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TITLE: Radiation Dosimetry of Intratumoral Injection of
Radionuclides into Human Breast Cancer

PRINCIPAL INVESTIGATOR: Franklin C. Wong, MD PhD

CONTRACTING ORGANIZATION: The University of Texas
M. D. Anderson Cancer Center

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13. ABSTRACT (Maximum 200 Words) This study has been designed to evaluation the spatial and temporal distribution as well as the radiation dosimetry of intratumoral injection of proprietary radiopharmaceuticals Ga-67 and Ga-68 Galium Iron Macroaggregates (GIMA). Our team has adopted a 2-prong approach for this study. While the refinement of the human protocol is ongoing, we continue on basic studies to investigate in vivo imaging methods and effects of other drugs under revised versions of our approved animal protocol. During this last year, we have gained the approval of our human protocol by our institutional IRB. We also worked along with recommendations from the Army for the approval of our human protocol by the Army Human Subject Protection Committee. We made revisions the approved human protocol and received preliminary approval from the Army since March 2005, awaiting the final approval of the revisions by the MDACC IRB. However, because of developments in our own institution, the committee overseeing our use of radioactive drug is no longer in operation. Our team has started to redirect our efforts to seek other approved avenues to conduct the human study in compliance with FDA regulation. Currently we are working on IND-approval processes and expect to initiate human studies within the next year.				
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Introduction

The efforts of our laboratory in this project continue to proceed in a two-prong approach. We continue to refine the human protocol (the IRB approved version of 7/2004 is enclosed in the Appendix I) to conform to the US Army IRB requirements. We have revised the single IRB-approved protocol into 2, one for each radiopharmaceuticals of Ga-67 GIMA and Ga-68 GIMA, respectively. In fact, both of the revised protocols (2005-0219 and 2005-0220 in Appendice II and III) have gained the preliminary approval from the Army Human Subject Protection Committee, pending final approval from our IRB. However, developments within our institution between 11/04 and 2/05 rendered the Radioactive Drug Research Committee inactive. This is the FDA-approved committee to oversee the conduct of human studies without the need for IND using certain radiopharmaceuticals, including both Ga-67 GIMA and Ga-68 GIMA. Our efforts have thus concentrated on compliance of FDA requirements to conduct these 2 protocols. Such changes are reflected on a revision of statement of work (Appendix IV) which has gained approved by the Army. We have started tightening the manufacturing processes for more detailed quality assurance. We have also worked with the institutional research administration to pursue the IND processes. We expect to do the first human study within the next year, once the approval processes are completed.

In the meantime, we conducted basic research studies to support future clinical applications. The basic research work includes preparation of different radiopharmaceuticals, imaging methods (ultrasound, MRI, PET and scintigraphy) for in vivo visualization of these radiopharmaceuticals, radiation dosimetry simulation, as well as toxicity and efficacy studies of intratumoral injection in the suppression of tumor growth in animals. While the refinement of the human protocol is still ongoing, the basic research efforts has lead to the publication of 3 abstracts (in the Appendices) and the continuing prosecution of our non-provisional patent application (US Patent Application Serial No.10/724,027) to the US Patent and Trademark Office. When the feasibility of using Gallium Iron Macroaggregates (GIMA) in human breast cancer is confirmed by the clinical studies, the results of our basic research will further guide the optimal choice of radiopharmaceuticals and strategies for the locoregional treatment of solid tumors.

Body

1) Revision of our IRB-approved protocol ID03-0070 into 2 protocols (2005-0219 and 2005-0220) which have gained preliminary approval from the Army Human Subject Protection Committee, pending our IRB approval. Initiation of IND processes to gain FDA approval of the studies, in lieu of the unfortunate loss of our institutional overseeing Radioactive Drug Research Committee.

2) Recruitment of patients- Because of the ongoing refinement of our IRB-approved protocols to conform to the FDA requirements, no patients has been recruited. However, we had worked with the collaborating breast surgeons, MRI radiologists and ultrasound radiologists to establish the logistics of the imaging work flow and recruitment.

3) Establishing MR imaging scheme for measurement of Iron contents- Although no study has been performed on human subjects. Under revisions of our institutional ACUF-approved animal protocol (Appendix V), we have performed rats experiments and confirm that the routine clinical protocol of T2-weighted GRE sequence is able to demonstrate the iron content after interstitial injection. (Abstract #1 in Appendix).

4) PET/scintigraphy for spatial and temporal changes- repeated animal experiments using Ga-67 GIMA have shown persistent retention of GIMA in the injection site (Abstract #2 and #4). However, because of the small size of a rat and the lower resolution of positrons (Ga-68) with PET, possible spatial change has not been evaluated. Such observation will be made with the human PET studies.

5) We have performed tumor suppression experiments on rats with selected radiopharmaceuticals including Ga-67 GIMA, Y-90 labeled Iron Macroaggregates (YIMA) and In-111 labeled Iron Macroaggregates InIMA, which were prepared similarly to GIMA (Abstract #3)

Key Research Accomplishments

- ❑ Confirmation of the feasibility of MRI and ultrasound in the detection of interstitially injected GIMA in rats
- ❑ Preparation of radiopharmaceuticals with therapeutic potentials and establishment of quality assurance procedures
- ❑ Demonstration of efficacy of intratumoral GIMA or YIMA in the suppression of tumor growth in rats
- ❑ Revision of IRB-approved human protocol ID03-0070 (Appendix I) into 2 protocols (Appendix II and III), pending approval by IRB and then the Army
- ❑ Development of Institutional ACUF approved animal protocol (Appendix V)

Reportable Outcomes

Presentations:

September 2004, Annual Meeting of European Association of Nuclear Medicine, Helsinki, Finland.

Wong, F. C. and Sparks R. B. Simulated Dosimetry Profiles of Locoregional Radionuclide Therapies using Radioactive Gallium Iron macroaggregates (GIMA). Eur. J. Nucl. Med. Imaging, 31(2 Suppl.): S293, 2004.

Wong, F. C. and Wang, S. Locoregional ablation of rat breast cancer using Ga-67 Iron macroaggregates (GIMA) and Y-90 Iron Macroaggregates (YIMA). Eur. J. Nucl. Med. Imaging, 31(2 Suppl.):S297, 2004.

Wong, F. C. and Wang, S. Paramagnetic Radiopharmaceuticals of Iron and Gadolinium Macroaggregates. Eur. J. Nucl. Med. Imaging, 31(2 Suppl.): S387, 2004.

June 2005

Wong FC, Wang Y, Fonnegra A, Kim EE. Sequestration of Particulate Radiopharmaceuticals after Intratumoral Injection into Breast Cancer Grafts in Rats. Era of Hope 2005, Department of Defense Breast Cancer Research No. 44. June, 2005

Patent Applications:

11/2003 US Patent Application Serial No.10/724,027 F. Wong and Shuang Wang. Radiopharmaceuticals and Radioactive Microspheres for Locoregional Ablation of Abnormal Tissues.- converted into non-provisional application in 7/2004- continuing prosecution.

Conclusions

We are proceeding with our 2-prong approach with different degree of success. The development of the human protocol for the conformance with FDA requirements is still ongoing, while we are still to overcome the unfortunate loss of the overseeing committee. Our preparatory animal studies have shown interesting and rewarding findings that will provide invaluable information for the design of radiopharmaceuticals and optimal choice of locoregional strategies for the treatment of solid tumors. Once our human study confirms the feasibility of locoregional radionuclide treatment in the breast, similar strategies can be designed for other radiopharmaceuticals and for other solid tumors.

References

Abstract #1

Wong, F. C. and Wang, S. Locoregional ablation of rat breast cancer using Ga-67 Iron macroaggregates (GIMA) and Y-90 Iron Macroaggregates (YIMA). Eur. J. Nucl. Med. Imaging, 31(2 Suppl.):S297, 2004.

Abstract #2

Wong, F. C. and Sparks R. B. Simulated Dosimetry Profiles of Locoregional Radionuclide Therapies using Radioactive Gallium Iron macroaggregates (GIMA). Eur. J. Nucl. Med. Imaging, 31(2 Suppl.): S293, 2004.

Abstract #3

Wong, F. C. and Wang, S. Paramagnetic Radiopharmaceuticals of Iron and Gadolinium Macroaggregates. Eur. J. Nucl. Med. Imaging, 31(2 Suppl.):S387, 2004.

Abstract #4

Wong FC, Wang Y, Fonnegra A, Kim EE. Sequestration of Particulate Radiopharmaceuticals after Intratumoral Injection into Breast Cancer Grafts in Rats. Era of Hope 2005, Department of Defense Breast Cancer Research No. 44. June, 2005



Protocol Page

Radiation Dosimetry of Intra-tumoral Injection of Radionuclides in Human Breast Cancer
ID03-0070

Core Protocol Information

Short Title	Intratumoral Injection of Radionuclides into Human Breast Cancer
Study Chair:	Edmund Kim
Additional Contact:	Franklin Wong
Department:	Nuclear Medicine
Phone:	713-794-1052
Unit:	59
Full Title:	Radiation Dosimetry of Intra-tumoral Injection of Radionuclides in Human Breast Cancer
Protocol Type:	Standard Protocol
Protocol Phase:	N/A
Version Status:	Activated 07/27/2004
Version:	06
Submitted by:	Franklin Wong--7/12/2004 4:04:14 PM
OPR Action:	Accepted by: Marion Olson -- 7/19/2004 2:37:07 PM

Protocol Body

1.0 Objectives

The Objectives are:

1. Use MRI to measure the spatial and temporal profiles of GIMA after intratumoral injection into breast cancer
2. Use Positron Emission Tomography (PET) for Ga-68 GIMA and high resolution gamma scintigraphy for Ga-67 GIMA to measure the spatial and temporal profiles of the radioactivity of GIMA after intratumoral injection into breast cancer;
3. Use the imaging data from MRI and nuclear imaging to calculate whole-body, organ, and locoregional radiation dosimetry to evaluate safety and efficacy factors for intratumoral GIMA.

The Hypotheses are:

1. After intratumoral injection, GIMA will be dispersed but remain contained in the tumor.
2. The radiation absorbed doses will be high within the tumor but low in the body and surrounding organs.

