VIRGINIA COMMONWEALTH UNIVERSITY
AMERICAN COLLEGE OF RADIOLOGY IMAGING NETWORK

ACRIN 6688

PHASE II STUDY OF FLUORINE-18 3'-DEOXY-3'-FLUOROTHYMIDINE (F-18-FLT) IN INVASIVE BREAST CANCER

Agent Name: Fluorine-18 3'-deoxy-3'-fluorothymidine (18F-FLT) **Agent NSC Number: 743144**

IND Number: 71,260

VCU Study Chair- Funding PI
Paul R. Jolles, MD
Department of Radiology
PO Box 980001
Gateway Building, 2nd Floor
Virginia Commonwealth University
Richmond, VA 23298-0001
Phone: 804-828-7975
Fax: 804-828-4181
Email: prjolles@vcu.edu

VCU Study Co-Chair
Harry D. Bear, MD, PhD
Department of Surgery
PO Box 980011
West Hospital, 7th Floor
Virginia Commonwealth University
Richmond, VA 23298-0011
Phone : 804-828-9325
Fax : 804-828-4808
Email: hdbear@vcu.edu

VCU Study Co-Chair
Michael O. Idowu, MD, MPH
Department of Pathology
PO Box 980662
Gateway Building, 6th Floor
Virginia Commonwealth University
Richmond, VA 23298-0662
Phone: 804-828-9739
Fax: 804-828-9749
Email: midowu@mcvh-vcu.edu

ACRIN Study Co-Chair
Primary NCI Point of Contact PI
Lale Kostakoglu, MD, MPH,
Mount Sinai School of Medicine
One Gustave L. Levy Place,
Box: 1141
New York, NY 10029
Phone: 212-241-6319
Fax: 212-831-2851
Email: lale.kostakoglu@msnyuhealth.org

ACRIN Study Co-Chair
David Mankoff, MD, PhD
Professor of Radiology (Nuclear Medicine)
Seattle Cancer Care Alliance
825 Eastlake Avenue East, G4-836
Seattle, WA 98109
Phone: 206-598-4240
Fax: 206-288-6556
Email: dam@u.washington.edu

ACRIN Study Statistician
Fenghai Duan, Ph.D.
Center For Statistical Sciences
Brown University, Box G-S121-7
121 South Main Street
Providence, RI 02912
Phone: 401-863-2175
Fax: 401-863-9182
Email: fduan@stat.brown.edu

PARTIAL PROTOCOL: PLEASE CONTACT ACRIN PROTOCOL DEVELOPMENT AND REGULATORY COMPLIANCE FOR A COMPLETE VERSION OF THE PROTOCOL

ACRIN CDUS Reporting Point of Contact
Lucy Garfield Hanna, PhD
Center For Statistical Sciences
Brown University, Box G-S121-7
121 South Main Street
Providence, RI 02912
Phone:401-863-9184
Fax: 401-863-9182
lhanna@stat.brown.edu

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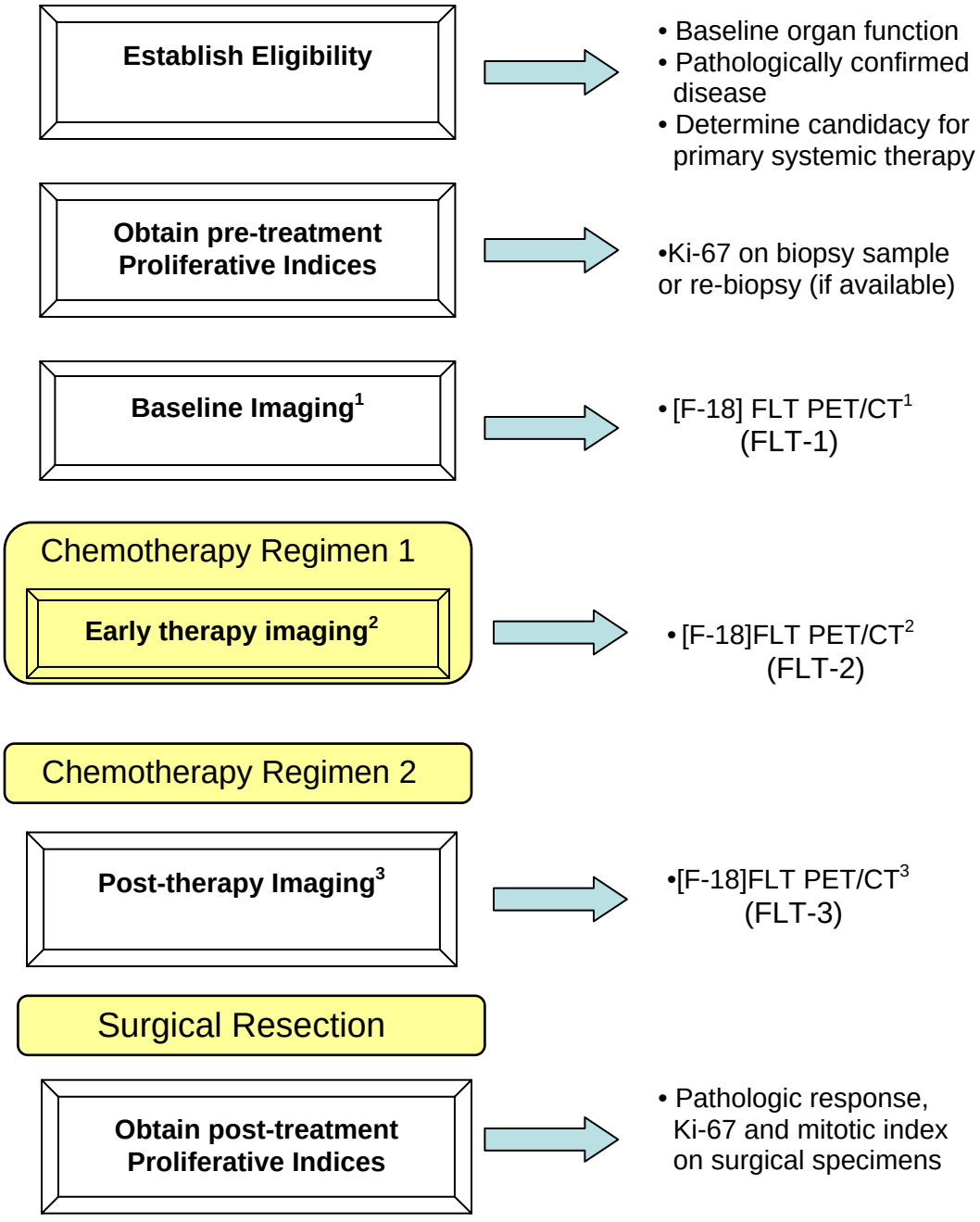
PHASE II STUDY OF FLUORINE-18 3'-DEOXY-3'-FLUOROTHYMIDINE (18F-FLT) IN INVASIVE BREAST CANCER

SCHEMA



[1] Baseline 3'-deoxy-3'-^{18}F fluorothymidine, also known as [F-18] FLT PET/CT imaging (FLT-1) must be done within 4 weeks prior to therapy initiation of therapy protocol.

[2] Early therapy [F-18] FLT PET/CT imaging (FLT-2) will be done 5-10 days after the initiation of the first cycle of the chemotherapy.

[3] Post therapy imaging [F-18] FLT PET/CT (FLT-3) will be performed after the completion of chemotherapy and within 3 weeks prior to surgery.

There is no specific neoadjuvant chemotherapy regimen required for this protocol. Several neoadjuvant therapy protocols are currently used at participating institutions and subjects for the study may be recruited from the participating institutions with their prospective neoadjuvant chemotherapy regimens, which may also include targeted agents, such as trastuzumab. However, patients on primary systemic hormonal neoadjuvant therapies are not eligible.

HYPOTHESIS

[F-18] FLT uptake parameters will predict pathologic complete response to neoadjuvant therapy of the primary tumor in patients with locally advanced breast cancer.

ELIGIBILITY *(see Section 6.0 for details)*

Breast cancer patients with locally advanced disease who are candidates for primary systemic (neoadjuvant) chemotherapy and for whom subsequent definitive surgery is planned, according to current clinical guidelines are eligible for this study. This includes all patients with locally advanced breast cancer, not Stage IV and a tumor size ≥2cm (as measured on imaging or estimated by physical exam), and patients for whom neoadjuvant therapy is indicated to make breast conservation surgery feasible. Participant will be required to have three FLT imaging sessions to evaluate therapy response at baseline, early-treatment, and post-treatment.

SAMPLE SIZE

Total of fifty-four (54) eligible and evaluable patients with locally advanced breast cancer will be enrolled in this study. It is anticipated that the accrual will be completed in 18 months with minimum of 36 patients enrolled in a year. The trial will continue to accrue until the target of 54 eligible and evaluable participants has been met. "Evaluable" is defined as: all required image series have been completed; local site has determined that all images are adequate and have been submitted to ACR Imaging Core Lab; and submitted images have passed quality control review by the ACR Imaging Core Lab. Ineligible and inevaluable cases will be replaced.

1 ABSTRACT

Systemic therapy has become an integral component of the multimodality management of primary breast cancer[1] and clinical trials evaluating adjuvant chemotherapy have demonstrated a substantial reduction in odds for recurrence and death.[2] Neoadjuvant therapy, the administration of systemic chemotherapy prior to surgical management provides enhanced disease free survival. Initial tumor response in patients receiving neoadjuvant chemotherapy in breast cancer is generally determined at the completion of therapy. This evaluation is determined by either presence or absence of palpable tumor as a clinical response and/or presence or absence of tumor cells in surgical specimen as a pathological response. The ability to evaluate the effectiveness of neoadjuvant therapy during treatment would be of significant importance. While there is some promising evidence that mid treatment [^{18}F]FDG PET imaging may be predictive of subsequent tumor response,[3] the tendency of [^{18}F]FDG to accumulate in inflammatory tissues can complicate the interpretations of mid-therapy images. Preliminary data suggests that early [F-18]FLT PET is better able to predict response to therapy, as [F-18]FLT uptake has been shown to correlate with cellular proliferation[4-7], and not significantly accumulate in inflammatory tissue[8]. The primary objective of this study is to correlate the percentage change in SUV between baseline and early therapy [F-18] FLT uptake in the primary tumor with pathologic response in patients with locally advanced breast cancer. Additionally, [F-18]FLT PET parameters will be compared with proliferative indices from the initial biopsy and residual tumor surgical samples via Ki-67 immunostaining and mitotic index. During this study, potential safety issues and the physiologic effects associated with [F-18] FLT administration will be evaluated.

2 BACKGROUND

2.1 Neoadjuvant Therapy (Primary Systemic Therapy)

Over the past thirty years, systemic therapy has become an integral component of the multimodality management of primary breast cancer.[1] Clinical trials evaluating adjuvant chemotherapy have demonstrated a substantial reduction in odds for recurrence and death regardless of hormone receptor or nodal status.[2] Even greater reduction in odds of recurrence and death has been shown in trials evaluating long term hormonal therapy in women with estrogen receptor positive breast cancer, regardless of menopausal or nodal status.[9]

Administration of systemic chemotherapy prior to surgical management (neoadjuvant therapy) provides disease free survival (DFS) and overall survival (OS) equivalent to that of post-surgical adjuvant therapy and improves the rate of breast conserving therapy relative to mastectomy. Importantly, patients who achieve a pathologic complete response (pCR) with neoadjuvant therapy enjoy a substantially lower risk of treatment failure than women with residual disease found at surgery. [10,11] As a result, pCR is now considered a valid primary end-point for assessing the efficacy of new agents or treatment regimens in the neoadjuvant treatment of early stage breast cancer.

Women diagnosed with locally advanced breast cancer (LABC) present substantial management challenges. The large local tumor burden makes local/regional therapy difficult and often ineffective. The majority of these women harbor occult metastatic disease, and many develop systemic failure within several years of treatment even if aggressive local procedures are successful at achieving local control of the cancer.[12]

Neoadjuvant chemotherapy has improved the prognosis of women with LABC. The only Phase III trial evaluating the effect of neoadjuvant systemic therapy in LABC was conducted by the European Organization for Research and Treatment of Cancer. The study randomized 363 women with inoperable LABC to receive radiation therapy alone, radiation therapy with hormonal therapy, radiation therapy with chemotherapy, and radiation therapy with both hormonal therapy and chemotherapy. Chemotherapy consisted of twelve cycles of CMF (cyclophosphamide, methotrexate, 5-FU). Hormonal therapy was delivered by ovarian irradiation and prednisone in premenopausal and tamoxifen in postmenopausal women. Systemic therapy improved time to local/regional recurrence and distant progression.[13] A survival advantage with hormonal therapy was demonstrated after long term follow-up.[14]

The vast majority of neoadjuvant chemotherapy research in LABC has been conducted in Phase II trials, the majority of which employed anthracycline-based therapy. As a result, Level I evidence for improved DFS or OS with anthracycline-based neoadjuvant chemotherapy is not available. However, the MD Anderson Cancer

Center (MDACC) has conducted a series of multi-modality trials for decades. They compared outcomes from eras when only local/regional therapy was utilized in Stage IIIA and IIIB disease with current regimens of local/regional treatment incorporating anthracyclines. Ten-year relapse-free survival appeared to be improved from approximately 18% to over 30% in patients with IIIB disease. An apparent improvement from approximately 50% to 60% was also noted in IIIA disease.[15] Though not definitive, this information is consistent with benefit from incorporation of anthracycline-based chemotherapy in the treatment of women with LABC.

