparation for FDG-PET/CT Scan: You will be asked not to eat anything for 4 – 6 hours before you appointment and to drink only water. Check with your study doctor to make sure it is o.k. to take medications, especially if you are diabetic.

During the Exam: On the day of your scan, a small intravenous (IV) catheter will be inserted into a vein of your hand or arm. This IV catheter will be used to draw a small blood sample (less than 1 teaspoon) to check the amount of sugar (glucose) present in your blood stream. <<Change to fingerstick and describe, if that is the site

[procedure>>](#). If your blood sugar is o.k., you will receive a small injection of the radioactive drug FDG (fluorodeoxyglucose – a radioactive form of sugar). The FDG will need to circulate around in your body for 50-70 minutes before your scan. During this time you will be asked to rest comfortably. FDG travels to places in the body where glucose is used for energy. It shows up in cancer because cancer cells use more glucose than normal tissues. Before the start of your scan you will be asked to use the restroom. <<[Describe Foley catheterization, if that is site procedure>>](#). You will be asked to lie on the imaging table with your arms resting comfortably above your head or secured to your sides. You will move slowly through the scanner so that your body can be scanned from the head through the thighs. You will be asked to remain still for the scan which will take between 20 – 45 minutes to complete. The total amount of time you will need for the FDG PET/CT scan is approximately 2-3 hours.

Preparation for a ^{64}Cu -ATSM-PET/CT Scan: There are no eating or drinking requirements for ^{64}Cu -ATSM-PET/CT scanning. You may also take your medications according to your normal schedule. You will be asked to arrive to the PET center approximately 60 minutes before the start of your imaging scan.

During the Exam Day: On the day of your ^{64}Cu -ATSM PET/CT scan, an IV catheter will be placed (usually in a vein in your arm) to allow for injection of ^{64}Cu -ATSM. Then you will be given an injection of a small amount of a radioactive drug through the IV catheter. The ^{64}Cu -ATSM will travel through your body and accumulate in the cancer cells with low oxygen. About 30 minutes later, you will lie down on the PET/CT scanner table and a 30-minute scan will be performed over your pelvis at the site of your tumor. You will be able to rest your arms comfortably at your side or across your chest. The scan will take approximately 35 minutes to complete. At the completion of the ^{64}Cu -ATSM study, you will be removed from the scanner.

Time Required: The entire ^{64}Cu -ATSM-PET/CT scan procedure is expected to take about 2 hours.

Treatment

After you have had your ^{64}Cu -ATSM PET/CT scan, you will receive chemotherapy and radiation treatment within 4 weeks. The chemotherapy and radiation that you will receive is not a part of this study. It will be the standard of care treatment for your type of cancer. Your chemotherapy and radiation treatment will take about 1.5 months. Then three (3) months after your chemotherapy and radiation treatment, you will have an FDG-PET/CT scan.

You will continue to see your treating radiation and/or gynecologic oncologist once the chemotherapy and radiation treatments have ended. You will see your treating doctor at regular intervals according to her/his recommendations and usual practice—every 3 months for the first 2 years and every 6 months for the next 3 years—for total of 5 years. Information gathered by your treating doctor as part of your normal follow-up visits will be given to your study doctor(s) for approximately 3 years after your participation in the study so they can find out more about your health. Your follow-up care will be decided between you and your treating doctor.

Study Chart

What you do if you are in the study:

<p>At Baseline Visit – Eligibility and Registration</p>	<ul style="list-style-type: none"> • Review and sign an informed consent form; • Provide medical history, including allergy history; • Provide list of medication; • Have a physical examination; • Have a blood or urine pregnancy test, if applicable; • Have a blood sample collected (optional); • Have an FDG-PET/CT scan, if necessary. You will have a glucose testing done prior to the scan.
<p>Visit 1: Within 14 Days From Baseline Visit—Day of ⁶⁴Cu-ATSM-PET/CT</p>	<p>You may be asked to have another pregnancy test if the pregnancy test was not completed within 7 days before Visit 1. You must have a negative pregnancy test to participate in the study.</p> <ul style="list-style-type: none"> • Provide list of medication; • Have an injection of ⁶⁴Cu-ATSM, radioactive drug; • Have vital signs taken before the injection, at 15 minutes and at 75 minutes after having the injection. • Have the ⁶⁴Cu-ATSM PET/CT scan. • The day after your scan, a researcher will call you to see how you are feeling.
<p>Visit 2: Within the 4 Weeks After the ⁶⁴Cu-ATSM-PET/CT</p>	<ul style="list-style-type: none"> • Begin therapy (chemotherapy and radiation also called chemoradiotherapy).
<p>Visit 3: 4 Weeks After Chemoradiotherapy Treatment</p>	<ul style="list-style-type: none"> • Provide an update to medical history; • Have a physical examination.
<p>Visit 4: 3 Months (±4 Weeks) After Chemoradiotherapy Treatment</p>	<ul style="list-style-type: none"> • Provide an update to medical history; • Have a physical examination; • Have a blood sample collected (optional); • Have glucose testing; • Have an FDG-PET/CT scan.
<p>Visit 4B – For Outback Trial Arm B participant ONLY: 3 Months After Completing Chemoradiotherapy AND AT Least 2 Weeks, But Within 6 Weeks After Completing Adjuvant Chemotherapy</p>	<ul style="list-style-type: none"> • Provide an update to medical history; • Have a physical examination; • Have a blood sample collected (optional); • Have glucose testing; • Have an FDG-PET/CT scan.
<p>Routine Follow-up Visits: Every 3 Months After Visit 4 for the First 2 Years</p>	<ul style="list-style-type: none"> • Provide an update to medical history; • Have a physical examination; • Have a CT scan of your abdomen and pelvis at the discretion of your study or treating doctor, if necessary.
<p>Routine Follow-up Visits: Then Every 6 Months During the 3rd Year</p>	<ul style="list-style-type: none"> • Provide an update to medical history; • Have a physical examination; • Have a CT scan of your abdomen and pelvis at the discretion of your study or treating doctor, if necessary.

HOW LONG WILL I BE IN THE STUDY?

You will be actively participating in the study for approximately for about one (1) year. During this study period, you will have your chemotherapy and radiation treatment which will take about 1.5 months. Per standard of care for your cancer, you will have routine follow-up visits with your treating doctor for total of 5 years as part of your standard of care. Information gathered by your treating doctor as part of your normal follow-up visits will be given to your study doctor(s) for approximately 3 years so they can find out more about your health.

You can stop participating in this study at any time. However, if you decide to stop participating, we encourage you to talk to the your study doctor(s) and your treating doctor(s) first.

WHAT SIDE EFFECTS OR RISKS CAN I EXPECT FROM BEING IN THE STUDY?

In addition to the side effects of your chemoradiotherapy treatment, you may have side effects while on the study. Everyone taking part in the study will be watched carefully for any side effects, but you should be sure to inform your doctor of any side effects that you may have. Doctors don't know all the side effects that may happen. Side effects may be mild or very serious. Your health care team may give you medicines to help lessen side effects. Many side effects go away soon after the ⁶⁴Cu-ATSM-PET/CT scan and FDG-PET/CT scan.

Risks Associated with Blood Sample Collection (optional):

Likely:

Minor discomfort;
Pain at the site of needle.

Less likely:

- Bruising;
- Bleeding;

Rarely:

- Infection at the site of injection.

Risks Associated with Intravenous (IV) Catheter Placement:

Likely:

- Minor discomfort;
- Pain at the site of catheter placement.

Less likely:

- Bruising;
- Bleeding;
- Phlebitis - swelling, redness, heat, and pain in the vein at the injection site;
- Infection at the site of injection.

Risks Associated with ⁶⁴Cu-ATSM (investigational radioactive drug):

- **Rare:** Possible allergic reaction or allergic-like reaction. While none have been encountered to date, such a reaction could be serious or even cause death.