2.0 Background

Locoregional Radiation Therapy of Breast Cancer - a beginning

Multiple trials of breast conservation in patients treated with and without whole breast radiation have found that the majority (> 90%) of local recurrences occur at the site of surgical resection [1]. Clinical trials have confirmed the usefulness of sealed radionuclides as internal radiation sources for locoregional adjuvant treatment of breast cancer, as demonstrated by the recent FDA approval of MammoSite using Iridium-192 [2]. Therefore, conventional radiation treatment to the whole breast following breast conserving surgery may not be a necessary approach for the majority of women. More directed local treatment with radiotherapy appears to be safe and effective treatment. Conventional brachytherapy involves the implanting of sealed radiation sources implanted into the post-surgical field for several weeks [3, 4]. Recent clinical trials have reported favorable outcomes treating brain and breast cancer patients using a single implanted catheter filled with Iodine-125 Iotrex and Iridium-192 seeds irradiating the tissues around the post-surgical cavity (by Proxima Therapeutics, Inc.). This approach has recently gained FDA approval (GlialSite for brain tumor and MammoSite for breast cancer [1, 5, 6, 7]). Locoregional radionuclide therapy offer several desirable features: predictable dosimetry, the capability of being monitored, and short duration. Ablating breast tumors using intratumoral injection of radionuclides without has not been explored. This is due to the lack of requisite information on radionuclide dispersion and on radiation dosimetry in the tumor and surrounding tissues to establish efficacy and safety. This proposed study aims to explore the feasibility of using intratumoral injection of unsealed radionuclides as internal radiation sources.

Breast Lymphoscintigraphy - an opportunity to study radionuclides in human tissues

Breast lymphoscintigraphy is a nuclear medicine procedure that is increasingly important in the identification of sentinel lymph node(s). Typically, aliquot(s) of about 1cc containing 0.5 mCi of Technetium-99m (Tc-99m) labeled sulfur colloid (SC) is injected percutaneously into the tumor or breast tissues around the tumor. Smaller sizes (<0.22 micron) of SC allow better lymphatic drainage and therefore better visualization of the sentinel lymph node(s). Only a small fraction (<1%) [8, 9] of the SC injected ever drains via the lymphatics to allow visualization of the sentinel lymph node(s). Conversely, particles of larger sizes (>0.22 micron) or direct intratumoral (IT) injection of SC into the breast tumor reveals even less lymphatic drainage. Although unsealed, radionuclides injected into the tumor or surrounding tissues are indeed subject to spatial sequestration. The injection site appears spherical and unchanged (for days) on scintigrams. Although difficult to quantify, ultrasound guidance during selected breast lymphoscintigraphy shows that injections of SC into the breast tissue result in a larger dispersed volume which has not been adequately assessed. Radiation dosimetry of breast lymphoscintigraphy have shown variations up to ten-fold [10, 11, 12], partly because of the imprecision in determining the volume of the dispersed injectate. An injection of 0.5 mCi Tc99m SC delivers about 40 cGy to the injection site and 4 cGy to the sentinel lymph node. When standard guidelines are observed, there is good margin for radiation safety and the radiation absorbed dose to the sentinel lymph node is about one tenth that of the injection site [13]. The Medical Internal Radionuclide Dosimetry (MIRD) schemes require accurate determination of volume and residence time of dispersed radionuclides [14]. A recent report directly measured the injectate volume using the full-width half maximum (FWHM) of the injection site from the scintigram. The accuracy of this volume estimate was limited by the system resolution of 2 cm [12]. The search for an accurate measurement of the dispersed injectate volume for dosimetry has been futile because, besides the radioactivity, there is no other physical signal from the injected radionuclide for external imaging.

A paramagnetic radiopharmaceutical Gallium-Iron Macroaggregate (GIMA) has been identified to provide both radioactive and paramagnetic signals for external measurement. This study is designed to evaluate the volume of dispersion and radiation dosimetry of GIMA after intratumoral injection into untreated human breast tumor.

Radionuclide Dosimetry of Unsealed Sources- Simulated Radiation doses to tumor and surrounding tissues

Earlier general internal dosimetry schemes including MIRDose3 (an established Medical Internal Radiation Dosimetry program) do not provide depth dosimetry to account for surrounding tissues. Earlier reports of simulation are limited to specific radionuclides in specific configurations [15, 16]. In our study, Monte Carlo simulation for Y-90 Zevalin was applied and found helpful in defining regions of toxicity [17]. A simulation project using sphere and shell models with common core volumes of 0.4, 2, 10, 50 and 250 cc is continuing and we reported radiation dosimetry in the core and 30 concentric layers from 19 radionuclides [18]. As predicted before, the radiation absorbed doses to the sentinel lymph nodes will be about one tenth of those to the injection sites in the tumor. The extremes of heterogenous distribution of radionuclides in the lesion were reported using shell models assuming that all the radionuclide was confined to the first layer around the central cavity. There was little dosimetry difference

from the sphere models (<10%) in tissues beyond 1 cm. These sphere [18] and shell models [19] provide estimates of dosimetry ranges. Although the exact radiation dosimetry has yet to be determined, the radiation doses to the tumor can be estimated from the published biological half-life of 30 hours [20]. The 3 groups (0.25 mCi Ga-67 GIMA, 0.5 mCi Ga-67 GIMA and 0.5 mCi Ga-68 GIMA) of patients will receive estimated doses of 315, 632 and 463cGy respectively in the injection site, with a 10% isodose range of 0.02cm, 0.02 cm and 0.20 cm from the injection site edge respectively. Based on preclinical studies suggesting a total of 2% leakage of radiogallium in the form of free Ga(+3), the MIRDOSE3 models predict low radiation absorbed doses to the vital organs in units of cGy/mCi:

Simulated Dosimetr	Ga-68 GIMA	0.5 mCi Ga-68 GIMA	Ga-67 GIMA	0.2 mCi Ga-67 GIMA
		For this study		for this study
Organ	rad/mCi	Total rads	rad/mCi	Total rads
Adrenals	0.0130	0.0065	0.1200	0.0240
Brain	0.0025	0.0013	0.0140	0.0028
Breast w/injectate	1.0000	0.5000	22.0000	4.4000
Breast wo/injectate	0.0550	0.0275	0.5900	0.1180
Gallbladder Wall	0.0100	0.0050	0.0940	0.0188
LLI Wall	0.0030	0.0015	0.0290	0.0058
Small Intestine	0.0091	0.0046	0.0270	0.0054
Stomach	0.0140	0.0070	0.1500	0.0300
ULI Wall	0.0084	0.0042	0.0450	0.0090
Heart Wall	0.0560	0.0280	0.5900	0.1180
Kidneys	0.0072	0.0036	0.0590	0.0118
Liver	0.0170	0.0085	0.1700	0.0340
Lungs	0.0400	0.0200	0.4500	0.0900
Muscle	0.0110	0.0055	0.1000	0.0200
Ovaries	0.0026	0.0013	0.0150	0.0030
Pancreas	0.0140	0.0070	0.1400	0.0280
Red Marrow	0.0140	0.0070	0.1200	0.0240
Bone Surfaces	0.0120	0.0060	0.1700	0.0340
Skin	0.0170	0.0085	0.1600	0.0320
Spleen	0.0130	0.0065	0.1100	0.0220
Testes	0.0011	0.0006	0.0053	0.0011
Thymus	0.0530	0.0265	0.5800	0.1160
Thyroid	0.0090	0.0045	0.0790	0.0158
Urin Bladder Wall	0.0026	0.0013	0.0140	0.0028
Uterus	0.0028	0.0014	0.0170	0.0034
Total Body	0.0600	0.0300	0.3200	0.0640
EFF DOSE EQUIV	1.2000	0.6000	5.1000	1.0200
EFF DOSE	0.4200	0.2100	1.9000	0.3800

Using human breast tumor as a model system, dosimetric measurement will be achieved by acquiring the spatial and temporal distribution of injected GIMA, measured from MRI and nuclear imaging. Ga-68 GIMA (with a physical half-life of 1.1 hours) is used to take advantage of the higher resolution and sensitivity of PET to measure the short-term spatial distribution of GIMA; while Ga-67 GIMA (physical half-life of 78 hours) is used to measure the prolonged distribution of radioactivity using a gamma camera. Confirmation of the sequestration and

derivation of radiation dosimetry will permit variations to achieve high radiation dose for therapeutic effects. For instance, larger amounts of radioactivities may be achieved by using larger volumes of GIMA while maintaining the Ga/Fe ratio; alternatively, larger radioactivities may be delivered by increasing the Ga/Fe ratio while maintaining the volume of the injectate. Results from this dosimetric study will provide bases for the design of future phase I and II clinical trials to use this class of radiopharmaceuticals to treat selected subgroups of patients with breast cancers and to correlate with biologic markers.

3.0 Background Drug Information

A known radiopharmaceutical Ga-68 /Fe macroaggregates (GIMA) [20] that may provide paramagnetic signals for volume measurement by MR imaging and simultaneously emit gamma rays for nuclear imaging was identified . It has a biologic half-life of 30 hours, a physical half-life of 1.2 hours and measures 10-30 micron in size. It was used in human lung perfusion imaging in the 1970's until the advent of the current imaging agent of Tc-99m -macroalbumin aggregates. It was produced in a carrier-added (additional nonradioactive gallium) preparation (0.12 Ci/mole) containing large amounts of additional nonradioactive gallium which in turn caused dose-limiting toxicity [20]. Following similar steps while deleting the toxic nonradioactive gallium (carrier), our laboratory has managed to produce carrier-free GIMA (with Ga-68 and Ga-67, respectively) of good stability (>98% after incubation in PBS for 24 hours) and confirmed the large sizes (99%>0.5 microns). Additionally, we have demonstrated decreases in Gradient Echo (GRE) signals on MRI with increasing Fe contents in the concentration range intended for intratumoral injection. Ga-68 GIMA is a positron emitter with a physical half-life of 1.1 hours with which the short-term organ distribution will be monitored using PET to exploit the high sensitivity and good spatial resolution (advantages over gamma-camera). Ga-67 GIMA is a gamma-ray emitter with a physical half-life of 78 hours during which the long-term organ distribution of GIMA can be monitored using gamma-cameras.

3.1 Supplier/How Supplied

- 3.1.1 Carrier-free Gallium-68 GIMA will be prepared according to the method of Colombetti [20] with the exception that no non-radioactive gallium (carrier) will be added. The 1-hour short-lived isotope Ga-68 will be obtained from a Ga-68 generator (Du Pont Radiopharmaceuticals, N, Billerica, MA). The product is a colloid suspended in saline. Aseptic procedures will be followed and pyrogenicity test will be performed and negativity will be confirmed before injection. Waterproof gloves will be worn by the personnel during preparation procedures. All vials will be brought to room temperature immediately prior to use. Part of the contents in the vials will be tested for the evaluation of pyrogenicity using the LAL assay (Whittaker Bioproducts, Walkersville, MD) which will last approximately 30 minutes. The synthesis and testing procedures typically last 80 minutes. Therefore, about three-fold more radioactivity will be prepared for each vial. The suspended colloid is available in screw-cap vials with radioactivity ranging from 0.1 to 2 mCi per vial. The total iron content is approximately 2 milligrams. The radioactivity purity is greater than 99% at the

time of calibration. The sterility of the products will be tested and monitored for 10 days for aerobic and anaerobic pathogens using BD Bactec Plus/F and Thioglycolate cultures (Becton and Dickinson, Sparks, MD), as a standard testing procedure of radiopharmaceuticals of short half-lives.