In patients with LABC, pCR in the breast also appears to correlate with long term outcome, justifying the use of breast pCR as a primary efficacy endpoint in these patients. However, with LABC, the extent of residual disease in the axilla also appears to be an independent predictor of long term outcome. [16-19]

Because systematic phase III evaluation of specific regimens in LABC has not been possible due to the relative infrequency of this stage of breast cancer, a clear standard of treatment has not been identified. However, review of results from published Phase II trials provides a frame of reference for evaluation of new regimens in this patient population. In a series of fifteen trials conducted in 1,048 patients and reported from 1995 through 2003, clinical response rates varied from 53% with 3 to 4 cycles of CMF or cyclophosphamide/ doxorubicin/5-FU (CAF), to 98% with a regimen of high dose doxorubicin/ cyclophosphamide (AC) for 6 cycles. Clinical complete response rates (cCR) ranged from 4% with 4 to 6 cycles of doxorubicin/docetaxel (AT) to a high of 50% with high dose AC for six cycles. [16, 18, 20-32] Pathologic complete response rates (pCR) varied from 8% with 4 to 6 cycles of cyclophosphamide, doxorubicin, cisplatin (CAP) to 32% with a regimen of cyclophosphamide, doxorubicin, methotrexate and 5-FU (CAMF) administered to maximal response and administered with concurrent hormonal therapy. [10, 23-25] Not all trials reported five-year disease free survival but, among those reporting, a range of 44% to 73% was described. [22, 26, 28, 32] The higher cCR and pCR rates were noted with regimens that employed a longer duration of neoadjuvant chemotherapy. [16, 21, 22, 27, 28, 30]

2.1.1 Role of sequential anthracycline/taxane regimens in treatment of locally advanced breast cancer

Sequential use of anthracycline and taxane based chemotherapy regimens has become a standard for both neoadjuvant and adjuvant chemotherapy. NSABP Protocol B-27 showed a doubling of pCR to 26.1% with addition of four cycles of docetaxel 100mg/m2 every three weeks to four cycles of AC in women with operable breast cancer. [33] The Aberdeen trial evaluated the role of four cycles of docetaxel 100 mg/m2 following CVAP (cyclophosphamide, vincristine, doxorubicin, prednisone) in patients with primary tumors greater than 3 cm or LABC. Patients failing to respond to initial CVAP were crossed over to docetaxel, and only one of forty-six patients in this group achieved pCR. Patients responding to CVAP were randomized to four more cycles of CVAP or four cycles of docetaxel. The pCR rate was increased from 16% to 34% in the patients receiving docetaxel. Three year DFS was also improved from 77% to 90% and overall survival increased from 84% to 97% with the cross-over to docetaxel. [34]

MDACC has reported results of a randomized Phase III trial evaluating two schedules of paclitaxel for twelve weeks followed by FAC (5-FU, doxorubicin, cyclophosphamide) for four cycles as neoadjuvant therapy for women with operable breast cancer. Randomization was between a schedule of paclitaxel 250 mg/m2 over twenty-four hours every three weeks and one of two weekly regimens of paclitaxel depending on clinical nodal status. The pCR rate was doubled from 13.6% to 28.8% with weekly paclitaxel. [35]

These results demonstrate that current sequential anthracycline taxane regimens administered over twenty-four weeks can achieve pCR rates in the range of 25%-30%, which suggests that they are among the most active regimens studied to date. This regimen has now become standard care at VCU and other centers for primary chemotherapy of breast cancer, both for patients with LABC and for those with large tumors that require shrinkage before being considered candidates for breast conservation therapy (BCT). It is highly doubtful that the sequence of treatments would significantly affect the efficacy of treatment, especially in light of the results from MDACC, in which weekly paclitaxel followed by an anthracycline based regimen produced results every bit as good as those observed in NSABP B-27. [35]

2.2 [F-18]FLT PET Imaging

3'-deoxy-3'-^{18}F fluorothymidine ([F-18]FLT) is a structural analog of the DNA constituent, thymidine (Figure 1). It is a radiolabeled imaging agent that has been proposed for investigating cellular proliferation with positron emission tomography (PET). Although [F-18]FLT is not incorporated into DNA, it is trapped in the cell due to phosphorylation by thymidine kinase, a part of the proliferation pathway. As such, it has potential as a marker of proliferating tumor in proportion to the DNA synthesis rate. Therefore, [F-18]FLT is proposed as a radiolabeled imaging probe for *in vivo* assessment of cellular proliferation in malignant tumors using PET.

Figure 1 (from Investigator's Brochure, Edition Number 7; Edition Date: 2009)

Although [F-18]FLT studies are designed to characterize [F-18]FLT as a tracer of cellular proliferation in the primary tumor, the comparison of [F-18]FLT images with other clinical imaging and with surgical staging will provide initial data about [F-18]FLT's ability to depict regional tumor proliferation and distant metastases.

2.3 Rationale

Initial tumor response in patients receiving neoadjuvant chemotherapy in breast cancer is generally determined at the completion of therapy by either clinical response (presence or absence of palpable tumor) and/or pathological response (presence or absence of tumor cells in surgical specimen). While there is some promising evidence that mid treatment [F-18]FDG PET imaging may be predictive of subsequent tumor response,[3] the tendency of [F-18]FDG to accumulate in inflammatory tissues can complicate the interpretations of mid-therapy images. Preliminary data suggests that early [F-18]FLT PET is better able to predict response to therapy, as [F-18] FLT uptake has been shown to correlate with cellular proliferation[4-7], and not uptake significantly in inflammatory tissue[8]. Following the completion of therapy, the standard of care is surgical resection of residual tumor/ tumor bed. The surgical specimen will be assessed to determine pathologic response, and proliferative indices of any remaining tumor cells will be determined.

The primary objective of this study is to correlate the percentage change in SUV's, specifically SUV60, between baseline (FLT-1) and early-therapy (FLT-2) with pathologic complete response to neoadjuvant chemotherapy of the primary tumor in patients with locally advanced breast cancer. Using the imaging schema presented earlier, the tumor uptake in the initial [F-18]FLT PET/CT study will be correlated with the proliferative index of the pre-treatment biopsy (or re-biopsy) tissue, when available. The correlation between the [F-18]FLT PET imaging parameters and the Ki-67 immunostaining and mitotic index for proliferation will provide preliminary evidence that [F-18]FLT is imaging its molecular target.

The [F-18]FLT imaging parameters at all three time points [pre-therapy or baseline (FLT-1), post-one week or early-therapy (FLT-2), and post-therapy (FLT-3)] will be correlated with post-therapy tumor response as determined by: clinical assessment, pathological assessment, anatomic tumor size as measured by the transmission CT (if measurable), and. the Ki-67 immunostaining and mitotic index for proliferation These comparisons will determine the ability of [F-18]FLT to predict tumor response. The percentage change in [F-18] FLT uptake between pre-therapy and early-therapy studies (between FLT-1 and FLT-2) will serve as the

primary parameter of interest, and the predictive value will be based on pathological complete response (preliminary efficacy). The data obtained after completion of therapy (FLT-3) will also be analyzed with respect to prediction of pathological response and will be correlated with the Ki-67 immunostaining and mitotic index for proliferation in the residual tumor, if present.

By analyzing the imaging data from all three [F-18] FLT studies, further safety data and parameters for optimal image acquisition and analysis methods in breast cancer can be established.

The patient population for this study will include all patients for whom neoadjuvant chemotherapy is indicated and for whom subsequent definitive surgery is planned, according to current clinical guidelines. This includes all patients with locally advanced breast cancer (Stage IIIB and some IIIA), all patients with Stage IIIC disease (supraclavicular node involvement), and patients for whom primary chemotherapy is indicated to make breast conservation surgery feasible. The last group would be expected to have a median tumor diameter of approximately 4 cm. Based on prior experience, we expect 80-90% of patients to have an objective clinical response to chemotherapy treatment (> 50% reduction in tumor size); 35-60% to have a clinical complete response (no palpable tumor); and 15-30% to have a pathologic complete response (no invasive tumor in the breast on surgical specimen pathology).

3 OBJECTIVES

3.1 Primary objective

3.1.1 To correlate the percentage change in SUV60 between baseline (FLT-1) and early-therapy (FLT-2) with pathologic complete response to neoadjuvant chemotherapy of the primary tumor in patients with locally advanced breast cancer;

3.2 Secondary objectives

3.2.1 To demonstrate correlation between FLT-1 and FLT-3 uptake parameters and tumor proliferation markers in locally advanced breast cancer;

3.2.2 To evaluate the relationship between FLT-1, FLT-2 and FLT-3 uptake parameters and pathologic complete response of the primary tumor and residual cancer burden (RCB);

3.2.3 To evaluate the relationship between FLT-1, FLT-2 and FLT-3 uptake parameters and non-response of the primary tumor (stable or progressive disease) to therapy;

3.2.4 To evaluate the relationship between FLT-1, FLT-2 and FLT-3 uptake parameters and pathologic complete response to neoadjuvant chemotherapy in patients with regional disease in the lymph nodes in patients with locally advanced breast cancer;

3.2.5 To compare the changes of FLT-2 and FLT-3 uptake parameters to changes in tumor sizes from other serial imaging modalities such as mammograms, MRI, and ultrasound, as available;

3.2.6 To compare the changes of FLT-2 and FLT-3 uptake parameters to metabolic changes from [^{18}F]FDG- PET, as available;

3.2.7 To continue to monitor for potential safety issues and define any physiologic effects associated with [^{18}F] fluorothymidine (FLT) administration.

4 IMAGING AGENT INFORMATION

For complete information, please refer to the Investigator's Brochure:
"3'-deoxy-3'-[F-18] fluorothymidine: [F-18]FLT, An Investigational Positron Emission Tomography (PET) Radiopharmaceutical for Injection and intended for use as an in vivo diagnostic for imaging active cellular proliferation of malignant tumors", Edition Number 7, Edition date 2009.

4.1 Pharmacology and Toxicology

The pharmacology of FLT is based on its action as an inhibitor of DNA synthesis.[36-38] Intracellular metabolism of FLT produces nucleotides that inhibit endogenous DNA polymerases because they lack a 3'-hydroxyl substituent. This results in premature chain termination of DNA synthesis.[39, 40] These biochemical properties can account for FLT's prominent hematological and liver toxicity.[40-42] The pharmacology of FLT closely parallels that of the widely used prescription HIV-antiviral drug azidothymidine (AZT).[43, 44] Both FLT and AZT are 3'-deoxythymidine analogs that act as inhibitors of DNA synthesis and are cleared from the body in the same way. However, FLT is significantly more cytotoxic than AZT in test cell lines.[42] Cellular uptake of FLT and thymidine is greater than that of AZT. Transport of FLT and thymidine across cell membranes occurs by active transport and passive diffusion.[44]

4.2 Toxicity of FLT in Humans

FLT was investigated as an anti-AIDS drug in humans.[41] Toxic effects and death were reported for some subjects who received FLT during randomized concentration-controlled trials during a 16-week treatment of oral multi-dosing. Doses of 0.125 mg/Kg every 12h produced a mean cumulated drug exposure (AUC12: area under curve) of 417 ng-h/mL. At this level, serious (grade 3) hematologic toxicity occurred in 6 of 10 subjects. At 300 ng-h/mL, grade 2 or greater (fall in hemoglobin to < 9.4 g/dL) developed within 4 weeks in 9 of 12 subjects. At 200 ng-h/mL almost no clinically significant anemia developed, but dose-limiting granulocytopenia (< 750 granulocytes/mm3) occurred in 5 of 15 subjects. Mild peripheral neuropathy occurred in 2 of 10 subjects at 50 ng-h/mL, but was not dose-limiting. FLT drug trials were terminated after the unexpected death of 2 subjects of hepatic failure. One patient assigned to 200 ng-h/mL developed progressive liver failure and died after 12 weeks of FLT therapy. A second subject, who received a fixed dose of 10 mg/day, developed progressive liver failure and died at about the same time. All surviving subjects were followed closely for 4 weeks after stopping FLT and none had evidence of clinically significant liver disease or other adverse effects. Overall, 25 of the 44 subjects receiving at least two doses of FLT completed the 16 week study without clinically significant adverse effects.

FLT (Alovudine) was withdrawn from development for several years, and then reinvestigated for multi-drug resistant HIV infection. Fifteen patients with multi-drug resistance HIV received 7.5 mg each day for 28 days along with their ongoing therapy.[72] No serious adverse events were observed. In a randomized, double-blind, placebo-controlled study by the same group, 51 patients received 0.5 mg, 1.0 mg, or 2.0 mg daily for 28 days in addition to their routine therapy; 21 patients received placebo.[73] No unexpected adverse events were observed, and no serious AEs were attributed to the study drug.

4.3 Dosimetry

The dose of FLT to be administered in this imaging trial is 1400-fold lower than the dose that led to serious toxicity in the studies described above. A summary of the relevant human dosimetry for 2 different voiding scenarios from the investigator's brochure is included in Table 1.

Table 1. Human dosimetry estimates

Organ of Interest	Men mGy/MBq (mrad/mCi)			Women mGy/MBq (mrad/mCi)		
Total Body Dose	Scenario 1	1.23E-02	(46)	Scenario 1	1.56E-02	(58)
	Scenario 2	1.26 E-02	(47)	Scenario 2	1.59 E-02	(59)
Bladder	Scenario 1	1.79E-01	(662)	Scenario 1	1.74E-01	(646)
	Scenario 2	7.91E-02	(293)	Scenario 2	7.76E-02	(287)
Liver	Scenario 1	4.51E-02	(167)	Scenario 1	6.42E-02	(238)
	Scenario 2	4.54 E-02	(168)	Scenario 2	6.45 E-02	(239)

Scenario 1: Single bladder voiding at 6 h after [F-18]FLT administration with a 10% post-voiding bladder residual decayed to infinity. This scenario assumed no urine reaccumulation after 6 h.