Risks Associated with FDG:

- **Rare:** Possible allergic reaction or allergic-like reaction. While none have been encountered to date, such a reaction could be serious or even cause death.

Risks Associated with PET/CT Scans:

- Discomfort from lying still on the enclosed scanning table;
- Claustrophobia;
- *Rare:* Malfunction of worn or implanted electronic medical devices.

If you have wear or have electronic medical devices implanted such as a pacemaker or a drug pump, please make sure you tell your study doctors and research staff. It was recently reported by the FDA that the CT scan may cause the malfunction of electronic medical devices.

Risks Associated with ⁶⁴Cu-ATSM-PET/CT Radiation and FDG-PET/CT Radiation:

This research study involves exposure to radiation from the injection of an investigational radioactive drug, ⁶⁴Cu-ATSM. In addition, you will be exposed to x-rays for the CT scan. The radiation exposure you will receive from the ⁶⁴Cu-ATSM-PET/CT scan procedure is equal to a uniform whole-body exposure of up to approximately 48 mSv (a measure of radiation exposure), which is about 96% of the annual allowable exposure for occupational workers. The radiation exposure you will receive from each FDG-PET/CT scan procedure is equal to a uniform whole-body exposure of approximately 17 mSv (34 mSv from 2 FDG-PET/CT scans), which is about 34% of the annual allowable exposure for occupational workers (68% for two FDG-PET/CT scans). The risk from this level of radiation exposure is too small to be measured and is small when compared with other everyday risks. In addition, the radiation dose from these imaging studies is a small fraction of the radiation dose that you will be receiving for treatment of your cervical cancer. If you would like more information about radiation exposure, please speak with your study doctor.

Reproductive Risks:

Because PET/CT scans can be harmful to an unborn baby, you should not become pregnant while on this study. (The risks of fetal injury, however, are far greater from the chemotherapy and radiation that will be used to treat your cancer.) There is not enough medical information to know what the risks might be to an unborn child in a woman who takes part in this study. It is important that you understand that you need to use birth control while on this study. Ask your study doctor about what kind of birth control methods to use and how long to use them. If you are a woman who can become pregnant, you must agree to a pregnancy test (blood or urine test) before the ⁶⁴Cu-ATSM-PET/CT scan and FDG-PET/CT scan.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?

You may not benefit directly from taking part in this study. The information from this study will help your study doctors learn whether the investigational radioactive drug, ⁶⁴Cu-ATSM, and the ⁶⁴Cu-ATSM PET/CT scan can help identify cervical cancer that has low oxygenation levels, and that is therefore likely to be more aggressive disease. In the future, this knowledge may help doctors decide on the best treatment for patients with cervical cancer with low oxygenation levels.

WHAT OTHER CHOICES DO I HAVE IF I DO NOT TAKE PART IN THIS STUDY?

You may choose not to participate in this study. If you choose not to participate, there will be no penalty or loss of benefits to which you are otherwise entitled. Your doctor can tell you the different options available for treatments of your cervical cancer.

The study doctor may stop you from taking part in this study at any time if he/she believes it is in your best interest; if you do not follow the study rules; or if the study is stopped.

WILL MY MEDICAL INFORMATION BE KEPT PRIVATE?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Records of your participation on this study, your progress, specimens, and images submitted while you are on the study will be kept in a confidential form at this institution and in a computer file at the headquarters of the American College of Radiology Imaging Network (ACRIN) in Philadelphia, PA. Your personal information may be given out if required by law.

Authorized representatives of ACRIN, Center for Statistical Sciences at Brown University, Washington University School of Medicine, the Food and Drug Administration (FDA), the Institutional Review Board (IRB) of <<Institution>>, and other groups or organizations that have a role in this study will have access to and may inspect and/or copy both your medical and research records due to your participation in this study. This access is necessary to ensure the accuracy of the findings and for your safety and welfare. If any publication or presentations result from this study, you will not be identified by name. Results will be reported in a summarized manner such that you cannot be identified.