3.1.2 The South Texas Nuclear Pharmacy has agreed to provide carrier-free Ga67-GIMA in pyrogen-free (LAL test-negative) conditions and monitor sterility tests for each dose preparation for 10 days.

3.2 Determination of radioactivity of GIMA.
The radioactivity of either Ga-68 and Ga-67 will be measured by a Capintec dose calibrator and the volume will be noted.

3.3 Storage and Disposal

Unopened vials of Ga-68 GIMA and Ga-67 GIMA will be stored at room temperature and shielded from sunlight behind lead blocks.

3.4 Toxicity.

From the published results of human lung scanning, no adverse effects have been attributed to GIMA. Published toxicity of gallium compound has been correlated with the nonradioactive free gallium (carrier) with a limiting dose of 1mg, corresponding to the about 0.1 mCi of the low-specificity GIMA. The high specific activity GIMA prepared by our method contains no nonradioactive gallium and the physical amount of gallium (Ga-68) is one-billionth that of the earlier preparation [20] and is therefore well below the toxicity threshold. In fact, cancer patients injected with larger systemic doses up to 10 mCi carrier-free Ga-67 (as used in routine tumor localization imaging) do not have signs of toxicity.

4.0 Patient Eligibility

All Study patients must meet the eligibility criteria:

4.1 Eligible Patients

- 4.1.1 Patients must have signed the informed consent.
- 4.1.2 Patients must be 18 years of age or older
- 4.1.3 Patients must have breast cancer diagnosed by histopathology but no surgical resection of the tumor.
- 4.1.4 Patients should have received no previous focal external beam radiation therapy to the thorax.
- 4.1.5 Patients who have not received systemic or cytotoxic chemotherapy for the breast cancer under study. Patient under hormonal therapy alone will be eligible.
- 4.1.6 Patients with adequate platelets to avoid excessive bleeding and adequate white cells to avoid infection.
 - Granulocytes $\geq$ 1000 cells/mcl

- Platelets $\geq 40,000/\text{mcl}$
 - 4.1.7 Patients with Zubrod performance scale of 2 or below.
 - 4.1.8 Patients with breast tumor > 2 cm compressed thickness on mammogram but no tumor necrosis by MRI.
 - 4.1.9 Patients must have scheduled surgical resection (either mastectomy or conservation surgery) of the breast tumor within 2 weeks after injection.
- 4.2 Ineligible Patients
- 4.2.1 Patients of child-bearing age who have positive pregnancy test or are lactating.
 - 4.2.2 Patients with septicemia, severe infection or acute hepatitis.
 - 4.2.3 Patients who had radiation therapy or chemotherapy of the breast cancer prior to the planned surgery.
 - 4.2.4 Patients who had residual radiation from previous radionuclide administration, from the day of injection:
 - F-18 agents of more than 10 mCi within 2 days for the Ga-68 GIMA and Ga-67 GIMA groups;
 - In-111, Ga-67 or I-131 agents of more than 1 mCi within 14 days for the Ga-67 GIMA groups.
 - 4.2.5 Patients who cannot undergo MRI procedures (including nonvisualization of tumor on MRI and implants incompatible with MRI)
 - 4.2.6 Patients with claustrophobia cannot be entered for the Ga-67 GIMA groups because of the requirements of repeated MRI requiring repeated conscious sedation. Entry into Ga-68 GIMA will be acceptable with only one session of conscious sedation.
 - 4.2.7 Patients who have scheduled surgical resection of the breast tumor in less than 7 days are not eligible to enter the Ga-67 GIMA groups

5.0 Treatment Plan

Since breast cancer is predominantly a disease of the adults and some teenage pediatric patients and GIMA is expected to stay within the tumor, no dose adjustment is made for younger patients because of statistical requirements of uniformity for small sample sizes.

5.1 Human Study:

Patients will be recruited from female breast cancer patients scheduled for surgery at least one-week from the planned day of injection. One of the inclusion criteria will be a tumor size of $> 2\text{cm}$ in diameter but no tumor necrosis as determined by MRI. No spillage outside of the tumor is expected from an injection of 1 cc. A total of 10 patients in 2 groups of 5 patients each will be studied. The MRI and nuclear imaging studies will follow routine clinical procedure.

The first group of 5 patients will receive 0.5 mCi of Ga-68 GIMA (in 1 cc saline) intratumorally under MRI guidance and imaged with MRI and then undergo PET/CT studies at 1 hour after injection on the first day. The second groups will receive 0.2 mCi intratumoral injections of Ga-67 GIMA (in 1cc saline) under MRI guidance and then undergo whole-body scintigraphy, MRI and ultrasound at 2, 4, 24 hours and one of the 2nd, 3rd or 4th day after injection. Blood sample collection will

take place during each of the scintigraphy session. Although the exact radiation dosimetry has yet to be determined, the radiation doses to the tumor can be estimated from the published biological half-life of 30 hours [20]. These 2 groups of patients will receive estimated doses of 463 cGy and 262 cGy respectively in the tumor, with a 10% isodose range of 0.20 cm and 0.02 cm from the injection site edge respectively.

5.1.1 Patient Entry Requirements

Patients entered into this patient treatment study must meet eligibility requirements and sign the informed consent form. Each patient will be given a standard medical examination including a breast MRI with a medical history and laboratory work to determine eligibility. Patients will be accrued from the breast oncology clinics. When a patient with a breast cancer larger than 2 cm and who needs to undergo surgery is identified, the patient will be interviewed and provided information about this study. Signed informed consent will be obtained by Dr. Edmund Kim or other physician investigators (except Dr. Franklin Wong because of potential conflict of interest) no earlier than the next day after the initial interview to provide adequate time for the patient to consider participation.

5.2 MRI and Injection

The patients will be scanned using a GE Signa Lx 1.5 Tesla MRI scanner equipped with a high performance gradient system (amplitude = 22mT/m; slew rate = 120 T/m/s). A phased-array bilateral breast RF coil will be used to maximize the signal-to-noise ratio. A breast positioning system with two compression plates and Vitamin E markers will be used to hold the breast in a reproducible position. Image slice thickness will be approximately 6mm and the scan will be positioned to also include the axilla as much as possible.

Before entry into the study, patients will have been asked about metallic implants such as pacemakers and other devices as well as history of claustrophobia. Patient with these conditions will have been excluded as determined by the MRI radiologist collaborators. Specific and easily accessible tumors will be preferred over other locations. An MR-compatible 20-gauge needle of 7 cm in length will be used. If sedation is required and indicated, oral dose of 1 mg of Ativan or 5 mg of valium will be administered as per established procedures in the MRI suite.

The breast tumor will first be localized using a fast gradient echo T1-weighted 3D pulse sequence in the sagittal plane. An MR-compatible disposable sterile needle will be placed intra-tumorally. Areas of necrosis, if any (developed after the pre-injection MRI), will be avoided. An MR scan will be performed to ensure the proper location of the needle. Prior to injection, a high-resolution baseline image will be obtained using a gradient echo (GRE) pulse sequence with parameters selected to be sensitive to T2*. Injection may be preceded with surface or subcutaneous local anesthesia at the needle entrance through the surface. The GIMA will be injected in one single intratumoral injection into the tumor over 1 minute. The needles will then be slowly removed. This procedure is similar to routine breast lymphoscintigraphy. Immediately after injection, images (without breast compression) from multi-phase T2*-weighted MRI will be acquired using the same pulse sequence in quick

successions up to 1 hour. All subsequent MRI images will be without breast compression. The volume of the injectate will be determined from manual segmentation.

5.3 Nuclear imaging using PET/CT and High Resolution Scintigraphy

The earlier biodistribution of GIMA sequestration in the tumor and lymph nodes will be studied with Ga-68 GIMA using PET in the first group of 5 patients. Accurate localization and quantitation of radioactivity will be derived from the superior accuracy and resolution of PET. However, delayed PET studies will not be useful because Ga-68 decays rapidly (1.1 hour half-life). The second groups of patients will receive 0.2 mCi of Ga-67 GIMA to assess the prolonged phase (up to 5 days) of radioactivity distribution using whole-body gamma camera scanning.

After MR guided injection and imaging, the patient will be sent to the Nuclear Medicine/PET Clinic. The radioactivity residence time in the tumor and lymph nodes will be derived from serial scintigrams or PET scans. For patients injected with Ga-67 GIMA, scintigrams will be acquired in a Siemens dual-head ECAM gamma camera equipped with ultra-high resolution collimators. This combination will be able to achieve a system resolution of 7mm FWHM (tested with Tc-99m at a distance of 10 cm). One transmission scan will be performed before injection. Then, whole-body and planar imaging will continue at 2, 4, 24 hours and one of the 2nd, 3th or 4th day after injection. Patients injected with Ga-68 GIMA will undergo PET scans with attenuation correction using a high resolution Seimens HR Plus or a GE DST PET/CT scanner with spatial resolution of 6 mm covering the thorax. Images will be reconstructed in 2D/3D mode and the Ga-68 voxel concentration will be measured in the tumor and in the lymph node and be correlated with volumes from anatomic imaging including MRI and CT.

5.3.1 Urine and Blood Collection.

For the Ga-67 GIMA group, patients will be asked to provide urine samples at the following time intervals: before injection and during scintigraphy sessions at 2, 4, 24 hours and one of the 2nd, 3rd or 4th day after injection. Patients will be instructed and provided containers to collect all urine output up to the last day of imaging and urine samples will be collected during the scintigraphy sessions. Urine samples will have volume, time of excretion, and radioiodine content measured. All these patients will be asked to provide blood samples (3-4 cc each) at nuclear imaging sessions to characterize the systemic Ga-67 clearance and whole-body and organ radiation dosimetry. Blood samples will be collected by a physician, nurse or nuclear medicine technologist.

5.3.2 Ultrasonography

The injectate volumes will be monitored using ultrasonography using parameters for routine breast imaging, following each MRI session.