Scenario 2: First bladder voiding at 2 h after [F-18]FLT administration with a 10% post-voiding residual; urine reaccumulation between 2 and 6 h at a rate determined by the bladder curve fit; second bladder voiding at 6 h with a 10% post-voiding residual decayed to infinity. This scenario assumed no urine reaccumulation after 6 h. The first scenario is conservative, whereas the second has a more realistic voiding scheme.

4.4 Previous human [F-18]FLT imaging studies

Several preliminary studies using [F-18]FLT imaging in human subjects have been performed in Germany and the United States (UCLA, University of Washington in Seattle, Wayne State University). The imaging protocols were pre-approved by their respective regulatory committees and conducted under the RDRC process or under NCI IND, with patients receiving between 1.4 and 13 mCi of [F-18]FLT. Some of the imaging results have been published (Table 2). The group in Seattle, which has the most experience with this agent in the US, has performed numerous studies in patients with lung cancer as well as a few in patients with primary brain tumors. Their findings demonstrate the feasibility and merit of tumor imaging with [F-18]FLT. [F-18]FLT PET showed increased uptake in tumor lesions outside the liver or bone marrow (standardized uptake value (SUV) 4-7), which were delineated from surrounding tissue (SUV 0.5-2).

Table 2. Summary of published manuscripts reporting [F-18]FLT human imaging studies

Year	Organ System	N	mCi injected	MBq Injected Range (Mean)	Specific Activity	Reference (#)
2008	Brain	12 [a]	4.2 – 5.2	185	>1.25 Ci/umol	Spence [74]
2008	Bone & Soft Tissue	22		350-425	120 GBq/mmol	Buck [75]
2008	Pancreas	5	5.2 - 7		Not reported	Quon [76]
2008	Lung	55		300-400	Not reported	Tian [77]
2008	Brain	13 [b]		111-370 (322±85)	Not reported	Ullrich [78]
2008	Lung	54 [c]		101-238 (158)	Not reported	Yamamoto [79]
2008	Lung	34 [c]		3.5 /kg	Not reported	Yamamoto [80]
2008	Brain	18	3.5 - 6.4	129-236 (161)	Not reported	Hatakeyama [81]
2007	Sarcoma	10		320-430 (399 med), 120-430 (363 med)	>10 TBq/mmol	Been [82]
2007	Brain	21 [d]		2.0 /kg	Not reported	Chen [83]
2007	Lymphoma	22		300-370	Not reported	Herrmann [84]
2007	Gastric	45		270-340	Not reported	Herrmann [85]
2007	Lymphoma	48	3.9	148.6	Not reported	Kasper [86]
2007	Breast	15		153-381	15-227 GBq/umol	Kenny [87]
2007	Brain	9 [d]		1.5 kg	Not reported	Schiepers [88]
2007	Head/Neck	10	6.76	250	10 TBq/mmol	Troost [89]
2007	Lung	20 [e]	0.07 /kg	2.59 kg	0.12 -1.6 Ci/umol	Turcotte [90]
2007	Rectal	10	8.1	300	Not reported	Wieder [91]
2007	Lung	18 [c]		145 ± 26	Not reported	Yamamoto [92]
2006	Bone marrow	18	10.8	400	10 TBq/mmol	Agool [93]
2006	Brain	12 [a]	5	185	7.4 GBq/umol	Muzi [94]

2006	Brain	25	10	370	Not reported	Saga [95]
2006	Brain	10	4	104-202	37-222 GBq/umol	Yamamoto [45]
2006	Lymphoma	34	9.3	265-370	Not reported	Buck [46]
2006	Lung & SPN	22	5	185.2	Not reported	Yap [47]
2006	Breast	14	4	150	74 TBq/mmol	Pio [48]
2005	Lung	17	5	2.6/kg; max 185	>37 GBq/mmol	Muzi [96]
2005	Multiple	33	8.4-9.7	310-360 (350)	>220 GBq/mmol	Shields [97]
2005	Breast	15	401-10.5	153-380	25-465 GBq/umol	Kenny [98]
2005	Brain	23 [d]	8.6	111-370 (321)	Not reported	Jacobs [49]
2005	Esophageal	10	11.1	340-450 (410)	>10 TBq/mmol	vanWestreenen [50]
2005	Lung	47 [f]	9.5	265-370	Not reported	Buck [51]
2005	Breast	10		390-420 [3 pts] 60-250 [7 pts]	>10 TBq/mmol	Been [52]
2005	Brain	25	4.7	141-218 (174)	~74 Bq/mmol	Chen [32]
2005	Brain	26 [d]	10	370	3.2-7.7 Ci/umol	Choi [53]
2004	Lymphoma	7 [g]	4.3 -13.2	159-489 (324)	Not reported	Buchmann [54]
2004	Lung	17	5.7	130-420 (210)	>10TBq/mmol	Cobben [55]
2004	Lung	28 [h]	9	265-370 (334)	Not reported	Halter [56]
2004	Breast	12	8.1-12.1	300-450	Not reported	Smyczek-Gargya [33]
2004	Colorectal	11 [i]	9.7	360 ± 25	Not reported	Visvikis [57]
2004	HEENT	21	9.2	165-650 (340)	>10TBq/mmol	Cobben [58]
2004	Soft Tissue	19	10.8	115 -430 (400)	>10TBq/mmol	Cobben [59]
2003	Lymphoma	11	7.5	280	Not reported	Wagner [60]
2003	Colorectal	10 [i]	9.5	351± 52	Not reported	Francis [61]
2003	Colorectal	17 [i]	9.4	312-412 (360)	Not reported	Francis [62]
2003	Lung	18	5	185 max	37 GBq/umol	Vesselle [63]
2003	Lung	16	5.4 -10.8	200 - 400	Not reported	Dittmann [64]
2003	Melanoma	10	10.8	185-430 (med 400)	>10TBq/mmol	Cobben [65]
2003	SPN	26 [j]	9	265-370 (334)	Not reported	Buck [66]
2002	SPN	30 [j]	9	265-370 (334)	Not reported	Buck [67]
2002	Lung	10 [k]	5	185 max	37 GBq/umol	Vesselle [68]
Total # Subjects:		1045				

(a) There appears to be an overlap of two patients reported in the Muzi 2006 and Spence 2008 manuscripts.
(b) There appears to be an overlap of two patients reported in the Ullrich 2008 and Jacobs 2005 manuscripts.
(c) Comparison of the three Yamamoto lung cancer manuscripts indicates that they represent 72 unique patients.
(d) The Chen 2005, Chen 2007, and Schiepers manuscripts appear to represent a total of 34 unique patients.

(e) Turcotte appears to include an additional 2 patients who were not reported in the Vesselle 2003 manuscript.
(f) Only 13 unique patients can be confirmed against the previous manuscripts from this group.
(g) All 7 patients were previously reported in Wagner 2003.
(h) There appear to be 10 additional unique patients who were not described in the Buck 2002 and 2003 manuscripts.
(i) It is unclear if the same patients are being described for both of the Francis manuscripts and the Visvikis manuscript.
(j) It is unclear if the same patients are being described in the Buck 2002 and 2003 manuscripts.
(k) It appears that these patients were described in the 2003 Vesselle manuscript.

Due to the possibility that the same patients are being described in some reports, the total number of unique patients represented in the published studies appears to be 827. As evidenced in Table 2, many of the published studies did not report the specific activity of the [F-18]FLT, so it is not possible to determine the amount of FLT that was actually administered to the patient.

No adverse events have been reported for [F-18]FLT at the strength to be used for this study. As described in section 4.2, non-radioactive FLT has been investigated as an anti-AIDS drug, and reversible peripheral neuropathy was observed in subjects exposed to 50 ng-h/mL plasma over a course of 16 weeks (15µg/kg q12h). The FLT dose anticipated for this study will be <6.1µg for a single injection. Assuming a 70kg individual, the maximum concentration of FLT would be expected to be equivalent to 0.29 ng-h/mL. The radiation exposure associated with this study is described in section 4.3 and is comparable to the dose for other widely used clinical nuclear medicine procedures.

In a 2007 study performed at the University of Washington, Turcotte et al[89] assessed the toxicity of [F-18]FLT in 20 patients with proven or suspected diagnosis of non-small cell lung cancer. Blood samples from multiple timepoints before and after [F-18]FLT-PET were assayed for comprehensive metabolic panel, total bilirubin, complete blood and platelet counts. A standard neurological examination was also performed by a qualified physician for each patient before and immediately after [F-18]FLT-PET. No side effects were reported by patients or witnessed. No change in the neurological status of patients was observed.

The group in Seattle also recently published the results from safety studies performed in patients with recurrent glioma.[74] Twelve patients were injected with 0.07 mCi/kg (5mCi maximum) of [F-18]FLT (specific activity 1.25 Ci/umol) and were closely monitored for three hours afterward. Additional follow-up was performed at 1 day post- and 1 month post-injection. Their findings showed no evidence of toxicity at this dose. Monitoring of vital signs and ECG, review of systems, neurological assessments, and laboratory evaluations revealed no evidence of adverse effects. Notably, no signs or symptoms of peripheral neuropathy were observed in any patient at any time.

4.5 [F-18]FLT Administered Dose

The administered dose will be 0.07 mCi/kg with a maximum of 5 mCi. The drug solution is stored at room temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial and has an expiration time of 8 hours. The injectable dose of [F-18]FLT for most studies will be ≤ 0.07 mCi/kg of fluorine-18, not to exceed 5 mCi with a specific activity greater than 200 Ci/mmol at the time of injection. In the dose of [F-18] FLT, only a small fraction of the FLT molecules are radioactive. The amount of injected drug is ≤ 6.1 µg (≤ 25 nmol per dose) of FLT. [F-18]FLT is administered to subjects by intravenous injection of ≤ 10 mL. There is no evidence that nonradioactive and radioactive FLT molecules display different biochemical behavior.

4.6 Quality Assurance, Quality Control and Storage

In accordance with regulations, the radioisotope vendor conducts several quality control tests on the [F-18] FLT product prior to release for human administration. Once delivered to the participating institution, doses will be stored in the appropriate storage area in the nuclear medicine facility until they are administered to the patient.

4.7 Supplier of [F-18]FLT

4.7.1 Drug Ordering

[F-18] FLT will be purchased from a commercial vendor of radioisotopes in most cases. The vendor must be authorized within the NCI IND. The investigator or the investigator-designee will order patient doses of [F-18] FLT. The investigative radiopharmaceutical [F-18]FLT solution will be shipped to the site the same day the participant is to be injected.

The investigational pharmacist or qualified nuclear medicine technologist at the participating institution will be the responsible party designated by the investigator.

[F-18]FLT can only be synthesized on site if the chemistry manufacturing and control procedures are filed within the NCI IND.

4.7.2 Drug Returns

If for any reason the study imaging is unable to be completed, sites will allow the radioactivity of the [F-18]FLT solution to decay and then discard it appropriately per site's policies and procedures. A copy of the policy should be available upon request.

4.7.3 Drug Accountability

The investigator or the investigator-designee must maintain a detailed record of receipt, disposition, and destruction dates of [F-18]FLT solution, using the Drug Accountability Record form available on the ACRIN web site (www.acrin.org/6688_protocol.aspx) or by calling the ACRIN 6688 project manager.

5 STUDY OVERVIEW

Breast cancer patients with locally advanced disease who are candidates for primary systemic (neoadjuvant) chemotherapy and definitive surgery will be enrolled in this prospective phase II trial. As there is no specific neoadjuvant chemotherapy regimen required for this protocol, participants will receive standard of care treatment at their participating institutions. A total of 54 eligible women with evaluable images will be accrued in the study and will include women with locally advanced breast cancer, not Stage IV, and a tumor size ≥2cm (as measured on imaging or estimated by physical exam), and patients for whom neoadjuvant therapy is indicated to make breast conservation surgery feasible. At least four (4) ACRIN-qualified institutions may participate and accrue for this study. Accrual is expected to be completed within 18 months from site Institutional Review Board (IRB) approval after study activation. Eligible participants will be actively involved in the trial for approximately one (1) year.

The overall goal of this study is to evaluate the relationship between [F-18]FLT uptake parameters and pathologic complete response to neoadjuvant therapy of the primary tumor in patients with locally advanced breast cancer. The participants will receive FLT-PET/CT prior to neoadjuvant chemotherapy as a baseline measurement, a second (early) FLT-PET/CT 5-10 days after the initiation of the first cycle of the chemotherapy, and a third (post therapy) FLT PET scan will take place after the completion of chemotherapy and within 3 weeks prior to surgery. Following completion of neoadjuvant chemotherapy, the participant will undergo surgical resection of residual tumor. A portion of this residual tumor will be used for pathological analysis and proliferation assays.