Your research records, specimens, and images will be kept permanently on file at ACRIN and may be used for future research. All personal identifiers are removed and replaced with a unique identifying number. The research that may be done with the information will not specifically help you. But, it might help people who have cancer and other diseases in the future.

WHAT ARE THE COSTS OF TAKING PART IN THIS STUDY?

Taking part in this study will not lead to added costs to you or your insurance company.

You will not be charged for the following that are part of this research study:

- Blood sample collection
- FDG-PET/CT before start of therapy, if not already done as standard clinical procedure, or if repeated
- ⁶⁴Cu-ATSM PET/CT scan
- FDG-PET/CT 3-month after completion of therapy

You or your insurance company will be charged for any other portion of your care that is considered standard of care for the treatment of your cervical cancer. You may be responsible for any co-payments and deductibles that are standard for your insurance coverage.

You will not be paid for taking part in this study.

WHAT HAPPENS IF I AM INJURED BECAUSE I TOOK PART IN THIS STUDY?

It is important that you tell your study doctor, <<insert name>>, if you feel that you have been injured because of taking part in this study. You can tell the study doctor in person or call him/her at <<insert telephone number>>.

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment related to injury during the study.

WHAT ARE MY RIGHTS IF I TAKE PART IN THIS STUDY?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you, and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

WHO CAN ANSWER MY QUESTIONS ABOUT THE STUDY?

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor, <<insert name>>, at <<insert telephone number>>.

For questions about your rights while taking part in this study call the <<insert name IRB contact person>> at <<insert name of the IRB>> Institutional Review Board (a group of people who review the research to protect your rights and safety) at <<insert telephone number>>.

WHERE CAN I GET MORE INFORMATION?

You may call the NCI's Cancer Information Service at **1-800-4-CANCER (1-800-422-6237)** or **TTY: 1-800-332-8615**; or visit the NCI's Web sites for clinical trials information <http://cancertrials.nci.nih.gov>, or for cancer information visit <http://cancernet.nci.nih.gov>.

ACRIN's Web site is www.acrin.org. For more information on PET/CT scans you can go to ACRIN's Web site at: www.acrin.org/PATIENTS/ABOUTXRAYSCANSDSCANS/tabid/135/Default.aspx. You or your doctor can print a description of PET/CT scans from this Web site.

ACKNOWLEDGEMENT

When you sign this document, you are agreeing to take part in this study. This means you have read all the above information, asked questions regarding your participation, and received answers that you understand to all your questions. You have also had the opportunity to take this consent form home for review or discussion if you want to. A copy of the signed consent will be given to you.

Signature of Participant (or Legal Representative)

Date

<Insert other signature and date lines as appropriate per local IRB policies and procedures>

Appendix II

ACRIN 6682 Eligibility Checklist

The ACRIN 6682 Eligibility Checklist is available on the ACRIN web site through the ACRIN 6682 protocol-specific web page: www.acrin.org/6682_protocol.aspx. For more detailed information, contact the ACRIN 6682 Data Manager at ACRIN. Contact information can also be found on the above-mentioned web page.

Appendix III

ACRIN 6682 Participating Institutions

At a minimum of three (3) participating institutions will participate in this trial. Additionally, ACRIN will collaborate with Gynecologic Oncology Group (GOG) and the leadership for the Australian New Zealand Gynaecological Oncology Group (ANZGOG) on the (ANZGOG)-0902 OUTBACK trial (<http://clinicaltrials.gov/ct2/show/NCT01414608>). Eligible participants randomized on this trial to either the Control Group (Arm A) or Intervention group (Arm B) is also eligible for recruitment to ACRIN 6682.

Site Principal Investigators will be identified upon review and approval of completed ACRIN Protocol Specific Application (PSA), which is available on the ACRIN web site through the ACRIN 6682 protocol-specific web page: www.acrin.org/6682_protocol.aspx.