5.4 Dosimetry modeling of beta and gamma emissions from radionuclides

There are three components necessary for the estimation of absorbed doses to tissues

surrounding the injected activity: 1) The energy deposited in the surrounding tissues will be determined using radiation transport analysis [21], 2) the geometry of the activity distribution (source region) will be determined using MR image data, and 3) the total number of radioactive transitions that occur in the region will be determined using data from the scintigram. Both beta and gamma emissions will be evaluated. The total radiation absorbed doses will be derived for the tumor and surrounding tissues.

The volumetric data measured from MRI will be used to derive the S-values of the tumors using voxel-based simulation [22] to calculate the radiation absorbed doses to the injection sites and the surrounding tissues. Radiation dose rates, or S-values, will be compared with those from the sphere [18] and shell models [19] to evaluate the effects of potentially heterogeneously distributed injectate in the tumor. Such comparison will establish the boundaries of the models and aid choices of dosimetric methods in future studies.

5.5 Pathologic and Autoradiographic Evaluation

Histopathologic data will be collected from the surgical specimen obtained during the scheduled tumor resection (about 7-14 days from injection). If present, the histologic changes from radiation effects [23, 24] in and around the tumor/lymph nodes will be correlated with predicted and measured dosimetry. Selected histopathologic slices, as revealed from the reddish-brown appearance of GIMA, from patients injected with Ga-67 GIMA will be temporarily secured for autoradiography (overnight) to visualize geographic distribution. The fraction of injected radioactivity in the tumor, lymph nodes and injection sites will be determined, without physical damage to the specimens. The histologic changes and GIMA distribution from autoradiography will be used to correlate with MRI-derived volumetric data and with radioactivity data from nuclear imaging.

5.6 Potential Radiation Effects and Radiation Safety to the personnel

Because of the rapid decay of Ga-68 GIMA with half-life of 1.1 hours, negligible residual radioactivity (0.2 nano Ci) is expected 1 day after injection. Therefore, the planned surgery can be performed without health risk to the personnel. The surgical specimens will not undergo autoradiography although histopathologic correlations will be performed. With a biologic half-life of 30 hours and effective half-life of 21 hours, the residual Ga-67 GIMA will be less than 2% of the original dose after the 5 days of imaging (or 6 effective lives). At 7 days after injection, there will be essentially negligible residual amount (2 micro Ci, in total) of Ga-67 and health risk is minimal to health personnel, as long as general body-fluid precaution is followed including washing hands.

6.0 Pretreatment evaluation

Prior to the imaging procedures, subjects will be questioned to obtain a medical history, and given a complete physical examination including a mammogram, breast MRI and laboratory tests including CBC to determine eligibility. Patients who have not previously received a breast MRI (with contrast if necessary) will have one performed (at the cost of the study) prior to entry into the study to evaluate the tumor size and assure absence of tumor necrosis. Women with child-bearing potential will receive a pregnancy test.

7.0 Evaluation During Study

For the groups injected with Ga-67 GIMA, blood and urine samples will be taken from the patients at nuclear imaging time to measure radiogallium clearance and retention up to 5 days.

8.0 Evaluation of Toxicity

8.1 Toxicity

The radiation absorbed doses to the body and organs are low for both the low doses (0.5 mCi, maximum) of Ga-67 and Ga-68, compared with the routine dose of 8 mCi of Ga-67 citrate for tumor localization studies. The residual of 1 mg of Fe in the tissue is also not expect to present significant toxicity, considering the routine intramuscular injection of up to 1000 mg of iron sulfate for the treatment of anemia.

8.2 End-points of Study

End-point will be defined as grade III/IV toxicity (NCI criteria) in 2 or more of the 5 patients in a particular dose level.

9.0 Criteria for Removal from the Study

- 9.1 All patients will be followed with reasonable efforts until 1 month after injection. Any patient initially accepted into the study, but who subsequently is determined to be ineligible for radionuclide evaluation will be removed from the study. The reason and time of removal will be documented.
- 9.2 The development of unacceptable toxicity is defined as unpredictable, irreversible or grade 4 toxicity.
- 9.3 Non-compliance by patient with protocol requirements.
- 9.4 Patients have the right to withdraw from the study at any time without consequence. If a patient withdraws from the study, reasonable attempts will be made to document the reason for withdrawal.
- 9.5 Any patient can be removed at the discretion of the investigator or sponsor.

10.0 Number of Patients

This is a study of biodistribution. Two groups of 5 patients each will be studied with 0.2 mCi Ga-67 GIMA and 0.5 mCi Ga-68 GIMA respectively. Therefore, a total of 10 patients will be entered into the study. Only descriptive statistics (mean, variance, ratios and diagrams) will be applied to analyse the results of dispersed volumes by MRI and ultrasound, tumor and organ percentage of injected doses, dosimetry, potential toxicity and histologic changes.

11.0 Reporting Requirements

- 11.1 Any life-threatening and/or unexpected and serious (grade 3 or 4) adverse reaction will be reported immediately to the study chairman who, in turn, must notify the Surveillance Committee.
- 11.2 All patients experiencing an adverse reaction must have an adverse reaction form completed.

12.0 References

1. Vaidya JS, Baum M., Clinical and biological implications of the Milan breast conservation trials. *Eur J Cancer* Jul;34(8):1143-4, 1998.
2. Edmundson GK, Vicini FA, Chen PY, Mitchell C, Martinez AA. Dosimetric characteristics of the MammoSite RTS, a new breast brachytherapy applicator. *Int. J. of Radiat. Oncol. Biol. Physics.* 52(4):1132-9, 2002.
3. Nag S, Kuske RR, Cicini FA, Arthur DW and Wwicker RD. Brachytherapy in the treatment of breast cancer. *Oncology (Huntington).* 15(2): 195-202, 2001.
4. King TA, Bolston JS, Kuske RR, Fuhrman GM, Scroggins TG and Jiang XZ Long-term results of wide-field brachytherapy as the sole method of radiation therapy after segmental mastectomy for T(is, 1,2) breast cancer. *Amer. J. Surg.* 180(4):299-304, 2000.
5. Vicini FA, Baglan KL, Kestin LL, Mitchell C, Chen PY, Frazier RC et al. Accelerated treatment of breast cancer. *J. Clin. Oncol.* 19(7):1993, 2001.
6. deGuzman AF, Karvelis SKC, Shaw EG, et al. Brachytherapy Of Re-Resected Malignant Glioma Cavity Margins Using An Inflatable Balloon Catheter And Liquid 125I Source: A Phase I Study. *J.Nucl.Med.* 41(5 Suppl): 274P, 2000.
7. Dempsey JF, Williams JA, Stubbs JB, Patrick TJ and Williamson JF. Dosimetric properties of a novel brachytherapy balloon applicator for the treatment of malignant brain-tumor resection-cavity margins. *Int. J. Radiat. Oncol. Biol. Phys.* 42(2): 421-429, 1998.
8. Johnson N, Soot L, Nelson J, Franzini MD, Vea H, Gruner S, Kulawiak L, Young K.

Sentinel node biopsy and internal mammary lymphatic mapping in breast cancer. American Journal of Surgery. 179(5):386-8, 2000.

9. Doting MH, Jansen L, Nieweg OE, Piers DA, Tiebosch AT, Koops HS, Rutgers EJ, Kroon BB, Peterse JL, Olmos RA, de Vries J. Lymphatic mapping with intralesional tracer administration in breast carcinoma patients. *Cancer*. 88(11):2546-52, 2000.
10. Bergqvist L, Strand SE, Persson B, et al. Dosimetry in lymphoscintigraphy of Tc-99m antimony sulfide colloid. *J Nucl Med* 1982; 23:698-705.
11. Glass EC, Basinski JE, Krasne DL, et al. Radiation safety considerations for sentinel node techniques. *Ann Surg Oncol* 1999; 6:10-11.
12. Hung JC, Wiseman GA, Wahner HW, et al. Filtered technetium-99m-sulfur colloid evaluated for lymphoscintigraphy. *J Nucl Med* 1995; 35:1895-1901.
13. Kim, E. E., Wong, F. C. Radiation Dosimetry and Safety in Lymphoscintigraphy. *In*: K. Hunt, M. Ross (eds), *The Mastery of Lymphatic Mapping*. New York. Springer-Verlag, (in press).
14. Loevinger R, Budinger TF, Watson EE. *MIRD Primer*. Society of Nuclear Medicine, 1991.
15. Evans JF, Stabin MG, Stubbs JB. Specific absorbed fractions of energy from internal photon sources in brain tumor and cerebrospinal fluid. *Medical Physics*. 22(3):331-40, 1995.
16. Ferrari M, Cremonesi M, Bartolomei M, Stabin M, Agosteo S, Chinol M, et al. Dosimetric Modeling for locoregional treatment of brain tumors with 99-Y-Biotin. *Eur. J. Nucl. Med.* 27(8):925, 2000.
17. Sparks, R.B., Crowe, E. A., Wong, F. C., Toohey, R. E., Siegel, J.A. Radiation dose distributions in normal tissue due to adjacent tumors containing I-131 or 90Y: *J Nucl Med*. 43(5):91P, 2002, Suppl.
18. Wong, F.C., Kim, E. E., Sparks, R., Wong, W-H, Yang, D.J. and Podoloff, D.A. Three Sphere Dosimetry Models (0.4 cc, 2cc and 10cc) of 12 Commonly Available Radionuclides for Locoregional Therapy or Extravasation. *J. Nucl. Med.* 42:243P, 2001.
19. Wong, F.C., Sparks, R., Kim, E. E., Yang, D. J., Podoloff, D. A. Simulation of radiation dosimetry in intra-cavitary injection of eight radionuclides in five shell models. *J Nucl Med*, 43(5):90P, 2002, Suppl.
20. Colombetti LG, Goodwin DA, Togami E. Ga-68-labeled macroaggregates for lung studies. *J. Nucl. Med.* 11(12): 704-707, 1970

21. MCNP manual, Monte Carlo N-Particle Transport Code System. RSIC 1994.
22. Yoriyaz H, Stabin MG, dos Santos A. Monte Carlo. MCNP-4B-based absorbed dose distribution estimates for patient-specific dosimetry. J Nucl Med. 42(4):662-9, 2001
23. McCormick B, Strom E., Craighead PS, et al, Radiation Therapy Oncology Group. Research Plan 2002-2006. Breast Cancer Working Group. Int. J. of Radiation Concol, Biol, Physics. 51(3Suppl 2):56-7, 2001.
24. Mirza AN, Fornage BD, Sneige N, uerer HM, Newman LA, Ames FC and Singletary SE. Radiofrequency ablation of solid tumors. Cancer J. 7(2):95-102, 2001.