6 PARTICIPANT SELECTION/ELIGIBILTY CRITERIA

6.1 Inclusion Criteria

6.1.1 Pathologically confirmed breast cancer, determined to be a candidate for primary systemic (neoadjuvant) therapy and for surgical resection of residual primary tumor following completion of neoadjuvant therapy;

6.1.2 **Locally advanced breast cancer, not stage IV, and with a** tumor size ≥ 2cm (as measured on imaging or estimated by physical exam);

6.1.3 No obvious contraindications for primary chemotherapy;

6.1.4 Residual tumor planned to be removed surgically following completion of neoadjuvant therapy;

6.1.5 Able to lie still for 1.5 hours for PET scanning;

6.1.6 Age 18 years or older;

6.1.7 ECOG Performance Status ≤ 2 (Karnofsky ≥ 60%; see Appendix II);

6.1.8 Normal organ and marrow function as defined below at first visit:

-leukocytes ≥ 3,000/μl;

-absolute neutrophil count ≥ 1,500/μl;

-platelets ≥ 100,000/μl;

-total bilirubin within normal institutional limits;

-AST(SGOT)/ALT(SGPT) ≤ 2.5 times the institutional upper limit of normal;

-creatinine within normal institutional limits;

OR

-creatinine clearance ≥ 30 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal;

6.1.9 If female, postmenopausal for a minimum of one year, OR surgically sterile, OR not pregnant, confirmed by institutional SOC pregnancy test, and willing to use adequate contraception (hormonal or barrier method of birth control; abstinence) for the duration of study participation;

6.1.10 Able to understand and willing to sign a written informed consent document and a HIPAA authorization in accordance with institutional guidelines.

6.2 Exclusion Criteria

6.2.1 Previous treatment (chemotherapy, radiation, or surgery) to involved breast; including hormone therapy;

6.2.2 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements;

6.2.3 Medically unstable;

6.2.4 Condition requiring anesthesia for PET scanning and/or unable to lie still for 1.5 hours;

6.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to [F-18] F-18 fluorothymidine;

6.2.6 Under age 18. *Because no dosing or adverse event data are currently available on the use of [F-18] FLT in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric single-agent trials, if applicable.*

6.2.7 Pregnant or nursing. *Pregnant women are excluded from this study because the effects of [^{18}F]FLT in pregnancy are not known. The literature includes a report of tumor cells incubated with 10 μM FLT for extended periods that showed chromosome damage, which suggests that [F-18] FLT may have mutagenic properties. Because there is an unknown but potential risk for adverse events in nursing infants secondary to administration of [^{18}F]FLT in the mother, breastfeeding should be discontinued if the mother receives [F-18] FLT.*

6.2.8 Previous malignancy, other than basal cell or squamous cell carcinoma of the skin or in situ carcinoma of the cervix, from which the patient has been disease free for less than 5 years.

6.2.9 Currently on hormone therapy as the primary systemic neoadjuvant therapy

6.3 Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. However, because breast cancer is far more common in women, it is unlikely that any patients enrolled will be male.

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	2	0	2
Not Hispanic or Latino	52	0	52
Ethnic Category: Total of All Subjects *	54**	0	54**
Racial Categories			
American Indian/Alaska Native	1	0	1
Asian	1	0	1
Native Hawaiian or Other Pacific Islander	1	0	1
Black or African American	4	0	4
White	47	0	47
Racial Categories: Total of All Subjects *	54**	0	54**

* The "Ethnic Category: Total of All Subjects" must be equal to the "Racial Categories: Total of All Subjects."
** Total of fifty-four (54) eligible and evaluable patients with locally advanced breast cancer will be enrolled in this study. It is anticipated that the accrual will be completed in 18 months with minimum of 36 patients enrolled in a year. The trial will continue to accrue until the target of 54 eligible and evaluable participants has been met. "Evaluable" is defined as: all required image series have been completed; local site has determined that all images are adequate and have been submitted to ACR Imaging Core Lab; and submitted images have passed quality control review by the ACR Imaging Core Lab. Ineligible and inevaluable cases will be replaced.

6.4 Recruitment and Screening

The investigative team at each participating site should include medical oncologists, radiologists and/or nuclear medicine physicians, as part of the standard care to treat breast cancer. At the time of the patient's visit, the standard of care treatment will be discussed along with possible participation in the ACRIN 6688 trial. If the patient agrees to participate, the consent will be obtained by the site principal investigator or investigator-designee.

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

1. Documentation of the number of patients treated in the previous two 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 provided materials provided by ACRIN. Site investigators will be responsible for obtaining IRB approval.

7 SITE SELECTION

7.1 Institution Requirements

The potential sites for this study are ACRIN-participating institutions that meet qualifications for participating in this study. Each institution must complete a Protocol Specific Application (PSA); available online at www.acrin.org/6688_protocol.aspx) and have the PET scanner approved prior to the institution participating in the study. Detailed information for PET Qualification Procedures and its application to become qualified, as well as the PSA can be accessed at www.acrin.org/6688_protocol.aspx.

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

All institutions must have study-specific Institutional Review Board (IRB) approval for the protocol and site-specific informed consent form (ICF). The ICF 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 1572, a copy of the IRB approval letter, and a copy of the IRB-approved, institutional study-specific informed consent must be on file must be delivered to the trial monitor to review the approved form and to keep on file at ACRIN Headquarters (fax: 215-717-0936, ATTN: Protocol Development and Regulatory Compliance Department) prior to enrolling the first study participant.

7.3 Accrual Goals and Monitoring of Participant Accrual

The ACRIN Biostatistics and Data Management Center (BDMC) will monitor participant accrual. Total target accrual for this study is 54 evaluable participants in 18 months. During the first year, accrual will be reviewed monthly with the 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. If a site's actual accrual falls below 60% of what is reported in the PSA, the Protocol Support Enrollment Committee (PSEC), comprised of the trial PI and his or her designees, will determine a follow-up action plan to identify site accrual barriers and develop strategies to support the site in meeting accrual goals.

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. Accrual and safety information will be presented to the ACRIN Data and Safety Monitoring Committee (DSMC) at regularly scheduled meetings thereof; the DSMC may, at its discretion, re-evaluate the study with respect to feasibility or the need for additional participating institutions.

7.4 Institutional Review Board

Sites must obtain initial local IRB approval to participate in ACRIN trials. Prior to participant registration, a copy of the IRB approval letter for the protocol and the ICF must be sent to ACRIN, along with a copy of the IRB-approved, site-specific ICF. Investigator will provide a copy(s) of IRB approval letter(s) for any amendment(s), and copy(s) of annual renewal(s).

8 STUDY PROCEDURES

8.1 Visit 1: Screening visit

- A screening assessment will occur to determine eligibility for the study.
- A signed consent form will be obtained prior to study trial procedures.
- Screening labs will be obtained within the four-week period prior to administration of [F-18] FLT. These include CBC with differential and serum chemistry, and platelets. *If patient data are available from clinical records in the appropriate time window, they need not be repeated for the pre-study evaluation.*

- Medical history, demographics, height, weight, and physical exam will be obtained. *If patient data are available from clinical records in the appropriate time window, they need not be repeated for the pre-study evaluation.*
- Tissue samples from biopsy will be sent to VCU Pathology

8.2 Visit 2: FLT PET Imaging Studies (FLT-1)

Note: Per inclusion criteria 6.1.9 and exclusion criteria 6.2.7, any non-sterile, pre-menopausal women MUST not be pregnant as general practice and per institution's standard of care prior to performing any scans.

- The baseline [F-18] FLT PET (FLT-1) scan will take place prior to chemotherapy.
- Specific image acquisition and reconstruction protocols can be found on the ACRIN web site under Imaging Materials at www.acrin.org/6688_protocol.aspx.
- An adverse event evaluation will be performed at 24 hours (+/- 4 hours).

8.3 Visit 3: FLT PET Imaging Studies (FLT-2)

Note: Per inclusion criteria 6.1.9 and exclusion criteria 6.2.7, any non-sterile, pre-menopausal women MUST not be pregnant as general practice and per institution's standard of care prior to performing any scans.

- The early therapy [F-18]FLT PET (FLT-2) scan will take place 5-10 days after the initiation of the first cycle of the chemotherapy.
- An adverse event evaluation will be performed at 24 hours (+/- 4 hours).

8.4 Visit 4: FLT PET Imaging Studies (FLT-3)

Note: Per inclusion criteria 6.1.9 and exclusion criteria 6.2.7, any non-sterile, pre-menopausal women MUST not be pregnant as general practice and per institution's standard of care prior to performing any scans.

- The post therapy [F-18]FLT PET (FLT-3) scan will take place after the completion of chemotherapy and within 3 weeks prior to surgery.
- An adverse event evaluation will be performed at 24 hours (+/- 4 hours).

8.5 Visit 5: Surgery

- Following completion of neoadjuvant chemotherapy, the subject will undergo surgical resection of residual tumor.
- A portion of this residual tumor should be sent for to VCU Core Pathology for pathological analysis and proliferation assays within two weeks post surgery. If no viable tumor remains, a pathological complete response will be documented.

8.6 Sharing of Results

The final results of the study will be shared with the participating centers at the completion of the study. With agreement between the patient and the treating physician, and at the discretion of the participating center, the treating physician may share the experimental results of the FLT imaging scans with the participant. The sharing of the informal results should be done with caution, as the FLT-PET is still experimental and the significance of these results is unknown. The results from the FLT-PET scans should not be part of the patient's medical record and should not be used to direct or change therapy. We emphasize that sharing of imaging results with patients remains optional and at the discretion of the participating center, the patient and the treating physician

8.7 Study calendar

	<4 weeks Pre-Study[1]	Pre-therapy Imaging (FLT-1)[2]	After 1 week (5-10 days) Imaging (FLT-2)[3]	Post-therapy Imaging (FLT-3)[4]	Surgery
[F-18]**FLT PET/CT**[5]		X[5]	X[5]	X[5]	
Informed Consent	X				
Demographics[1]	X				
Medical History[1]	X	X			
Height[1]	X	X			
Weight[1]	X	X	X	X	X
Physical Exam[1]	X				
CBC w/Diff, Plts[1]	X				
Serum Chemistry[1,6] (to include AST, ALT, creatinine, total bilirubin)	X				
Adverse Event Evaluation		X	X	X	

[1] If patient data are available from clinical records in the appropriate time window, they will not be repeated for the pre-study evaluation.

[2] Baseline [F-18]FLT PET/CT imaging (FLT-1) must be done within 4 weeks prior to therapy initiation of therapy protocol.

[3] Early [F-18]FLT PET/CT imaging (FLT-2) will be done 5-10 days after the initiation of the first cycle of the chemotherapy .

[4] Post-therapy [F-18]FLT PET/CT imaging (FLT-3) will be done after the completion of chemotherapy and within 3 weeks prior to surgery

[5] Note: Per inclusion criteria 6.1.9 and exclusion criteria 6.2.7, any non-sterile, pre-menopausal women MUST not be pregnant as general practice and per institution's standard of care prior to performing any scans.

[6] Serum Chemistry to include AST, ALT, creatinine, and total bilirubin.

9 DATA MANAGEMENT/ONLINE REGISTRATION

9.1 General

9.1.1 The ACRIN web address is www.acrin.org**.**

9.1.2 Data collection and management will be performed by the 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.

9.1.3 Participant enrollment and data collection occurs through a series of programmed screens accessed through the Oracle Remote Data Capture C3D System to register participants, collect participant data, and maintain patient casebook for each participant. Participant registration and data entry is available to clinical sites 24 hours a day, seven days a week. A DMC contact list is located on the ACRIN web site for each protocol.

9.2 Clinical Data Submission

9.2.1 The investigative site is required to submit data according to protocol via the patient casebook, 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.

9.2.2 To submit data via the Oracle C3D System, the appropriate investigator-designated research staff will log onto the C3D System and supply the pre-assigned user name and password. Each visit is separated into modules. The user selects the appropriate visit and enters data directly into the eCRF. As information is entered into the eCRF, various validation checks will be performed. These checks look for data that are missing, data that are out of range, and data that are 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. Forms that are not completed in one sitting can still be submitted and completed at a later date.

9.2.3 Once data entry of a form is complete, the investigator or the research staff presses the "Save Complete" button on the screen and the data is transferred into the clinical database. Should a problem occur during transmission, the investigator or research associate should contact the DMC for resolution of the submission.

9.2.4 If a temporary problem prevents access to the C3D system, 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.

9.3 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.

9.4 Electronic Data Management

9.4.1 Uniquely collected elements from the eCRF forms will be periodically transferred to BDMC. 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 data entry screens. Data elements that fail validation are followed up by the DMC research associate. 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 is 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.

9.4.2 If checks at DMC or BC detect missing or problematic data, the DMC personnel assigned to the protocol will initiate a query for the site RA or investigator specifying the problem and requesting clarification.

9.5 Missing and Delinquent Data Submission

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.

9.6 Data Quality Assurance

9.6.1 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.

9.6.2 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 the 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.

9.6.3 In addition, the ACRIN QA Monitor will review case report forms and source documents at several different time points: after first few participants enrolled and during the conduct of the trial, including 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, participant's welfare and safety, and provides resources to sites for clarification to the protocol and guidance in completion of the case report forms.

10 IMAGING PROTOCOL

10.1 FLT PET Imaging Studies

Specific image acquisition and reconstruction protocols can be found on the ACRIN web site under Imaging Materials at www.acrin.org/6688_protocol.aspx.

10.2 [F-18]FLT Administration

[F-18]FLT will be synthesized according to the standard operating procedures provided by the NCI. A summary of the synthesis procedure and associated quality control can be found in the investigator's brochure.

[F-18]FLT will be administered in the PET imaging suite under physician supervision. The imaging technologist or nurse will administer the [F-18]FLT by intravenous infusion over one minute, followed by a saline flush. A fully equipped emergency cart and ACLS certified personnel will be available. Reported adverse events and potential risks are described in Section 11.1. The infusion and imaging procedure will be terminated in any patient who exhibits anaphylaxis, chest pain, dyspnea, or grand mal seizure.

10.3 Timing of FLT PET Studies

Three imaging sessions (pre-treatment (FLT-1), after one week (FLT-2), and post-treatment (FLT-3) will be performed (see Schema).