Appendix IV

ACRIN 6682 Protocol-Specific Application Information

Application Process

All participating institutions must be ACRIN-approved institutions prior to study participation and accrual. The approval process for ACRIN 6682 includes submitting an ACRIN Protocol Specific Application (PSA) and having the PET/CT scanner credentialed by ACRIN for study imaging. Detailed information is available on the ACRIN web site (www.acrin.org) under the list of current protocols. ACRIN 6682 is available at www.acrin.org/6682_protocol.aspx, and the complete Protocol-Specific Application can be found via this protocol-specific web page.

Appendix V

ACRIN Qualification Procedures for PET/CT Imaging

Details of the ACRIN Qualification Procedures for PET/CT Imaging are available on the ACRIN web site at <http://www.acrin.org/CORELABS/PETCORELABORATORY/PETQUALIFICATION.aspx>. For more detailed information, contact Adam Opanowski (aopanowski@acr.org/215-940-8890) at PET Core Lab (www.acrin.org).

Appendix VI

PET Imaging Acquisition Parameters and Image Data Analysis

Additional information for PET/CT Imaging Acquisition Parameters and Analyses are available on the ACRIN web site at (www.acrin.org/6682_protocol.aspx). For more detailed information, contact ACRIN Image Management Center (IMC) at PetCoreLab@acr.org.

Appendix VII

Detailed Criteria/Specifications for Performance of PET/CT Scans

FDG-PET/CT Scans

FDG-PET/CT scans will be performed according to ACRIN standard operating procedures (www.acrin.org). The baseline images will be evaluated at the ACRIN Core Laboratory for measurement of tumor volume.

Image Processing:

- In 3D analysis volumetric program, a large ROI will be drawn around the tumor, avoiding the urinary bladder, for determination of SUV_{max} . SUV_{max} will be recorded.
- Then 40% SUV_{max} will be used to threshold the image for measurement of tumor volume. Tumor volume will be recorded.

^{64}Cu -ATSM-PET/CT Scans

Acquisition and Analysis of ^{64}Cu -ATSM-PET/CT Scans

1. Participant Preparation

There are no preparations required for the participant for prior to this study.

- Upon arrival at the PET/CT facility, the participant's weight will be measured and recorded.
- Prior to positioning the participant on the PET/CT scanner the participant should be asked to urinate.
- The participant should be placed in a comfortable position, either supine or semi-recumbent.
- A large-bore intravenous line (typically, 20- or 22-gauge angiocath) should be placed in an arm or hand vein.

2. Injection of ^{64}Cu -ATSM

- The dose of ^{64}Cu -ATSM will be 18-25 millicuries (mCi).
- ^{64}Cu -ATSM will be synthesized and prepared in accordance with Investigational Drug Brochure (IDB) guidelines.
- The exact time of calibration of the dose should be recorded; the exact time of injection should be noted and recorded to permit correction of the administered dose for radioactive decay. In addition, any of the dose remaining in the tubing or syringe, or that was spilled during injection, should be recorded. The injection should be performed through an intravenous catheter.

3. ^{64}Cu -ATSM-PET/CT Imaging

- Scanning must begin 30 to 40 minutes after ^{64}Cu -ATSM injection. The exact time of scanning should be noted and recorded.
- Participants will generally be positioned in the PET/CT scanner with their arms on the chest or upper abdomen.
- A low-dose CT scan will be acquired for attenuation correction and anatomical localization of findings in the PET scan, as well as for positioning of the participant for emission scan in order to ensure the primary tumor is included in the field of view.

- Typical acquisition parameters for the low-dose CT scan that allows for attenuation correction are: kVp = 120; effective mAs = 30–80 (patient dependent); gantry rotation time \leq 0.5 sec; maximum reconstructed width = 3–5 mm without overlap; standard reconstruction algorithm, reconstruction diameter = outer pelvis to outer pelvis; and without oral or intravenous contrast agent administration.
- The axial field of view of the CT scan for attenuation correction will include the lower pelvis. Arm positioning will be the same as for the PET scan, over the chest or upper abdomen.
- The CT scan will be performed during “normal breathing.”
- After the CT scan, a PET scan covering the same axial field of view will be performed in the region of the primary tumor, which is determined according to the CT scan. This scan will start typically below the symphysis pubis. Typical parameters are 1 bed position and an acquisition of 30-minutes static image.