**Informed Consent****INFORMED CONSENT/AUTHORIZATION FOR PARTICIPATION IN RESEARCH****Radiation Dosimetry of Intra-tumoral Injection of Radionuclides in Human Breast Cancer
ID03-0070**

Subtitle: Intratumoral Injection of Gallium-67 GIMA for Gamma Camera Imaging and Magnetic Resonance Imaging

1.

Participant's Name

I.D. Number

You are being asked to take part in this clinical research study at The University of Texas M.D. Anderson Cancer Center (hereinafter referred to as "UTMDACC" or "the institution"). This research study is strictly voluntary. This consent form explains why we are performing this research study and what your role will be if you choose to participate. This form also describes the possible risks connected with being in this study. After reviewing this information with the person responsible for your enrollment, you should know enough to be able to make an informed decision on whether you want to participate in the study. This study complies with all laws and regulations that apply.

You are being asked to take part in this study because you have breast cancer and you have a surgery scheduled to remove the cancer.

DESCRIPTION OF RESEARCH**2. PURPOSE OF STUDY**

The goal of this clinical research study is to learn how special radioactive molecules called gallium-iron macroaggregates (GIMA) distribute (travel and spread) in the body after they are injected into breast cancer tissue.

3. DESCRIPTION OF RESEARCH

For the current diagnostic procedure called [lymphoscintigraphy](#), small radioactive particles (called colloids) are injected into a lymph node. Then, a special nuclear medicine scanner is used to watch these particles slowly distribute in the body. Researchers noticed the larger the molecule, the slower the particles move in the body. In fact, some of the larger colloid molecules seem to stay at the site of injection with very little movement. Since the amount of radiation on these colloids is very low, they cannot be used to treat cancer. New molecules have been developed called GIMA. These GIMA molecules have been designed to act like the colloid molecules (very slow-moving). However, the GIMA molecules were made to carry larger radioactive particles. Since these molecules are very slow-moving, they can be injected directly into a tumor without spreading out very far from the site of injection. In this way, radiation can be delivered directly to the tumor tissue without spreading to normal tissue.

For this study, small particles of radiation (Ga-67) will be attached to GIMA molecules. These molecules will then be injected into the breast cancer tumor. Special scanners will be used (gamma camera and MRI) to "see" how far the molecules move away from the injection site. If it is found that the molecules do not move very far, in the future, more radiation particles can be attached to the GIMA molecules. These GIMA molecules can then be used to treat cancer with radiation. The radiation level in GIMA (but not its size) can be measured from outside of the body by a gamma camera. The amount of iron in GIMA can be measured from outside of the body by a MRI scanner. The distribution of iron in GIMA can be used to determine the size of the GIMA collection by the appearance of darkening in the images around the injection site. It will be injected by a MRI-compatible needle to avoid potential injury during MRI. The small amounts of iron (one thousandth of a gram) in the GIMA injection will not be sufficient to cause physical movement of the particles because the particles are firmly dispersed within the tumor tissues. Ultrasound measurement is non-invasive and may provide similar size information and may in fact prove to be a more convenient method to monitor the size of GIMA in the future. Combining the radiation level and size measurements will allow researchers to [determine how](#) much radiation is delivered to the tumor and to other organs (if any).

Participants will receive a GIMA with a particle of radiation attached (Ga-67) that gives off a lower amount of radiation (0.2 mCi). The amount of radiation you will receive from the GIMA molecules is very low, about the same as a routine nuclear medicine scan.

The GIMA will be injected directly into the tumor tissue with the help of a MRI scanner. The MRI scanner will be used to make sure the needle is inserted directly into the tumor. Also, MRI scans will be done within one hour after the injection. An ultrasound examination of the injection site will also be performed to confirm the location of the GIMA particles

You will undergo nuclear imaging scans in the nuclear medicine clinic at 1, 2, and 4 hours after the injection as well as once a day for the next 4 days. You will also have MRI scans and ultrasound for the first 4 days after the injection. You will be asked to have blood (1 teaspoon) and urine samples collected during each imaging session.

Participants will have their regularly scheduled surgery. The tissue that is collected during the procedure will be studied to see the effect of the treatment on the tissue. Part of the tissue collected will be retained for analysis of the small amount of radioactivity left.

This is an investigational study. The research human use of radioactive Ga-67 GIMA has been approved by the UTMDACC Radiation Drug Research Committee which has been authorized by the FDA. The GIMA, nuclear imaging scans, ultrasound examinations and MRI scans performed for this study will be provided free of charge. Because participation in the Ga-67 GIMA group requires additional trips to UTMDACC over 5 days, you may be partially reimbursed for lodging expenses. If you live more than 50 miles from UTMDACC, and choose to stay in a local hotel during the study, you may be partially reimbursed up to 4 nights (up to \$70 per night). Up to 5 participants will take part in this Ga-67 GIMA portion of the research and up to a total of 10 participants will take part in this study. All will be enrolled at UTMDACC.

This protocol is partially funded by a research grant from United States Army Medical Research and Materiel Command. It should be noted that representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as a part of their responsibility to protect human subjects in research. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this study..

4. RISKS, SIDE EFFECTS, AND DISCOMFORTS TO PARTICIPANTS

While on this study, you are at risk for the side effects listed in this form. You should discuss these with the study doctor or your regular doctor. The known side effects are listed in this form, but they will vary from person to person. Many side effects go away shortly after the study drug is stopped, but in some cases, side effects may be serious, long lasting, and/or permanent and may even cause death.

Giving GIMA through the needle into the breast cancer tumor may result in pain at the injection site and or infection. GIMA have radiation particles attached to them. Radiation may increase the chance of developing new cancer. The radiation may also alter the cells in the tumor causing changes for the future planning of further treatment.

MRI (Magnetic Resonance Imaging) uses a large magnet instead of x-rays to take pictures of the inside of your body. People who have metal in their bodies (pacemakers, neurostimulators, certain clips, or staples from prior surgery) may not receive a MRI. The magnetic field used in MRI scanning may harm such people or cause problems with devices such as pacemakers. Part or all of the body will be passed into a long, narrow tube (scanner) which is open at both ends. The scanner has an intercom, which will allow you to speak to the doctors and staff during the procedure. The machine will produce a loud knocking noise. This is normal. You will be given earplugs to protect your ears. In addition, you may feel light vibrations throughout your body. Some people, especially those who have a tendency to feel uncomfortable in small or closed spaces, may feel "closed in" and become anxious while in the scanner. If you feel ill or anxious during scanning, doctors and the MRI staff will give comfort or the scanning will be stopped.

Ultrasound examination involves no ionizing radiation and is a safe routine procedure to evaluate breast tissues. In this study, it is used to confirm and follow the gross distribution of GIMA particles; while MRI is used to follow the refined disibution of GIMA.

Gamma camera scan is a medical technique that externally monitors the radioactivity in body and will be used to track the movement of the Ga-67 GIMA through the body. The gamma camera can take pictures of Ga-67 GIMA and "see" where it is in the body. By watching how Ga-67 GIMA travels through the body and studying where Ga-67 GIMA collects, researchers can learn if any radiation is deposited in certain organs in the body. Some people may feel "closed in" while lying in the scanner. However, the scanner is open at both ends and an intercom allows you to talk with doctors and staff. If you feel ill or anxious during scanning, doctors and/or technicians will give comfort or the scanning will be stopped.

For this study, the Ga-67 GIMA is radioactive substances of low radiation levels. The total amount of radiation you receive from this study is about the same as 1-2 chest x-rays.

You may experience pain, bleeding, and/or bruising from the blood draws. You may faint and/or develop an infection with redness and irritation of the vein at the site where blood is drawn

Adverse experiences that are both serious and unexpected will be immediately reported by telephone to the USAMRMC Deputy for regulatory compliance and quality (301-619-21650 (non-duty hours call 301-619-2165 **and** send information by facsimile to 301-619-7803). A written report will follow the initial telephone call within 3 working days. Address the written report to the U.S.Army Medical Research and Materiel Command, ATTN: MPMC-RCQ, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

This research study may involve unpredictable risks to the participants.

5. POTENTIAL BENEFITS

If this research study shows that GIMA stays at the injection site, future cancer therapies may be developed. This information may be of benefit to future patients. There are no benefits for you in this study.

6. ALTERNATE PROCEDURES OR TREATMENTS

You may choose not to take part in this study.

I understand that the following statements about this study are true:

7. According to the institutional conflict of interest policy, the principal investigator of this study and my primary physician cannot have a financial interest in any aspect of this research. However, in instances of medical emergency, it is possible that I may be cared for by a physician and/or administrator who has some form of financial interest in the sponsor of this study.

As of 06/10/2003, the following investigators on this study have disclosed an equity or stock option interest in the sponsor of this study: Through the University of Texas M. D. Anderson Cancer Center, Dr. Franklin C. Wong, a collaborator of this protocol has filed a patent application to the U.S. Patent and Trademark Office on radionulcide cancer therapies including the method of producing carrier-free GIMA. For these reasons, there is potential conflict of financial interest (intellectual properties) in this study involving Dr. Franklin C. Wong, The University of Texas, and UTMDACC. Dr. Franklin C. Wong is also the principal investigator of a U.S. Army Breast Cancer Research Grant supporting this study. Dr. E. Edmund Kim is the principal investigator who will supervise this study in UTMDACC. Either Dr. E. Edmund Kim or Dr. Gary Whitman will perform the injection of Ga-67 GIMA while you are in the MRI scanner.

The University of Texas M.D. Anderson Cancer Center has a financial interest in the sponsor of this study.

The University of Texas System has a financial interest in the sponsor of this study.

8. If I want to receive updated information regarding the financial interests of any physician and/or administrator at UTMDACC who has cared for me, I may call the Conflict of Interest Coordinator at (713) 792-3220. Upon request, I will be given

access to information disclosing the identity of all physicians and/or administrators who have a financial interest in the sponsor of this study.