10.3.1 Pre-chemotherapy (FLT-1) PET imaging must be completed within 4 weeks prior to beginning chemotherapy.

10.3.2 After one cycle of chemotherapy, (FLT-2) must be performed 5-10 days after initiation of first chemotherapy therapy cycle.

10.3.3 Post-chemotherapy imaging (FLT-3) must be performed after the completion of chemotherapy within 3 weeks prior to surgery.

10.4. Imaging Q/C Procedure

Participant must be scanned on PET scanners that have been qualified by the ACRIN PET Core Laboratory per the protocol-specific instructions posted on the ACRIN web site at: www.acrin.org/CORELABS/PETCORELABORATORY/PETQUALIFICATION/tabid/485/Default.aspx

A dedicated PET scan unit or hybrid PET/CT scanner is mandatory. The PET scanner must be capable of performing both emission and transmission images in order to allow for attenuation-corrected PET scan images. The ability to calculate standardized uptake values (SUVs) is also mandatory. All sequential imaging sessions will be performed on identical or technically equivalent PET/CT scanner.

The PET scanner must be kept calibrated in accordance with the manufacturer's recommendations. The scanner should routinely be assessed for quantitative integrity and stability by being tested using various imaging protocols on a standard phantom. For SUV measurements, this assessment should include a comparison against a dose calibrator to ensure accuracy; that is, a comparison of the absolute activity measured versus the measured activity injected, should be performed.

The PET scanner calibrations should be routinely verified according to manufacturer recommendations. The scanner should be assessed regularly for quantitative integrity and stability by scanning a standard quality control phantom with the same acquisition and reconstruction protocols used for study participants. The SUV verification measurements must include the dose calibrator used to measure the doses of study participants to ensure that the dose calibrator and PET scanner are properly cross calibrated, i.e. the dose measured in the dose calibrator and injected into the phantom matches the results obtained from analysis of the phantom images.

A daily QC check must be performed at the beginning of the day, including PET scanner and dose calibrator, in accordance with the manufacturer recommendations. If any of the quality control (QC) results are outside of the manufacturer's guidelines, the study must be rescheduled and the problem rectified before scanning any patients.

10.5 Imaging sessions

The participant will report to the PET suite and undergo [F-18]FLT injection, immediately followed by dynamic regional PET/CT imaging for 60 minutes, followed by a static whole body image (top of head to upper thigh; 5-7 bed positions depending on patient's size). The preferred imaging sequence for the PET scans is to obtain the dynamic PET imaging first, then followed by the torso survey using static PET imaging. For patients who are unable to tolerate lying in the scanner for dynamic imaging or for centers where scanner availability/scheduling is limited, it may not be feasible to obtain the dynamic PET imaging. In such cases, the acquisition of the standardized uptake value using static PET imaging starting 60 minutes after injection fulfills the needs of the study.

All adverse events occurring post [F-18]FLT infusion will be recorded with in a 24-hour period. The adverse events to be specifically monitored during the infusion include localized discomfort at the IV injection site, pain, respiratory difficulties, flushing, dizziness, pruritus/rash, and any other symptoms that could be secondary to an anaphylactic reaction. The subject will be instructed to report any subjective symptoms or sensory changes noted.

10.6 Supportive Care Guidelines

Any adverse effects, related or non-related to the injection of FLT, will be treated as clinically indicated with no study-related restrictions.

10.7 Surgery

Following completion of neoadjuvant chemotherapy, the subject will undergo surgical resection of residual tumor. A portion of this residual tumor will be used for pathological analysis and proliferation assays. If no viable tumor remains, a pathological complete response will be documented.

10.8 Dosing delays/ dose modifications

10.8.1 [F-18]FLT

The dose of [F-18]FLT is based on the radiation dosimetry estimates. Due to the potential of a poor radiosynthetic yield or unavoidable time delays, a lesser amount of radioactivity may be administered at the discretion of the site PI, based on whether clinically acceptable images can be acquired with the dose administered. Any such modifications of the agent infusion will be recorded.

10.8.2 Neoadjuvant Chemotherapy

Because the chemotherapy treatment is clinical standard of care, there will be no study-related restrictions or modifications on dosing. Chemotherapy agent and administered dose for each cycle will be recorded on the CRF.

10.9 Measurement of imaging agent safety and preliminary efficacy

10.9.1 Definitions

10.9.1.1 Positron emission Some radioactive compounds decay by positron emission. Once a positron is emitted, it encounters a nearby electron, resulting in an annihilation reaction that produces two 511 keV photons (gamma emissions) directed approximately 180 degrees apart. The distance that a positron travels prior to annihilation is dependent on the energy of the positron and the electron density of the local environment.

10.9.1.2 Positron Emission Tomography (PET) A PET imaging device measures the number of 511keV gamma emissions that occur in coincidence. In the simplest example, the location of the annihilation lies along the straight line connecting the two coincident photons. By making such measurements around the entire subject (i.e. a full ring detector configuration), the location of the original positron emission can be determined, and a three dimensional image (count density distribution) can be made. All sequential images for a given subject will occur on the same or technically equivalent PET scanner.

10.9.1.3 Computed Tomography (CT) CT is a tomographic x-ray technique that results in multiple 2D image slices based on the density of the tissues studied. These high resolution anatomic images are routinely used to identify normal and abnormal structures based on differences in x-ray attenuation.

10.9.1.4 Standardized Uptake Value (SUV) The SUV is a measurement of the radioactive counts within a region of interest (ROI) drawn on an image, normalized to the subject's body weight/ injected tracer activity.

10.9.1.5 Static PET Imaging Clinical PET imaging generally involves obtaining multiple static PET images at the appropriate number of bed positions to cover the body area of interest (generally mid-ear through upper thigh for a whole body study, ~5-7 bed positions). Each emission scan (bed position) takes 5 minutes to acquire, resulting in a ~25-35 minute scan time plus the time required for the transmission scan (<1min for whole body).

10.9.1.6 Dynamic PET Imaging Dynamic imaging of a single bed position including the primary breast tumor in the field of view can be performed so that the changes in radiotracer concentration can be measured over time. A time activity curve can be obtained by measuring the counts within a region of interest over the time imaged. Any number of the resultant dynamic frames can be summed to form a composite 'static' image. A portion of the left ventricle should be included in the field of view**.**

10.10 Guidelines for Defining Imaging Agent Signal

10.10.1 PET Image Acquisition [F-18]FLT

Participant must be scanned on PET scanners that have been qualified by the ACRIN PET Core Laboratory. Specific image acquisition and reconstruction protocols can be found on the ACRIN web site under Imaging Materials at www.acrin.org/6688_protocol.aspx

10.11 FLT PET/CT image analysis

Preliminary data suggests that [F-18]FLT is essentially irreversibly phosphorylated and trapped inside cells during the imaging period. Prior to this point, however, there is a flux of unphosphorylated [F-18]FLT (reversible component). If the rate of phosphorylation (k_3) is low, then the early contribution of perfusion to the measured tumor activity is high. Assuming the phosphorylation rate correlates to the proliferative rate, a high rate of phosphorylation means that the cumulative measured tumor activity should be representative of cellular proliferation.

Volumes of interest (VOIs) will be drawn around the target lesions on the [F-18] FLT images based on the tumor localization on the baseline CT. [F-18] FLT PET studies will be registered based on their corresponding CT transmission scans images, and time activity curves (TACs) will be calculated as described in section 10.6.

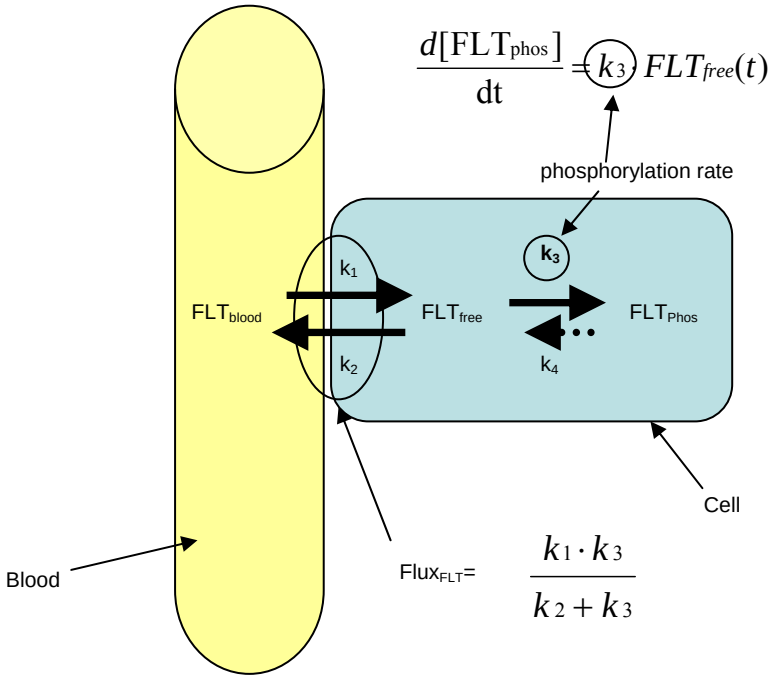


Figure 2. Kinetic model for [F-18]FLT. k_4 is assumed to be negligible at later time points. k_1 and k_2 are assumed to be equal, k_3 is assumed to be the dominate factor in [F-18]FLT accumulation in tumors over time.

10.11.1 Compartmental and Patlak Graphical Analysis

A 3-compartment data analysis will be performed to estimate values for the rate constants and [F-18] FLT flux parameters base on the model in Figure 2.

The Patlak graphical method enables estimation of the influx rate constant (K_i) and the volume of distribution (V_d) of unphosphorylated [F-18] FLT within the ROI from the data. The plot of ROI activity/Plasma Activity vs. $\int (PlasmaActivity * dt) / PlasmaActivity$ will become linear for irreversible systems. The slope of the linear portion is Ki and the y-intercept is V_d. The primary assumptions of these estimations are that the uptake of tracer is irreversible and that exchange of [F-18]FLT between the plasma and extracellular compartments has reached equilibrium. The rate of clearance of [F-18] FLT from the blood is equal to the Patlak influx rate constant (K_i) and is equal to $k_1k_3/(k_2+k_3)$.

10.11.3 Single Time point [F-18] FLT SUV

The optimal scenario would be that a single SUV measurement could be used to characterize the [F-18] FLT uptake. The amount of tracer present at a single imaging time point, though even less robust, may

be predictive, as it will represent the amount of [F-18] FLT phosphorylated from the time of injection to the time the image was acquired.

The uptake parameters listed in Table 3 will be determined from each imaging session. The change in these parameters between baseline and mid therapy sessions will then be calculated.

Table 3. [F-18]FLT PET Parameters from each imaging session

SUV_{30}	Patlak slope
ΔSUV_{30-60}	$Flux_{FLT}$
SUV_{60}	k_3

10.12 Other Comparators

10.12.1 Pathologic Response

As per accepted criteria (Wolmark, 2001), a pathologic complete response (pCR) is defined as the absence of viable invasive tumor at histopathologic examination of the post-therapy surgical specimen. This analysis will be performed at the treating site and reviewed at the central site at VCU. The presence of residual non-invasive cancer (DCIS) in the absence of viable invasive cancer is still considered a pCR. This is a dichotomous response assessment, either pCR or other than pCR.

A secondary related measure will also be assessed, the residual cancer burden (RCB) which will be used for secondary objectives and is described in Section 17.4.

10.12.2 Clinical Response

Clinical Response will be assessed as per the routine of the treating physician. This is based upon the percent change in anatomic tumor size between the pre-, early-, and post-treatment time points. The assessment of size will be made per routine of the treating physician and will typically be performed by one of the following: physical examination, mammography, ultrasound, or breast MRI. However, the same method should be used consistently for each patient throughout this study. The categorization of clinical response is categorized as described in Table 4.

Table 4. Clinical Response Criteria (http://ctep.cancer.gov/forms/quickrcst.doc)

Response Category	***Criterion***
Complete Response (CR):	Disappearance of the primary tumor
Partial Response (PR):	At least a 30% decrease in the LD of the primary tumor, from the baseline LD
Progressive Disease (PD):	At least a 20% increase in the LD of the primary tumor, taking as reference the smallest LD recorded since treatment started
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD

10.12.3 CT Transmission Imaging and Response

Since CT size assessment will be available on patients undergoing FLT PET by PET/CT devices, we will also compare FLT PET results to size changes based upon CT. The largest diameter and three-dimensional tumor volume measurements will be used to estimate tumor size when tumor is visualized on CT, when available. The percent change in tumor size between the pre- and post-treatment studies will be used as an additional indicator of treatment response.

10.13 Data Analysis

As described in Section 3.1, the primary goal of our endpoints will be the change in [F-18]FLT uptake parameters between the initial baseline and the early-therapy imaging studies as a predictor for pathological complete

response. Additional comparisons with imaging, pathologic, and clinical data will also be performed. These comparisons are summarized in Table 5.

Table 5. Planned Comparisons with FLT parameters

[F-18] FLT Parameters	*Compared To*
Pre-therapy (FLT-1) uptake parameters	Ki-67/ mitotic index of biopsy specimen Clinical Response Pathological Response (pCR and RCB)
After one cycle, (FLT-2) uptake parameters (absolute values and percent change from FLT-1)	Clinical Response Response from other imaging modalities, as available Pathological Response (pCR and RCB)
Post-therapy (FLT-3) uptake parameters (absolute values and percent change from FLT-1)	Ki-67/ mitotic index of post surgical specimen Clinical Response Response from other imaging modalities, as available Pathological Response (pCR and RCB)

* if available

10.14 Image Submission

The protocol-required images must be in DICOM format on CD/DVD-ROM or submitted via the internet using sFTP, and transmitted along with an Imaging Transmittal Worksheet (ITW) which can be found on the ACRIN web site (www.acrin.org/6688_protocol.aspx). The required images must be submitted to ACRIN Imaging Core Lab. ACRIN can provide electronic image submission and anonymity utilities for participating institutions via TRIAD software. For support in sending the images via the internet using TRIAD, contact the representatives of the Image Management Center (IMC) via email at Triad-Support@phila.acr.org or via phone: 215-940-8820.