4. Image Reconstruction

- The PET data will be corrected for dead time, scatter, random, and attenuation using standard algorithms provided by the scanner manufacturer.
- Image reconstruction will be performed as specified in the ACRIN certification of the PET/CT scanner.

5. Central Image Analysis

- Decay-corrected images should be provided for central analysis.
- Circular regions of interests (ROI) with a diameter of 1.0–1.5 cm will be centered at the site of maximum ^{64}Cu -ATSM-uptake within this lesion. ROIs of the same size will be placed in the slices above and below. These ROIs will be positioned to be adjacent to ROI, centered at the site of maximum tumor ^{64}Cu -ATSM-uptake (counts). Similar ROIs in the gluteal muscle regions will be drawn bilaterally.
- The maximum pixel counts within the tumor volume encompassed by these three ROIs will be determined and recorded. The average counts within the muscle volume encompassed by the ROIs will be determined and recorded. The T/M ratio will be determined by dividing the maximum counts of the tumor to the average counts of the muscles.

6. Local Image Interpretation

- Confirm that the primary tumor was in the field of view.
- Check image quality according to the checklist below.

Checklist for PET/CT Image Quality Control

7. T/M Calculations

7.1 Time of Injection and Scan Start Time

7.1.1 Data Correctly Recorded and Entered

Check whether the time of injection and the scan start time have been correctly recorded. If the PET scanner software performs decay correction for the time interval between injection and imaging, check whether the time of injection and the start time of the scanner have been correctly entered.

7.1.2 Data correctly recorded and entered: Proceed to 7.2

7.1.3 Injection Time Missing

7.1.3.1 Time between injection and start of scan known: Record time between injection and start of scan and proceed to 7.2

Appendix VIII

CLINICAL STAGING - CARCINOMA OF THE CERVIX UTERI FIGO CLASSIFICATION

PRE-INVASIVE CARCINOMA

STAGE 0: Carcinoma in situ, intraepithelial carcinoma.

(Cases of Stage 0 should not be included in any therapeutic statistics)

INVASIVE CARCINOMA

STAGE I: Carcinoma strictly confined to the cervix.

STAGE IA: Invasive cancer identified only microscopically. (All gross lesions, even with superficial staging, are stage IB cancers.)

Invasion is limited to measured stromal invasion with maximum depth of 5.0 mm and no wider than 7.0 mm.¹

STAGE IA1: Measured invasion of stroma no greater than 3.0 mm in depth and no wider than 7.0 mm.

STAGE IA2: Measured invasion of stroma greater than 3.0 mm and no greater than 5.0 mm and no wider than 7.0 mm.

STAGE IB: Clinical lesions confined to the cervix or pre-clinical lesions greater than stage IA.

STAGE IB1: Clinical lesions no greater than 4.0 cm in size.

STAGE IB2: Clinical lesions greater than 4.0 cm in size.

STAGE II: The carcinoma extends beyond the cervix but has not extended on to the pelvic wall. The carcinoma involves the vagina, but not the lower third.

STAGE IIA: No obvious parametrial involvement.

STAGE IIB: Obvious parametrial involvement.

STAGE III: The carcinoma has extended on to the pelvic wall. On rectal examination, there is no cancer-free space between the tumor and the pelvic wall. The tumor involves the lower third of the vagina. All cases with hydro-nephrosis or non-functioning kidney.

STAGE IIIA: No extension on to the pelvic wall.

STAGE IIIB: Extension on to the pelvic wall and/or hydro-nephrosis or non-functioning kidney.

STAGE IV: The carcinoma has extended beyond the true pelvis or has clinically involved the mucosa of the bladder or rectum. A bullous edema as such does not permit a case to be allotted to stage IV.

STAGE IVA: Spread of the growth to adjacent organs.

STAGE IVB: Spread to distant organs.