9. My participation is voluntary.
10. I may ask any questions I have about this study, including financial considerations, of my treating physician. I may contact the principal investigator for this study Dr. Edmund Kim at 713-794-1052 or the Chairman of the institution's Surveillance Committee at 713-792-2933 with any questions that have to do with this study.
11. I may withdraw at any time without any penalty or loss of benefits. I should first discuss leaving the study with my physician. Should I withdraw from this study, I may still be treated at UTMDACC.
12. I understand that the study may be changed or stopped at any time by my doctor, the principal investigator, the study sponsor, or the Surveillance Committee of UTMDACC.
13. I will be informed of any new findings that might affect my willingness to continue participating in the study.
14. The institution will take appropriate steps to keep my personal information private. However, there is no guarantee of absolute privacy. The Food and Drug Administration ("FDA"), and/or United States Army Medical Research and Materiel Command might review my record to collect data or to see that the research is being done safely and correctly. Under certain circumstances, the FDA could be required to reveal the names of participants.
15. If I suffer injury as a direct result of participation in this study, the institution will provide reasonable medical care. I understand that I will not receive reimbursement of expenses or financial compensation from the institution, the sponsor, the investigators or the United States Army Medical Research and Materiel Command for this injury. If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, (insert name and telephone number of principal investigator). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U. S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221. for this injury. I may also contact the Chairman of UTMDACC's Surveillance Committee at 713-792-2933 with questions about study related injuries.
16. Unless otherwise stated in this consent form, all of the costs linked with this study, which are not covered by other payers (HMO, Health Insurance company, etc.), will be my responsibility.
17. I recognize that there are no plans to provide any compensation to me for any

patents or discoveries that may result from my participation in this research.

Authorization for Use and Disclosure of Protected Health Information

- A. During the course of this study, the research team at UTMDACC will be collecting information about you that they may share with the FDA and/or United States Army Medical Research and Material Command. This information may include your treatment schedule and the results of any tests, therapies, or procedures that you undergo for this study. The purpose of collecting and sharing this information is to learn about how the treatment affects your disease and any side effects you experience as a result of your treatment.

Your doctor and the research team may share study information with certain individuals. These individuals may include representatives of the FDA and/or the above listed sponsor, clinical study monitors who verify the accuracy of the information, individuals with medical backgrounds who determine the effect that the treatment has on your disease, and/or individuals who put all the study information together in report form. The UTMDACC research team may provide this information to the FDA and/or the above listed sponsor at any time.

- B. There is no expiration date for the use of this information as stated in this authorization. You may withdraw your authorization to share this information at any time in writing. More information on how to do this can be found in the UTMDACC Notice of Privacy Practices (NPP). You may contact the Office of Protocol Research at 713-792-2933 with questions about how to find the NPP.
- C. If you refuse to provide your authorization to disclose this protected health information, you will not be able to participate in the research project.
- D. I understand that my personal health information will be protected according to state and federal law. However, there is no guarantee that my information will remain confidential, and may be re-disclosed at some point.

CONSENT/AUTHORIZATION

Having read and understood the above, and having had the chance to ask questions about this study and reflect and consult with others, I give _____ permission to enroll me on this study. I have been given a copy of this consent.

SIGNATURE OF PARTICIPANT

DATE

WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR

DATE

SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP

DATE

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language that is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks and that the participant understood this explanation.

SIGNATURE OF STUDY DOCTOR OR PERSON OBTAINING
CONSENT

DATE

Translator

I have translated the above informed consent into _____ for this participant.
(Name of Language)

NAME OF TRANSLATOR

SIGNATURE OF TRANSLATOR

DATE



Informed Consent



INFORMED CONSENT/AUTHORIZATION FOR PARTICIPATION IN RESEARCH

Radiation Dosimetry of Intra-tumoral Injection of Radionuclides in Human Breast Cancer ID03-0070

Subtitle: Intratumoral Injection of Gallium-68 GIMA for Imaging by Positron Emission Tomography and Magnetic Resonance Imaging

1. _____
Participant's Name I.D. Number

You are being asked to take part in this clinical research study at The University of Texas M.D. Anderson Cancer Center (hereinafter referred to as "UTMDACC" or "the institution"). This research study is strictly voluntary. This consent form explains why we are performing this research study and what your role will be if you choose to participate. This form also describes the possible risks connected with being in this study. After reviewing this information with the person responsible for your enrollment, you should know enough to be able to make an informed decision on whether you want to participate in the study. This study complies with all laws and regulations that apply.

You are being asked to take part in this study because you have breast cancer and you have a surgery scheduled to remove the cancer.

DESCRIPTION OF RESEARCH

2. PURPOSE OF STUDY:

The goal of this clinical research study is to learn how special radioactive molecules called gallium-iron macroaggregates (GIMA) distribute (travel and spread) in the body after they are injected into breast cancer tissue.

3. DESCRIPTION OF RESEARCH:

For the current diagnostic procedure called [lymphoscintigraphy](#), small radioactive

particles (called colloids) are injected into a lymph node. Then, a special nuclear medicine scanner is used to watch these particles slowly distribute in the body. Researchers noticed the larger the molecule, the slower the particles move in the body. In fact, some of the larger colloid molecules seem to stay at the site of injection with very little movement. Since the amount of radiation on these colloids is very low, they cannot be used to treat cancer. New molecules have been developed called GIMA. These GIMA molecules have been designed to act like the colloid molecules (very slow moving). However, the GIMA molecules were made to carry larger radioactive particles. Since these molecules are very slow moving, they can be injected directly into a tumor without spreading out very far from the site of injection. In this way, radiation can be delivered directly to the tumor tissue without spreading to normal tissue.

For this study, small particles of radiation (Ga-68) will be attached to GIMA molecules. These molecules will then be injected into the breast cancer tumor. Special scanners will be used (PET and MRI) to "see" how far the molecules move away from the injection site. If it is found that the molecules do not move very far, in the future, more radiation particles can be attached to the GIMA molecules. These GIMA molecules can then be used to treat cancer with radiation. The radiation level in GIMA (but not its size) can be measured from outside of the body by scanners such as a PET scanner. The amount of iron in GIMA can be measured from outside of the body by a MRI scanner. The distribution of iron in GIMA can be used to determine the size of the GIMA collection by the appearance of darkening in the images around the injection site. It will be injected by a MRI-compatible needle to avoid potential injury during MRI. The small amounts of iron (one thousandth of a gram) in the GIMA injection will not be sufficient to cause physical movement of the particles because the particles are firmly dispersed within the tumor tissues. Ultrasound measurement is non-invasive and may provide similar size information and may in fact prove to be a more convenient method to monitor the size of GIMA in the future. Combining the radiation level and size measurements will allow researchers to [determine how](#) much radiation is delivered to the tumor and to other organs (if any).

Participants in this group will receive a GIMA with Ga-68 attached at a radiation dose of 0.50 mCi. The amount of radiation you will receive from the GIMA molecules is very low, about the same as a routine nuclear medicine scan (1-2 chest X-rays).

The GIMA will be injected directly into the tumor tissue with the help of a MRI scanner. The MRI scanner will be used to make sure the needle is inserted directly into the tumor. Also, MRI scans and ultrasound examination will be done within one hour after the injection and also after the 1-hour PET scanning which will take place after the initial MRI and ultrasound imaging. In other words, the sequence of event will be: an initial MRI for the injection of Ga-68 GIMA, a MRI and ultrasound to measure the particle size distribution, PET scanning to measure Ga-68 radioactivity distribution followed by repeated MRI and ultrasound to measure the particles.

These procedures are expected to last about 3 hours totally.

Participants in all groups will have their regularly scheduled surgery. The tissue that is collected during the procedure will be studied to see the effect of the treatment on the tissue.

This is an investigational study. The human research use of radioactive Ga-68 GIMA has been approved by the UTMDACC Radiation Drug Research Committee which has been authorized by the FDA. The Ga-68 GIMA, PET scans, ultrasound examinations and MRI scans performed for this study will be provided free of charge. Five participants will take part in this group. All will be enrolled at UTMDACC.

This protocol is partially funded by a research grant from United States Army Medical Research and Materiel Command. It should be noted that representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as a part of their responsibility to protect human subjects in research. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this study..

4. RISKS, SIDE EFFECTS, AND DISCOMFORTS TO PARTICIPANTS:

While on this study, you are at risk for the side effects listed in this form. You should discuss these with the study doctor or your regular doctor. The known side effects are listed in this form, but they will vary from person to person. Many side effects go away shortly after the study drug is stopped, but in some cases, side effects may be serious, long lasting, and/or permanent and may even cause death.

Giving Ga-68 GIMA through the needle into the breast cancer tumor may result in pain at the injection site and or infection. Ga-68 GIMA have radiation particles attached to them. Radiation may increase the chance of developing new cancer. The radiation may also alter the cells in the tumor causing changes for the future planning of further treatment.

MRI (Magnetic Resonance Imaging) uses a large magnet instead of x-rays to take pictures of the inside of your body. People who have metal in their bodies (pacemakers, neurostimulators, certain clips, or staples from prior surgery) may not receive a MRI. The magnetic field used in MRI scanning may harm such people or cause problems with devices such as pacemakers. Part or all of the body will be passed into a long, narrow tube (scanner) which is open at both ends. The scanner has an intercom, which will allow you to speak to the doctors and staff during the procedure. The machine will produce a loud knocking noise. This is normal. You will be given earplugs to protect your ears. In addition, you may feel light vibrations throughout your body. Some people, especially those who have a tendency to feel

uncomfortable in small or closed spaces, may feel "closed in" and become anxious while in the scanner. If you feel ill or anxious during scanning, doctors and the MRI staff will give comfort or the scanning will be stopped.

Ultrasound examination involves no ionizing radiation and is a safe routine procedure to evaluate breast tissues. In this study, it is used to confirm and follow the gross distribution of GIMA particles; while MRI is used to follow the refined distribution of GIMA.

A PET (Positron Emission Tomography) scan is a medical technique that monitors the activity in the brain and other organs and will be used to track the movement of the Ga-68 GIMA through the body. The PET scanner can take pictures of Ga-68 GIMA and "see" where it is in the body. By watching how Ga-68 GIMA travels through the body and studying where Ga-68 GIMA collects, researchers can learn if any radiation is deposited in certain organs in the body. Some people may feel "closed in" while lying in the scanner. However, the scanner is open at both ends and an intercom allows you to talk with doctors and staff. If you feel ill or anxious during scanning, doctors and/or technicians will give comfort or the scanning will be stopped.

For this study, the Ga-68 GIMA is radioactive substance of low radiation levels. The Ga-68 GIMA PET scan procedure also exposes your body to low-level of radiation. The total radiation you receive from these procedures is about the same as 1-2 chest x-rays.

Adverse experiences that are both serious and unexpected will be immediately reported by telephone to the USAMRMC Deputy for regulatory compliance and quality (301-619-21650 (non-duty hours call 301-619-2165 **and** send information by facsimile to 301-619-7803). A written report will follow the initial telephone call within 3 working days. Address the written report to the U.S.Army Medical Research and Materiel Command, ATTN: MRMCMC-RCQ, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

This research study may involve unpredictable risks to the participants.