If required and part of the protocol, images maintained at ACRIN Headquarters Image Archive may be distributed to other participating sites, using sFTP, or CD-ROM where appropriate, for purposes of secondary review.

Removal of Confidential Participant Information: The header record on DICOM formatted image data, which often contains information identifying the participant by name, MUST be scrubbed before the image are transferred.

This involves replacing the following:

- Participant Name tag with the ACRIN Institution ID or number
- Participant ID tag with the ACRIN case number, and
- Other Participant ID tag with ACRIN Study Number.

sFTP Transfer: Digitally generated image files in DICOM v3.0 format can be transmitted to the ACRIN IMC via sFTP directly to the image archive. This can be performed using a customized software program or by using TRIAD software available from ACRIN. An Imaging Transmittal Worksheet (ITW) must be faxed at the time images are transmitted. Contact the ACRIN IMC for additional details at Triad-Support@phila.acr.org

A completed, signed ITW MUST accompany all imaging exams submitted to ACRIN for each time point. The ITW must be completed and faxed to 215-923-1737 at the time the images are being submitted. For exams submitted via media, this worksheet may be completed and included with the media shipment. Please affix a label to the jacket of the media to include: study name, site name, and case number, date of exam, time point, and type of imaging.

Please fax the ITW to:

ACRIN Core Lab at 215-923-1737

ATTN: ACRIN 6688 Imaging Specialist

In the event that the transfer of scrubbed image headers is not available, images may also be sent on a CD/DVD-ROM to the ACRIN IMC for transfer to the image archive. Please contact ACRIN prior to sending the media to confirm compatibility.

Images and the ITW may be mailed to:

American College of Radiology Imaging Network
PET/CT Core Laboratory
Attn: ACRIN 6688
1818 Market Street 16th floor
Philadelphia, PA 19103

10.15 Image Analysis

A central review and analysis of all PET/CT imaging will be performed at the Core Laboratory at the University of Washington. The centralized imaging analysis will be used for endpoint calculations. Radiologists are encouraged to review any images obtained at their local institutions and data will be shared at the culmination of the study.

11 ACRIN 6688 STUDY SPECIFIC RISKS/ADVERSE EVENT REPORTING

Qualifying Adverse Events (AEs), including Serious Adverse Events (SAEs), as defined herein, will be reported via the Adverse Event Expedited Reporting System (AdEERS) application. All Adverse Events, as defined herein, will, in addition, be reported via CDUS Complete, C3D, or other AE reporting system as specified below.

AdEERS is an electronic, internet based expedited Adverse Event reporting system operated by NCI/CTEP. It is generally used to capture and disseminate information on relatively significant Adverse Events, based upon trial stage, expectedness, severity, and attribution. However, it may be used to report adverse events of all types if AdEERS reporting is required per protocol.

For this study, Adverse Event reporting must follow the guidelines and timing requirements below. The latest version of the NCI/CTEP Adverse Event Reporting Requirements document, which is available at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/newadverse_2006.pdf

provides additional details, and may be consulted as a reference, but **does not supersede AE reporting as specified in this protocol.**

The electronic-AdEERS AE system is to be used for all 'expedited reporting' events as defined herein. If the system is temporarily unavailable, a paper and telephone/FAX based process is provided herein. If expedited AE data has been submitted via the manual (i.e. telephone/fax) process, it is to be re-submitted via the electronic AdEERS system as soon as is possible.

Recipients of faxed AdEERS Reports in case the system is temporarily unavailable:

To ACRIN
Attention: ACRIN PDRC Director
Maria Oh: moh@acr.org
Attention: ACRIN SAE Coordinator
Cornelia Worley: cworley@acr.org
RE: Adverse Event Report
ACRIN Protocol 6688

1818 Market Street
Suite 1600
Philadelphia, PA 19103

To Virginia Commonwealth University (VCU)
Paul R. Jolles, MD: prjolles@vcu.edu
Department of Radiology
PO Box 980001
Gateway Building, 2nd Floor
Virginia Commonwealth University
Richmond, VA 23298-0001

Megan M. Quinn: mmquinn@vcu.edu
Department of Radiology
1101 E Marshall Street (Room 4-065)
Richmond, Virginia 23298

CIP SAE Reporting Desk:
Phone: 301-897-1704
Fax : 301-897-7402
Email: CIPSAEReporting@tech-res.com
ATTN: CIP Support S&P Manager, Anna Edouard, MD

Adverse Event Reporting must follow the guidelines below. The ACRIN Adverse Event Reporting Manual [November, 2009 version or the most recent version available on the ACRIN web site] provides additional details and may be consulted as a reference, but does not supersede AE reporting as specified in this protocol.

11.1 General Definitions

Adverse Event (AE): For the purpose of this study, an Adverse Event is an untoward medical condition experienced by a study participant **during the Adverse Event reporting period defined in table in Section 11.9 of the protocol**, or by applicable guidance, regulation, or policy. An AE is any unfavorable or unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with participation in the study, **regardless of exposure to an agent or procedure, and regardless of whether it is considered to be caused by the agent, device, or process under investigation.**

If there is thought to be a conflict between the protocol and a regulatory or guidance source, consult the CIP Clinical Trials Branch. If a decision must be made pending final clarification, the stricter requirement should be applied.

Life-Threatening Adverse Event: A life-threatening AE is any adverse event that places the study participant, in the clinical opinion of the investigator, at immediate risk of death.

Serious Adverse Event (SAE): An SAE is defined as any untoward medical occurrence that meets any one of the following criteria:

- Results in death or is life-threatening at the time of the event
- Requires inpatient hospitalization, or prolongs a hospitalization
- Results in a persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (in a participant's offspring)
- Requires intervention to prevent any of the above, per the investigator/sponsor

NOTE: Any event that:

- Follows IND agent administration, AND

- Occurs within the Expedited AE reporting period defined in the Reporting Table [See Table in Section 11.8 of the protocol], AND
- Meets the definition of a Serious Adverse Event (SAE), as described above

MUST be reported through the AdEERS system.

All SAEs are to be followed by the investigator until resolution, stabilization, scientifically and clinically satisfactory explanation as to attribution and etiology, or until subject is lost to follow up.

Adverse Event Expedited Reporting System (AdEERS): AdEERS is a web-based system created by NCI for electronic submission of expedited AE reports & is to be used in this study.

Investigational Agent: An investigational agent is any agent held under an Investigational New Drug (IND) application. For purposes of this study, fluorine-18 3'-deoxy-3'- fluorothymidine (F-18 FLT) is an investigational agent.

Clinical Data Update System (CDUS/Complete CDUS): CDUS/CompleteCDUS is a data collection system used to capture clinical data. Complete CDUS is capable of capturing Adverse Event Data and is being used in this study. [See C3D, below.]

C3D: C3D is an integrated clinical trial data collection and AE reporting system for reporting of ALL Adverse Events, including those also requiring expedited reporting via (e)-AdEERS. Trials that will use C3D have been developed using C3D to create customized eCRFs.

This trial will use C3D. [See CDUS above.]

11.2 AE Reporting Requirements

The list of AEs [see Section 11.4 below], and the characteristics of an observed AE [see "Adverse Event Characteristics - Definitions" below, section 11.5 will determine whether the event requires expedited (via electronic-AdEERS) reporting **in addition** to routine reporting. For this study AdEERS reporting will be done electronically (via C3D/Complete CDUS) reporting.

NOTE: 24-Hour Notification for CIP IND Trials

The adverse event 24-hour notification requirement provides an early detection system for potential safety problems. Adverse events that must be reported within 24-hours of learning of the event are dependent upon the phase of trial, the agent/intervention (investigational or commercial), whether the event is expected or unexpected, the grade, and attribution. The table in Section 11.9 and footnotes to the table outline 24-hour notification requirements for AEs in trials utilizing an agent under a CIP IND. Adverse events that fulfill the 24-hour reporting requirement must be reported electronically via AdEERS. To ensure vigilance for AEs that require 24-hour notification, AdEERS is programmed to facilitate complete, timely submission.

11.3 Comprehensive Adverse Events & Potential Risks Lists (CAEPR)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single, complete list of reported and/or potential adverse events (AEs) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with bold and italicized text. **This subset of AEs (the ASAEL) contains events that are considered 'expected' for expedited reporting purposes only.**

CAEPR & ASAEL have been consulted in compiling the Adverse Events list in this protocol and the Informed Consent for the study.

Please refer to the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" at

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/newadverse_2006.pdf

for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information.

11.4 CAEPR for 3'-deoxy-3'-[F-18]fluorothymidine (NSC 743144)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_adeers for further clarification. The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for 3'-deoxy-3'-[F-18]fluorothymidine.

Version 1.0, July 1, 2010 [1]

Category (Body System)	Adverse Events[2] with Possible Relationship to 3'-deoxy-3'-[F-18]fluorothymidine (CTCAE v4.0 Term)	EXPECTED AEs FOR ADEERS REPORTING Agent Specific Adverse Event List (ASAEL)
	No AEs reported in human studies[2,3].	

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol, and the agent should be included in the e-mail.

[2]No adverse events have been attributed to Positron-Emission Tomography (PET) imaging/diagnostic administration of [3'-deoxy-3'-[F-18]fluorothymidine at the levels described in the Investigators Brochure. Therefore, no adverse events are expected as a result of the intravenous (IV) administration of 3'-deoxy-3'-[F-18]fluorothymidine for typical PET imaging applications.

[3]As with many intravenously administered agents, 3'-deoxy-3'-[F-18]fluorothymidine could cause an allergic reaction that could potentially pose a threat to life (anaphylaxis). This has not been observed in limited human exposure to date. Reasonable precautions should be taken, consistent with normal radiologic and clinical facility practice. The patient should be monitored until the PET procedure is completed, and trained personnel and emergency equipment should be available per facility standards.

For purposes of informed consent regarding reasonably foreseeable risks to subjects in trials utilizing 3'-deoxy-3'-[F-18]fluorothymidine, the following potential adverse events are considered extremely rare:

- **Injection-related risks that may include infection, or accidental extravasation of the dose that may lead to discomfort, localized pain, or infection.**
- **Risks related to allergic reaction/anaphylaxis that may be life threatening.**

Note: As with all PET imaging agents, 3'-deoxy-3'-[F-18]fluorothymidine is a radiopharmaceutical that decays with positron emission. As such, it poses an intrinsic radiation exposure risk. However, when administered in accordance with the Investigator's Brochure as a PET imaging agent, this risk is felt to be extremely small. The organ and total body doses associated with FLT PET imaging are comparable to or lower than those associated with other widely used clinical nuclear medicine procedures.

Note: 3'-deoxy-3'-[F-18]fluorothymidine in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Table of Estimated Effective Dose from Isotope and Scans associated with ACRIN 6688
(Sponsor: Cancer Imaging program, NCI, NIH)

Scans	Eligible Patients	PET Agent Dose	ED for maximum dose of isotope (mSv)	Equipment Type	PET/CT Image Acquisition	Attenuation (Transmission) Scan	ED from CT scan (mSv) (mean)	ED from CT scan (mSv) (Max)	ED from γ transmission scan (mSv) (max)	Total ED (mSv) (likely)	Total ED (mSv) (max)	ED (mSv) with transmis-sion scan
FLT prior to therapy (baseline)	All	2.6 MBq/kg (0.07 mCi/kg) maximum 185 MBq (5 mCi)	4.8	PET/CT or PET	Torso Scan 60 minutes dynamic post injection	low-dose CT scan or transmission	6.4	8.6	0.2	11.2	13.4	5
2nd FLT 5-10 days after initiation of therapy	All	2.6 MBq/kg (0.07 mCi/kg) maximum 185 MBq (5 mCi)	4.8	PET/CT or PET	Torso Scan 60 minutes dynamic post injection	low-dose CT scan or transmission	6.4	8.6	0.2	11.2	13.4	5
3rd FLT scan post therapy and pre-surgery	All	2.6 MBq/kg (0.07 mCi/kg) maximum 185 MBq (5 mCi)	4.8	PET/CT or PET	Torso Scan 60 minutes dynamic post injection	low-dose CT scan or transmission	6.4	8.6	0.2	11.2	13.4	5
3 FLT Scans	All	≤ 15 mCi	14.4				19.2	25.8	0.6	33.6	40.2	15

ED = Estimated Effective Dose in mSv
Likely dose = radionuclide + mean from CT scan (see report in Attachment A)

Adverse Events Related to Chemotherapy

AEs that occur within 24 hours (± 4 hours [approximately 10 half lives]) of FLT infusion will be reported, regardless of attribution/relatedness. Beyond this period, AEs that are determined to be definitely, probably, or possibly related to the subjects' underlying condition or therapy, that are ALSO determined to be unrelated or unlikely related to the agents and procedures specified by this protocol, will not be reported, Therefore, most AEs related to chemoradiation treatment are not expected to be reported for this trial. Sites will follow institutional policies and procedures for any reported side effects for chemoradiation therapy.