5. POTENTIAL BENEFITS:

If this research study shows that GIMA stays at the injection site, future cancer therapies may be developed. This information may be of benefit to future patients. There are no benefits for you in this study.

6. ALTERNATE PROCEDURES OR TREATMENTS:

You may choose not to take part in this study.

I understand that the following statements about this study are true:

7. According to the institutional conflict of interest policy, the principal investigator of this study and my primary physician cannot have a financial interest in any aspect of this research. However, in instances of medical emergency, it is possible that I may be cared for by a physician and/or administrator who has some form of financial interest in the sponsor of this study.

As of 06/10/2003, the following investigators on this study have disclosed an equity or stock option interest in the sponsor of this study: Through the University of Texas M. D. Anderson Cancer Center, Dr. Franklin C. Wong, a collaborator of this protocol has filed a patent application to the U.S. Patent and Trademark Office on radionulcide cancer therapies including the method of producing carrier-free GIMA. For these reasons, there is potential conflict of financial interest (intellectual properties) of this study involving Dr. Franklin C. Wong, The University of Texas, and UTMDACC. Dr. Franklin C. Wong is also the principal investigator of a U.S. Army Breast Cancer Research Grant supporting this study. Dr. E. Edmund Kim is the principal investigator who will supervise this study in UTMDACC. Either Dr. E. Edmund Kim or Dr. Gary Whitman will perform the injection of Ga-68 GIMA while you are in the MRI scanner.

The University of Texas M.D. Anderson Cancer Center has a financial interest in the sponsor of this study.

The University of Texas System has a financial interest in the sponsor of this study.

8. If I want to receive updated information regarding the financial interests of any physician and/or administrator at UTMDACC who has cared for me, I may call the Conflict of Interest Coordinator at (713) 792-3220. Upon request, I will be given access to information disclosing the identity of all physicians and/or administrators who have a financial interest in the sponsor of this study.
9. My participation is voluntary.
10. I may ask any questions I have about this study, including financial considerations, of my treating physician. I may contact the principal investigator for this study Dr. Edmund Kim at 713-794-1052 or the Chairman of the institution's Surveillance Committee at 713-792-2933 with any questions that have to do with this study.
11. I may withdraw at any time without any penalty or loss of benefits. I should first discuss leaving the study with my physician. Should I withdraw from this study, I may still be treated at UTMDACC.

12. I understand that the study may be changed or stopped at any time by my doctor, the principal investigator, the study sponsor, or the Surveillance Committee of UTMDACC.
13. I will be informed of any new findings that might affect my willingness to continue participating in the study.
14. The institution will take appropriate steps to keep my personal information private. However, there is no guarantee of absolute privacy. The Food and Drug Administration ("FDA"), and/or United States Army Medical Research and Materiel Command might review my record to collect data or to see that the research is being done safely and correctly. Under certain circumstances, the FDA could be required to reveal the names of participants.
15. If I suffer injury as a direct result of participation in this study, the institution will provide reasonable medical care. I understand that I will not receive reimbursement of expenses or financial compensation from the institution, the sponsor, the investigators or the United States Army Medical Research and Materiel Command for this injury. If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, (insert name and telephone number of principal investigator). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U. S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221. for this injury. I may also contact the Chairman of UTMDACC's Surveillance Committee at 713-792-2933 with questions about study related injuries.
16. Unless otherwise stated in this consent form, all of the costs linked with this study, which are not covered by other payers (HMO, Health Insurance company, etc.), will be my responsibility.
17. I recognize that there are no plans to provide any compensation to me for any patents or discoveries that may result from my participation in this research.

Authorization for Use and Disclosure of Protected Health Information

- A. During the course of this study, the research team at UTMDACC will be collecting information about you that they may share with the FDA and/or United States Army Medical Research and Material Command. This information may include your treatment schedule and the results of any tests, therapies, or procedures that you undergo for this study. The purpose of collecting and sharing this information is to learn about how the treatment affects your disease and any side effects you experience as a result of your treatment.

Your doctor and the research team may share study information with certain individuals. These individuals may include representatives of the FDA and/or the above listed sponsor, clinical study monitors who verify the accuracy of the information, individuals with medical backgrounds who determine the effect that the treatment has on your disease, and/or individuals who put all the study information together in report form. The UTMDACC research team may provide this information to the FDA and/or the above listed sponsor at any time. There is no expiration date for the use of this information as stated in this authorization.

- B. You may withdraw your authorization to share this information at any time in writing. More information on how to do this can be found in the UTMDACC Notice of Privacy Practices (NPP). You may contact the Office of Protocol Research at 713-792-2933 with questions about how to find the NPP.
- C. If you refuse to provide your authorization to disclose this protected health information, you will not be able to participate in the research project.
- D. I understand that my personal health information will be protected according to state and federal law. However, there is no guarantee that my information will remain confidential, and may be re-disclosed at some point.

CONSENT/AUTHORIZATION

Having read and understood the above, and having had the chance to ask questions about this study and reflect and consult with others, I give _____ permission to enroll me on this study. I have been given a copy of this consent.

SIGNATURE OF PARTICIPANT

DATE

WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR

DATE

SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP

DATE

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language that is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks and that the participant understood this explanation.

SIGNATURE OF STUDY DOCTOR OR PERSON OBTAINING
CONSENT

DATE

Translator

I have translated the above informed consent into _____ for this participant.
(Name of Language)

NAME OF TRANSLATOR

SIGNATURE OF TRANSLATOR

DATE

PDOL Draft

Radiation Dosimetry from Intratumoral Injection of Radionuclides into
Human Breast Cancer Ga 67 GIMA
2005-0219

Core Protocol Information

Short Title	Intratumoral Injection of Radionuclides (Ga 67 GIMA) into Human Breast Cancer
Study Chair:	Gary Whitman
Additional Contact:	Franklin Wong
Department:	Nuclear Medicine
Phone:	713-794-4649
Unit:	59
Full Title:	Radiation Dosimetry from Intratumoral Injection of Radionuclides into Human Breast Cancer Ga 67 GIMA
Protocol Type:	Standard Protocol
Protocol Phase:	N/A
Version Status:	Draft
Version:	00

Protocol Body

1.0 Objectives

Select Section Title: 1.0 Objectives

The Objectives are:

1. Use MRI to measure the spatial and temporal profiles of GIMA after intratumoral injection into breast cancer
2. Use high resolution gamma scintigraphy for Ga-67 GIMA to measure the spatial and temporal profiles of the radioactivity of GIMA after intratumoral injection into breast cancer;
3. Use the imaging data from MRI and nuclear imaging to calculate whole-body, organ, and locoregional radiation dosimetry to evaluate safety and efficacy factors for intratumoral GIMA.

The Hypotheses are:

1. After intratumoral injection, GIMA will be dispersed but remain contained in the tumor.
2. The radiation absorbed doses will be high within the tumor but low in the body and surrounding organs.

2.0 Background

Select Section Title: 2.0 Background

Locoregional Radiation Therapy of Breast Cancer - a beginning

Multiple trials of breast conservation in patients treated with and without whole breast radiation have found that the majority (> 90%) of local recurrences occur at the site of surgical resection [1]. Clinical trials have confirmed the usefulness of sealed radionuclides as internal radiation sources for locoregional adjuvant treatment of breast cancer, as demonstrated by the recent FDA approval of MammoSite using Iridium-192 [2]. Therefore, conventional radiation treatment to the whole breast following breast conserving surgery may not be a necessary approach for the majority of women. More directed local treatment with radiotherapy appears to be safe and effective treatment. Conventional brachytherapy involves the implanting of sealed radiation sources implanted into the post-surgical field for several weeks [3, 4]. Recent clinical trials have reported favorable outcomes treating brain and breast cancer patients using a single implanted catheter filled with Iodine-125 Iotrex and Iridium-192 seeds irradiating the tissues around the post-surgical cavity (by Proxima Therapeutics, Inc.). This approach has recently gained FDA approval (GliaSite for brain tumor and MammoSite for breast cancer [1, 5, 6, 7]). Locoregional radionuclide therapy offers several desirable features: predictable dosimetry, the capability of being monitored, and short duration. Ablating breast tumors using intratumoral injection of radionuclides without sealing (e.g. by a catheter) has not been explored. This is due to the lack of requisite information on radionuclide dispersion and on radiation dosimetry in the tumor and surrounding tissues to establish efficacy and safety. This proposed study aims to explore the feasibility of using intratumoral injection of unsealed radionuclides as internal

radiation sources.

Breast Lymphoscintigraphy - an opportunity to study radionuclides in human tissues

Breast lymphoscintigraphy is a nuclear medicine procedure that is increasingly important in the identification of sentinel lymph node(s). Typically, aliquot(s) of about 1cc containing 0.5 mCi of Technetium-99m (Tc-99m) labeled sulfur colloid (SC) is injected percutaneously into the tumor or breast tissues around the tumor. Smaller sizes (<0.22 micron) of SC allow better lymphatic drainage and therefore better visualization of the sentinel lymph node(s). Only a small fraction ($<1\%$) [8, 9] of the SC injected ever drains via the lymphatics to allow visualization of the sentinel lymph node(s). Conversely, particles of larger sizes (>0.22 micron) or direct intratumoral (IT) injection of SC into the breast tumor reveals even less lymphatic drainage. Although unsealed, radionuclides injected into the tumor or surrounding tissues are indeed subject to spatial sequestration. The injection site appears spherical and unchanged (for days) on scintigrams. Although difficult to quantify, ultrasound guidance during selected breast lymphoscintigraphy shows that injections of SC into the breast tissue result in a larger dispersed volume which has not been adequately assessed. Radiation dosimetry of breast lymphoscintigraphy have shown variations up to ten-fold [10, 11, 12], partly because of the imprecision in determining the volume of the dispersed injectate. An injection of 0.5 mCi Tc99m SC delivers about 40 cGy to the injection site and 4 cGy to the sentinel lymph node. When standard guidelines are observed, there is good margin for radiation safety and the radiation absorbed dose to the sentinel lymph node is about one tenth that of the injection site [13]. The Medical Internal Radionuclide Dosimetry (MIRD) schemes require accurate determination of volume and residence time of dispersed radionuclides [14]. A recent report directly measured the injectate volume using the full-width half maximum (FWHM) of the injection site from the scintigram. The accuracy of this volume estimate was limited by the system resolution of 2 cm [12]. The search for an accurate measurement of the dispersed injectate volume for dosimetry has been futile because, besides the radioactivity, there is no other physical signal from the injected radionuclide for external imaging.