11.5 Adverse Event Characteristics

Expected Adverse Event: An expected AE is an event that is listed in the protocol or the Investigator's Brochure.

Unexpected Adverse Event: An unexpected AE is an event that is NOT listed in the protocol or the Investigator's Brochure.

Attribution: Attribution is a clinical determination, by the investigator, as to whether an AE is related to a medical treatment or procedure. Attribution categories are:

- **Definite:** The AE is **clearly related** to a treatment or procedure
- **Probable:** The AE is **likely related** to a treatment or procedure
- **Possible:** The AE **may be related** to a treatment or procedure
- **Unlikely:** The AE is **likely unrelated** to a treatment or procedure
- **Unrelated:** The AE is **clearly not related** to a treatment or procedure

NOTE: Attribution is part of the assessment of an adverse event. Determining that an event is 'unlikely related' or 'unrelated' to a study agent or procedure does NOT make the event unreportable, or disqualify the event as an AE. As defined above, an AE is reportable as specified herein if it occurred:

"during the Adverse Event reporting period defined in section 11.9 in **the protocol**, or by applicable guidance, regulation, or policy."

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

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

NOTE: Severity is graded on a CTCAE based scale for each CTCAE event. For example, an abnormal hemoglobin value is graded for severity from 1 to 5 [death] based upon where that value falls on the CTCAE scale of abnormal Hemoglobin values. "Severity" is NOT the same as "Seriousness," which is an overall assessment [See SAE above] that determines reporting requirements.

11.6 CTCAE term (AE description and grade)

The descriptions and grading scales found in CTCAE Version 4.0, used by protocol the most recent release version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. All appropriate clinical areas should have access to a copy of the most current CTCAE. A copy of the CTCAE can be downloaded from the CTEP web site (http://ctep.cancer.gov).]

11.7 Expectedness

AEs can be 'Unexpected' or 'Expected' [see above] for expedited reporting purposes only. 'Expected' AEs (i.e., the ASAEL) are bold and italicized in the CAEPR.

11.8 Expedited Adverse Event Reporting

Expedited AE reporting for this study must use electronic AdEERS (Adverse Event Expedited Reporting System), accessed via the CTEP home page (http://ctep.cancer.gov). Site personnel will be trained in required AE identification and reporting procedures. These requirements are briefly outlined in the table in section 11.9 below.

In the rare event that Electronic AdEERS [internet] access is lost, an AE report may be submitted using the following process:

1. Sites should download reporting forms in advance and store them locally for access in the event of internet unavailability. They can be found at:

http://ctep.cancer.gov/protocolDevelopment/default.htm#adverse_events_adeers

2. Site chooses Single or Multiple Agent template as appropriate

3. Site completes appropriate sections of the SAE submission form.

   NOTE: For 24-hour notification, site follows up with a faxed SAE submission within 5 business days.

4. Site faxes SAE submission form and any additional information (source documents) necessary for thorough review of the event(s) along with the SAE submission form to 301-897-7402, attention CIP SAE Team. The CIP SAE Reporting Desk may be contacted for assistance with any part of this procedure (Tel. 301-897-7497), and should be contacted to confirm receipt of materials sent during any period of AdEERS unavailability, or to provide guidance with the process as appropriate.

5. Site follows up with an email to CIPSAEReporting@tech-res.com notifying the SAE Team that an SAE form and additional information (if available) has been faxed.

6. **For IND studies:** the submission process is not considered complete until an AdEERS report has been submitted electronically.

7. Once AdEERS access is restored, an AE report submitted by the backup process must be entered electronically into AdEERS by the original submitter at the site.

8. AdEERS will be programmed for automatic electronic distribution of reports to the following individuals:
   Maria Oh: moh@acr.org
   Cornelia Worley: cworley@acr.org
   Paul R. Jolles, MD: prjolles@vcu.edu
   Megan M. Quinn: mmquinn@vcu.edu

11.9 Expedited Reporting Guidelines

ADEERS TABLE for REPORTING PHASE 1 THROUGH EARLY PHASE 2 CIP IND AGENT

EVENTS:

(F-18) FLT Phase 1 to Early Phase 2 AdEERS Reporting Requirements for Adverse Events occurring within One Day (24 hours) +/- 4 hours of the last use of an agent held under CIP IND								
	Grade 1	**Grade 2**		**Grade 3**				**Grades 4 & 5**
				Unexpected		**Expected**		
	Unexpected and Expected	**Unexpected**	**Expected**	**with Hospital-ization**	**without Hospital-ization**	**with Hospital-ization**	**without Hospital-ization**	**Unexpected and Expected**
Unrelated Unlikely	Not Required	Not Required	Not	10 Calendar Days	Not Required	10 Calendar	Not Required	24-Hour; 5 Calendar

			Required			Days		Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

Hospitalization is defined as initial hospitalization or prolongation of hospitalization for ≥ 24 hours, due to adverse event.

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to agent administration or other cause must be provided.

[1] AEs reported through AdEERS must also be reported in routine study data submissions (i.e. ACRIN AE case report form). Please see section 11.11 for routine reporting requirements.

11.10 Expedited AE reporting timelines defined:

- "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event, followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.

- "10 calendar days" - A complete AdEERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via AdEERS if the event occurs following use of an agent under a CIP IND.

11.11 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine C3D/Complete CDUS study data submissions. **AEs reported through AdEERS must also be reported in routine study data submissions (i.e. completion and submission of an AE case report form).** The following adverse events **must** be reported in routine study data submissions (i.e. AE case report form).

- Grade 1 Expected and Unexpected AEs that occur within **(24 hours) +/- 4 hours of the last use of FLT** regardless of attribution (**unrelated, unlikely, possible, probable or definite)** require routine reporting.
- Grade 2 Expected and Unexpected AEs that occur within **(24 hours) +/- 4 hours of the last use of FLT** regardless of attribution (**unrelated, unlikely, possible, probable or definite)** require routine reporting.
  - [Refer to Section 11.9 for expedited reporting requirements].

- Grade 3 Expected and Unexpected AEs that occur within **(24 hours) +/- 4 hours of the last use of FLT** regardless of attribution (**unrelated, unlikely, possible, probable or definite)**require routine reporting.
    - [Refer to Section 11.9 for expedited reporting requirements].

- Grade 4 Expected and Unexpected AEs that occur within **(24 hours) +/- 4 hours of the last use of FLT** regardless of attribution (**unrelated, unlikely, possible, probable or definite)**require routine reporting.
    - [Refer to Section 11.9 for expedited reporting requirements].

- Grade 5 Expected and Unexpected AEs that occur within **(24 hours) +/- 4 hours of the last use of FLT** regardless of attribution (**unrelated, unlikely, possible, probable or definite)**require routine reporting.
    - [Refer to Section 11.9 for expedited reporting requirements].

12 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 Institutional Review Board (IRB) for 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 ICF. The investigator will provide a copy(s) of IRB approval letter(s) for any amendment(s), and copy(s) of annual renewal(s).

All study participants in this study will be given an IRB-approved, site-specific ICF describing the study and providing sufficient information for participants to make informed decisions about their participation in this study (see Appendix I for an ICF template). The ICF 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 ICF before the participant is subjected to any study procedures. The approved ICF MUST be signed and dated by the study participant or legally acceptable representative and the investigator-designated research staff obtaining the consent. Any revisions to the ICF at any time during the trial will need to be submitted to the IRB for approval and submission to ACRIN PDRC.

13 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 Conflict of Interest policies and applicable federal, state, and local laws and regulations.

14 PUBLICATION 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, the Study Chair, and/or ACRIN Publication Committee. Any investigator involved in this study is obligated to provide ACRIN with complete test results and all clinical data obtained from the participants in this protocol. Investigators will follow the ACRIN Publication Policy (available online at www.acrin.org/PublicationsPolicy.aspx).

15 INSTITUTIONAL MONITORING AND AUDITS

The investigator will permit study-related 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 sites' study-related facilities (e.g. imaging centers, 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 these visits.

Oversight for this study at all sites will be provided by the investigator with delegation of appropriate responsibilities to sub-investigators and designated study personnel. They will ensure all entry criteria are met prior to the initiation of the protocol and all study procedures and reporting of adverse events are performed according to the IRB-approved protocol.

15.1 Monitoring

Monitoring ensures that the rights, safety and well-being of the participants are protected. Monitoring also makes certain that the trial is in compliance with the currently approved protocol/amendments, with GCP and applicable regulatory requirements. It will provide the site an opportunity to verify that reported trial data are accurate, complete and verifiable from source documents. Institutional monitoring will be implemented at several different time points during the conduct of the study.

Monitoring instructions will be sent to the site prior to the implementation of monitoring to aid in preparation for the review. The instructions will specify regulatory documents and participant case records scheduled to be monitored. Case report forms (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.

15.2 Audits

All participating institutions which enroll participants will be audited. The timing of initial on-site audit will depend on several factors including the rate of accrual (both study-wide and site-specific), the number of evaluable participants enrolled at an individual site, the status of the protocol and pending amendments, and monitoring status. Generally, audits will be conducted after the number of evaluable participants reaches 30% of targeted accrual, either study-wide and/or site-specific. Audits are typically scheduled to occur at least 3 months after an institution has been monitored, provided that monitoring did not identify issues that mandate immediate auditing. This schedule may be altered in the event of pending protocol amendments. Closure of the study to accrual will trigger an audit of all participating institutions not yet audited. Additionally, site-specific circumstances may prompt an audit at any time. Subsequent audits will be scheduled per the outcome of the initial audit. Audits can be conducted more frequently at the discretion of the protocol team. 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 ICFs 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, ACRIN Headquarters 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.

15.3 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. If an item is not mentioned (e.g., history and physical examination alluding to a condition, but no mention of a psychological condition), it will be assumed it is not present.

Research records for each case should contain copies of the source documents for the data collected and reported to ACRIN. If data are 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.

15.4 Case Report Forms (CRFs)

CRFs, both web-based and paper forms, 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 and are 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 source data will be medical record documentation will be considered a major protocol deficiency.

16 CRITERIA FOR REMOVAL FROM STUDY

Participants will go off-study for the reasons identified below and will be replaced with other eligible participants. Participants going off-study will not undergo additional study-related imaging or follow-up visits.

16.1 Participants who are unable to complete chemotherapy and undergo primary tumor surgery because of disease progression will remain eligible for the primary analysis, but will be removed from secondary analysis. Disease progression must be confirmed by the clinical team using RECIST criteria or other serial imaging measurements to remain eligible for primary analysis.

16.2 Participants who experience any serious adverse event from the FLT PET imaging procedure (as listed in Section 11.0) will be removed from the primary analysis, but will remain eligible for secondary analysis. Participants must complete the baseline PET scan (FLT-1) to remain eligible for secondary aim correlations.

16.3 Participants who do not complete planned therapy for toxicity will be removed from primary analysis.

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18 TISSUE SPECIMEN COLLECTION

For each participant, the tumor tissue obtained from the diagnostic biopsy for breast cancer and post-treatment breast surgery will be collected and sent to the Core Laboratory at Virginia Commonwealth University for purposes of analysis for tumor proliferation markers for future correlative research. No additional biopsy will be obtained. The Core Laboratory will collect and analyze tissue for immunohistochemical analysis. It is preferred that participating institutions send a paraffin block containing tumor tissue to VCU. Upon completion of analysis, the tissue block will be returned to the institution. If the participating institution is unable to release tissue block(s), the Core Laboratory will accept five (5) unstained slides that have been sectioned at a thickness of 5 microns. These glass slides will not be returned. If the VCU Core Laboratory determines the quality of the unstained slides to be unsatisfactory, additional unstained slides may be requested. Additionally, a copy of the pathology report and the pre-treatment and post-treatment imaging report(s) should be sent to the Core Laboratory if available. Sites will be provided with specific detailed collection instructions at the initiation of the trial. Shipping address for tissue samples and reports:

Megan Quinn
Virginia Commonwealth University Health System
1101 East Marshall Street, Room 4-065
P.O. Box 980470
Richmond, VA 23298-0470
RE: ACRIN 6688 Pathology

18.1 Ki-67 (MIB-1 antibody) Immunohistochemical staining

Monoclonal antibody staining will be performed at the Core Laboratory at Virginia Commonwealth University/Department of Pathology's Immunohistochemistry section. Briefly, representative sections (5 μm) of formalin-fixed, paraffin-embedded tissue from the diagnostic biopsy for breast cancer and post-treatment breast surgery will be collected will be cut and mounted on glass slides. An index of cellular proliferation will be determined by immunohistochemistry (IHC) using the mouse monoclonal MIB-1 antibody, which binds to the Ki-67 antigen, a nuclear protein common to proliferating cells. Ki-67 immunohistochemical staining will be scored. A Ki-67 score is defined as the percentage of total number of tumor cells with nuclear staining (to the nearest 10%) over 10 high powered fields (at 40X).

18.2 Mitotic index

Cellular staining from the diagnostic biopsy for breast cancer and post-treatment breast surgery will be performed at the Core Laboratory at Virginia Commonwealth University/Department of Pathology's Immunohistochemistry section. A mitotic index will be determined by H&E staining of tumor per 10 high power fields (x40). Both the mitotic index (H&E) and Ki-67 will be reported as a percentage. Mitotic indices will also be obtained and used as a proliferative marker.

18.3 Routine Clinical Histopathology

Diagnostic biopsy and post-treatment surgical specimens will be reviewed for tissue histology characteristics according to standard clinical procedures at the Core Laboratory at Virginia Commonwealth University/Department of Pathology's Immunohistochemistry section.

18.4 Calculation of Residual Cancer Burden

Although pCR is a dichotomous variable, the reality of tumor response to chemotherapy is a continuous variable with non-response ranging from very small residual tumor burden to resistant tumors with progressive disease. Therefore, continuous measures of residual cancer burden (RCB) would be expected to be more predictive of clinical outcome than simple dichotomous classification as currently practiced. RCB determined from routine pathologic materials may be a significant predictor of distant relapse-free survival (98). Different parameters will be collected and submitted to the data collection center for calculation of RCB and will include:

- size of the tumor bed
- cellularity of residual primary tumor
- percentage of DCIS component
- number of positive nodes
- size of macrometastasis

This online tool is available at http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3.

The Residual Cancer Burden calculation will use clinical information obtained from participating institutions and pathological analysis at VCU. As the calculation of RCB is dependent on the parameters provided by participating institutions, the Core Laboratory at Virginia Commonwealth University will calculate the RCB as long as the parameters needed for the calculation are present in the reports.

The presence of any invasive tumor cells will be considered negative for pathologic complete response. Please to refer to the Symmans et al publication and Appendix 1 for more detailed pathology methods (note that this appendix is only accessible through the online version) (98).

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APPENDIX I

ACRIN 6688

SAMPLE CONSENT FOR RESEARCH STUDY

A PHASE II STUDY OF *[]* FLUOROTHYMIDINE (FLT) IN INVASIVE BREAST CANCER

[Note: The American College of Radiology Imaging Network (ACRIN) does not monitor compliance with the Health Insurance Portability and Accountability Act (HIPAA); that is the responsibility of local Institutional Review Boards (IRBs). Local IRBs may choose to combine the authorization elements in the informed consent. Information on ACRIN's HIPAA policy, as well as a template for HIPAA authorization, can be found at www.acrin.org.]

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part. Please take your time to make your decision. Discuss it with your friends and family. The National Cancer Institute (NCI) booklet "Taking Part in Clinical Trials: What Cancer Patients Need to Know" is available from your treating doctor.

You are being asked to be in this study because you have breast cancer. We are going to evaluate the usefulness of imaging using a contrast agent to determine the effectiveness of the chemotherapy in treating breast cancer.

WHY IS THIS STUDY BEING DONE?
This research study is being done to test the effectiveness of a new imaging agent, [F-18] fluorothymidine (FLT), in predicting the success of chemotherapy treatment (shrinkage of tumor). Imaging agents are drugs that are given before or during an imaging procedure (like medical x-rays) to improve the quality of the images that are obtained.

When [F-18] FLT is used during positron emission tomography (PET) imaging, it may produce images that will provide useful information about the tumor's response to treatment. It is hoped that [F-18] FLT PET imaging performed before and during the course of your chemotherapy treatments will help to monitor your tumor status and show whether your tumor is growing or shrinking. In the future, this may enable doctors to tell early in the course of treatment whether tumors are responding to a particular treatment. Several studies using [F-18]FLT at other institutions have shown promising results. You will not have a direct benefit from being in this study, but will be contributing to the knowledge that may benefit future breast cancer patients.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
54 participants will take part in this study who are eligible and images are evaluable

WHAT IS INVOLVED IN THE STUDY?
FLT is an investigational imaging agent, which means that it has not been approved by the U. S. Food and Drug Administration (FDA).

Screening Visit
If you choose to take part in the study, you will be asked to come to the medical center within 4 weeks of beginning the first imaging study to determine whether you meet the study entrance requirements. At this time, your medical history will be taken, you will have a physical exam, and your vital signs (temperature, blood pressure, heart rate and blood oxygen level) may be recorded. Some of this information may be taken from your medical records. Blood will be drawn to test your blood chemistry and liver function. If some of these tests have been done previously and the results are available in your medical records, they will not be repeated. Tissue samples from your biopsy will be sent to the VCU Pathology Lab (please see Tumor Tissue Collection below).

PET (Positron Emission Tomography)/ CT (Computerized Tomography)
There will be three imaging sessions and each imaging session will take one day.

First Imaging Session
For your first imaging session, you will report to the PET Imaging Center. If you are a woman of childbearing potential (i.e., able to become pregnant), you will be asked to take a pregnancy test as per your institution's standard of care. For PET imaging, you will be asked to lie still on your back in the PET scanner. You will receive an IV injection of [F-18] FLT through a catheter (a small, flexible tube) placed in a vein in your arm or hand. You will then be asked to remain still on your back for approximately one hour and a half while images of your chest and then your whole body are obtained. PET imaging does NOT require breast compression but does require you to lie on your back and to remain still.

The first image will take approximately 60 minutes. After completing the first image, you will then be given an opportunity to leave the scanner and use the toilet if you would like. After the end of the first imaging session, you will receive a whole body image PET scan, which will take approximately 30 minutes. During this imaging session, you will also receive a CT scan which should take about 5 minutes

If you feel unable to tolerate the entire imaging session (which takes 90 minutes on the scanner), you should discuss this issue with the study doctor. When unable to tolerate the 90 minute imaging session, you may remain on the study if able to tolerate a shortened imaging session (which takes 30 minutes on the scanner).

Second Imaging Session
The imaging session described above in the ***First Imaging Session*** will be repeated after one week after you start chemotherapy.

Third Imaging Session
The imaging session described above will be repeated after you complete your course of chemotherapy and prior to surgery. This will be the last imaging session.

Surgery
After the last PET imaging session, you will be scheduled to have surgery to remove any remaining tumor that is left after chemotherapy. This surgery is the standard of care for the treatment of your disease and is not a part of the imaging research.

Tumor tissue collection

After you complete your chemotherapy and your imaging sessions, you will have surgery to remove any remaining tumor. Your study doctors will test this surgical tissue and biopsy tissue to look for markers that will signify tumor growth and help identify tumor staging. All your personal information will be removed from the sample.

The tumor tissue will be given only to the approved researchers and will not be sold. The research to be done on the tumor tissue has been reviewed and approved by the researcher's Institutional Review Board (IRB). The research done with your tumor tissue will probably not help you but it may help patients who have breast cancer in the future.

HOW LONG WILL I BE IN THE STUDY?
Your participation in this study will last throughout the course of your chemotherapy treatment and is predicted to take up to 9 months, depending upon the length of chemotherapy treatment used in your center. Your direct participation will consist of a screening visit and three combined PET/CT imaging sessions at pre-treatment, after one week, and post-therapy.

Your participation in this study may be stopped at any time by the study doctor or the sponsor without your consent. The reasons might include:

- The study doctor thinks it necessary for your health or safety;
- You have not followed study instructions;
- The sponsor has stopped the study;
- Administrative reasons require your withdrawal.

WHAT ARE THE RISKS OF THE STUDY?

[F-18] FLT PET and PET/CT RELATED RISKS, STRESS, OR DISCOMFORT

There are a few possible risks of [F-18]FLT PET and PET/CT scans. The most common ones are not considered serious. Any serious risks of [F-18]FLT PET or PET/CT scans are considered very unlikely. All of the known risks are described below.

Possible risks from having an intravenous (IV) injection:
- Bruising, pain, or infection at the injection site
- Leaking of IV fluid into tissues near the injection
- Allergic reaction, which could be serious or life threatening

Possible risks from having a PET scan in general:
- Claustrophobia (feeling anxious and 'closed in')
- Discomfort from lying still on your back for a total of about [60] minutes for each scan

Possible risks from radiation exposure:
The PET/CT scans used in this study to monitor your tumor status and your response to treatment do involve exposure to radiation. Each dose of [F-18]FLT will expose you to about one half of the amount of radiation in a routine CT scan of a large body area such as your abdomen. Because we also need to do a CT, along with the [F-18]FLT PET, you could get up to another full CT dose of radiation at each scan depending on the scanner used.

If you live in the US, you receive about 300 millirem of radiation each year. It comes from space and the earth around you. This is called "background radiation." A "millirem" (mrem) is a unit used to measure doses of radiation. These scans will expose you to radiation. The radiation dose to your whole body from each of your PET scans will range from about 540 millirem (for PET only scanners) to 1745 mrem (for PET/CT scanners). So the total radiation for all three imaging sessions can range from 1620 mrem to 5,235 mrem depending upon the type of scanner and imaging approach used. This dose can vary from person to person**.** Dual PET/CT scanners are proposed for this study but sites without dual scanners may use single PET scanners.

The following potential adverse events are considered extremely rare for participants in trials utilizing 3'-deoxy-3'-[F-18]fluorothymidine (FLT).

- Injection-related risks that may include infection, or accidental extravasation of the dose that may lead to discomfort, localized pain, or infection.
- Risks related to allergic reaction/anaphylaxis that may be life threatening.

As with any experimental procedure, there may be unanticipated side effects. If you notice anything that concerns you please contact the investigators (contact number given below).

For more information on PET/CT scans you can go to ACRIN's Website at: http://www.acrin.org/PATIENTS/ABOUTXRAYSANDSCANS/tabid/135/Default.aspx . You or your treating physician can print a description of PET/CT scans from this website.

WILL I KNOW MY RESULTS?
The final results from this research study may be shared with your treating physician when the study is completed. Your experimental imaging results may be released to the treating physician for your review, at your and the

treating physician's request. As FLT imaging remains experimental, these early imaging results are not yet fully understood. The experimental imaging results should not and will not change your treatment course and will not be part of the medical record.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?
This is not a treatment study and you are not expected to receive any direct medical benefits from your participation in this imaging study. The information learned from this study may lead to better treatment in the future for patients with breast cancer.

WHAT OTHER OPTIONS ARE THERE?
This is not a treatment study and you may choose not to participate in this study. You will receive the current standard of care for the treatment of breast cancer and your doctor can tell you more about the possible benefits of different available treatments.

WHAT ABOUT CONFIDENTIALITY?
Every attempt will be made by researchers to keep all the information collected in this study strictly confidential. Your personal information will be removed from the research records and any publications or presentations. Your records will be accessed by authorized personnel only and all data sent over the Internet will be coded so that other people cannot read it. Records of your progress while on the study will be kept by your treating institution and at ACRIN headquarters. Other organizations that have a role in this study will have access to and may copy both your medical and research records. These groups include the Food and Drug Administration (FDA), the National Cancer Institute (NCI), the local Institutional Review Board (IRB), the Brown University Center for Statistical Science, or other groups. Your images from the breast examination and some physical information about you (such as your age, gender, and possibly symptoms) as well as your biopsy and surgical tissue will be kept permanently on file for evaluation and use in future research. Your personal information may be disclosed if required by law and we cannot guarantee absolute confidentiality.

WHAT ARE THE COSTS?
The National Cancer Institute (NCI) will provide all the [[^{18}F] FLT PET/CT studies free of charge for this study. There will be no charges to you for any visits or tests related solely to the imaging research study. You or your insurance will be billed for any treatments or procedures that are a part of the standard of care for your cancer (these are the costs that you would have whether or not you participated in this research study).

WHAT IS THE PAYMENT FOR PARTICIPATION
The National Cancer Institute (NCI) does not pay subjects to participate in its research studies. However, NCI has authorized reimbursement of reasonable travel expenses, parking and meals associated with participation in this study. If you complete all four of the research-related visits in this study, you will receive $180. If you do not complete all the visits, you will be reimbursed $45 per visit for the ones you did complete.

WHAT IS THE COMPENSATION FOR INJURY
In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charge. No funds have been set aside to compensate you in the event of injury.

WHAT ARE MY RIGHTS AS A PARTICIPANT?
Taking part in this study is voluntary. You may choose not to take part or may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. Participation in this study may delay the beginning or continuation of your chemotherapy by up to 1 week. If you decide to leave the study, please contact the study doctor so that he/ she can tell you how to stop the study safely

A Data Safety and Monitoring Board, an independent group of experts, may be reviewing the data from this research throughout the study. We will tell you about the new information from this or other studies that may affect your health, welfare, or willingness to stay in this study.

WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS? ***(This section must be completed)***
For additional information about your health, you may contact:

_____	_____
Name	Telephone Number

For additional information about this study, you may contact:

_____	_____
Name	Telephone Number

For information about your rights as a research subject, you may contact:
(*OHRP suggests that this person not be the investigator or anyone else directly involved with the research)*

_____	_____
Name	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.

Visit the NCI's Web sites for comprehensive clinical trials information http://cancertrials.nci.nih.gov or the American College of Radiology Imaging Network's website **www.acrin.org.**

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

SIGNATURE
I have read all the above, asked questions, and received answers concerning areas I did not understand. I have had the opportunity to take this consent form home for review or discussion. I willingly give my consent to participate in this program. Upon signing this form I will receive a copy.

_____	_____
Participant (or Legal Representative) Signature	Date

APPENDIX II

Karnofsky and ECOG Performance Criteria

KARNOFSKY ACTIVITY	SCORE %	ECOG GRADE	ECOG ACTIVITY
Normal, no complaints on all predisease activities	100		Fully active, able to carry
Normal, only minor signs/symptoms	90	0	without restrictions
Normal activity, but requires effort	80		No physically strenuous activity, but ambulatory
		1	and able to carry out light
Unable to do active work, but able to care for self	70		or sedentary work (eg., office work, light house work
Able to care for most needs, requires occasional help	60		Ambulatory/capable of all self-care, unable to
		2	perform any work activities Up and about more than
Requires frequent medical help and considerable assistance	50		50% of waking hours
Disabled, needs special assistance	40		Capable of only limited care and and self-care, confined to
		3	bed or chair for more than 50% of waking hours.
Severely disabled, needs hospitalization, death not imminent	30		
Very sick, hospitalized, active support needed	20		Completely disabled,
		4	totally confined to bed or chair.
Cannot carry on any self-care.	10		
Dead	0	5	