A paramagnetic radiopharmaceutical Gallium-Iron Macroaggregate (GIMA) has been identified to provide both radioactive and paramagnetic signals for external measurement. This study is designed to evaluate the volume of dispersion and radiation dosimetry of GIMA after intratumoral injection into untreated human breast tumor.

Radionuclide Dosimetry of Unsealed Sources- Simulated Radiation doses to tumor and surrounding tissues

Earlier general internal dosimetry schemes including MIRDose3 (an established Medical Internal Radiation Dosimetry program) do not provide depth dosimetry to account for surrounding tissues. Earlier reports of simulation are limited to specific radionuclides in specific configurations [15, 16]. In our study, Monte Carlo simulation for Y-90 Zevalin was applied and found helpful in defining regions of toxicity [17]. A simulation project using sphere and shell models with common core volumes of 0.4, 2, 10, 50 and 250 cc is continuing and we reported radiation dosimetry in the core and 30 concentric layers from 19 radionuclides [18]. As predicted before, the radiation absorbed doses to the sentinel lymph nodes will be about one

tenth of those to the injection sites in the tumor. The extremes of heterogeneous distribution of radionuclides in the lesion were reported using shell models assuming that all the radionuclide was confined to the first layer around the central cavity. There was little dosimetry difference from the sphere models (<10%) in tissues beyond 1 cm. These sphere [18] and shell models [19] provide estimates of dosimetry ranges. Although the exact radiation dosimetry has yet to be determined, the radiation doses to the tumor can be estimated from the published biological half-life of 30 hours [20]. This group (0.2 mCi Ga-67 GIMA) of 5 patients will receive estimated doses of 463cGy in the injection site, with a 10% isodose range of 0.02cm from the injection site edge. Based on preclinical studies suggesting a total of 2% leakage of radiogallium in the form of free Ga(+3), the MIRDose3 models predict low radiation absorbed doses to the vital organs in units of cGy/mCi:

SIMULATED DOSIMETRY	Ga-67 GIMA	0.2 Mcl Ga-67 GIMA for this study
ORGAN	Rad/mCi	Total rads
Adrenals	0.1200	0.0240
Brain	0.0140	0.0028
Breast w/ Injectate	22.0000	4.4000
Breast wo/Injectate	0.5900	0.1180
Gallbladder Wall	0.0940	0.0188
LLI Wall	0.0290	0.0058
Small Intestine	0.0270	0.0054
Stomach	0.1500	0.0300
ULI Wall	0.0450	0.0090
Heart Wall	0.5900	0.1180
Kidneys	0.0590	0.0118
Liver	0.1700	0.0340
Lungs	0.4500	0.0900
Muscle	0.1000	0.0200
Ovaries	0.0150	0.0030
Pancreas	0.1400	0.0280
Red Marrow	0.1200	0.0240
Bone Surfaces	0.1700	0.0340
Skin	0.1600	0.0320
Spleen	0.1100	0.0220
Testes	0.0053	0.0011
Thymus	0.5800	0.1160
Thyroid	0.0790	0.0158
Urine Bladder Wall	0.0140	0.0028
Uterus	0.0170	0.0034
Total Body	0.3200	0.0640
EFF DOSE EQUIV	5.1000	1.0200
EFF DOSE	1.9000	0.3800

Using human breast tumor as a model system, dosimetric measurement will be achieved by acquiring the spatial and temporal distribution of injected GIMA, measured from MRI and nuclear imaging. Ga-67 GIMA (physical half-life of 78 hours) is used to measure the prolonged distribution of radioactivity using a gamma camera. Confirmation of the sequestration and derivation of radiation dosimetry will permit variations to achieve high radiation dose for therapeutic effects. For instance, larger amounts of radioactivities may be achieved by using larger volumes of GIMA while maintaining the Ga/Fe ratio; alternatively, larger radioactivities may be delivered by increasing the Ga/Fe ratio while maintaining the volume of the injectate. Results from this dosimetric study will provide bases for the design of future phase I and II clinical trials to use this class of radiopharmaceuticals to treat selected subgroups of patients with breast cancers and to correlate with biologic markers.

3.0 Background Drug Information

Select Section Title: 3.0 Background Drug Information

A known radiopharmaceutical Ga-68 /Fe macroaggregates (GIMA) [20] that may provide paramagnetic signals for volume measurement by MR imaging and simultaneously emit gamma rays for nuclear imaging was identified . It has a biologic half-life of 30 hours, a physical half-life of 1.1 hours and measures 10-30 micron in size. It was used in human lung perfusion imaging in the 1970's until the advent of the current imaging agent of Tc-99m -macroalbumin aggregates. It was produced in a carrier-added (additional nonradioactive gallium) preparation (0.12 Ci/mole) containing large amounts of additional nonradioactive gallium which in turn caused dose-limiting toxicity [20]. Following similar steps while deleting the toxic nonradioactive gallium (carrier), our laboratory has managed to produce carrier-free GIMA (with Ga-68 and Ga-67, respectively) of good stability (>98% after incubation in PBS for 24 hours) and confirmed the large sizes (99%>0.5 microns). Additionally, we have demonstrated decreases in Gradient Echo (GRE) signals on MRI with increasing Fe contents in the concentration range intended for intratumoral injection. Ga-67 GIMA is a gamma-ray emitter with a physical half-life of 78 hours during which the long-term organ distribution of GIMA can be monitored using gamma-cameras.

The dry density of Iron Macroaggregates is about 2.66 gm/cc. However, with only 1 mg in the 1 cc solution, the density of the solution is only slightly higher than 1.0 gm/cc. With periodic shaking before injection, our team had no difficulty during injection in animal into small size tumors; we do not expect difficulty injecting into humans.

3.1 Supplier/How Supplied

- 3.1.1 Carrier-free Gallium-67 GIMA will be prepared according to the method of Colombetti [20] with the exception that commercially available radiopharmaceutical grade Ga-67 (nominal specific activities >30 Ci/mmol because of non-carrier added preparations) will be used and no non-radioactive gallium (carrier) will be added. The starting materials also involve ultra-high grade of iron chloride ((Iron (III) Chloride, anhydrous, powder, 99.99+% LOT # 04134TB SIGMA-ALDRICH, Inc, 3050 Spruce St. St Louis, MO 63103 USA.) and 0.22um-filtered sterilized PBS buffer and ammonia (Ammonium hydroxide, 28% NH3 in water,99.99% LOT# 07923LA SIGMA-ALDRICH, Inc, 3050

Spruce St Louis, MO 63103 USA). The final product (Ga-67 GIMA, synthesized according to Appendix A) is a colloid suspended in saline. Our previous experiments have consistently produced Ga-67 GIMA with >90% radiochemical yields. Only batches of >90% radiochemical yield will be used. Aseptic procedures will be followed and pyrogenicity test will be performed and negativity will be confirmed before injection. Waterproof gloves will be worn by the personnel during preparation procedures. Ga-67 GIMA will be stored in sterilized vials behind lead bricks. All vials will be brought to room temperature immediately prior to use. Part of the contents in the vial to be injected will be tested for the evaluation of pyrogenicity using the LAL assay (Whittaker Bioproducts, Walkersville, MD) which will last approximately 30 minutes. Unused vials or portions of Ga-67 products will be eliminated by nuclear decay in storage behind lead bricks for at least 4 weeks. The synthesis and testing procedures typically last 80 minutes. The suspended colloid is available in screw-cap vials with radioactivity ranging from 0.1 to 2 mCi per vial. The total iron content is approximately 2 milligrams. The sterility of the products will be tested and monitored for 10 days for aerobic and anaerobic pathogens using BD Bactec Plus/F and Thioglycolate cultures (Becton and Dickinson, Sparks, MD), as a standard testing procedure of radiopharmaceuticals of short half-lives.

3.1.2 Through a confidentiality disclosure agreement, the South Texas Nuclear Pharmacy has agreed to provide carrier-free Ga-67-GIMA in pyrogen-free (LAL test-negative) conditions and monitor sterility tests for each dose preparation for 10 days. They will follow procedures outlined in Appendix A.

3.2 Determination of radioactivity of GIMA.

The radioactivity of Ga-67 GIMA total products and individual patient dose will be measured by a Capintec dose calibrator in the MDACC Nuclear Medicine Nuclear Pharmacy (with daily quality assurance check) and the volume will be noted along with the time of measurement.

3.3 Storage and Disposal

Ga-67 GIMA will be handled only by our Clinical and Scientific staff (Physician, Nurse, Chemist or Nuclear Medicine Technologist). Unopened vials of Ga-67 GIMA will be stored for decay at room temperature and shielded from sunlight behind lead blocks in the nuclear pharmacy storage vault. After their radiation level fell to background level, they will be disposed. It is expected Ga-67 will take up to 4 weeks.

3.4 Toxicity.

From the published results of human lung scanning, no adverse effects have been attributed to GIMA. Published toxicity of gallium compound has been correlated with the nonradioactive free gallium (carrier) with a limiting dose of 1mg, corresponding to the about 0.1 mCi of the low-specificity GIMA. At multi-milligram levels of systemic administration, the typical symptoms include gastrointestinal discomfort and hepatic failure. The high specific activity GIMA prepared by our method contains no

nonradioactive gallium and the physical amount of gallium (Ga-67) is one-billionth that of the earlier preparation [20] and is therefore well below the toxicity threshold. In fact, cancer patients injected with larger systemic doses up to 10 mCi carrier-free Ga-67 (as used in routine tumor localization imaging) do not have signs of toxicity.

4.0 Patient Eligibility

Select Section Title: 4.0 Patient Eligibility

All Study patients must meet the eligibility criteria:

4.1 Eligible Patients

- 4.1.1 Patients must understand the procedures and the explanations in English and must provide informed consent by signing the informed consent form
- 4.1.2 Patients must be 18 years of age or older
- 4.1.3 Patients must have breast cancer diagnosed by histopathology but no surgical resection of the tumor.
- 4.1.4 Patients should have received no previous focal external beam radiation therapy to the thorax.
- 4.1.5 Patients who have not received systemic or cytotoxic chemotherapy for the breast cancer under study. Patient under hormonal therapy alone will be eligible.
- 4.1.6 Patients with adequate platelets to avoid excessive bleeding and adequate white cells to avoid infection.
 - Granulocytes ≥ 1000 cells/mcl
 - Platelets $\geq 40,000$ /mcl
- 4.1.7 Patients with Zubrod performance scale of 2 or below.
- 4.1.8 Patients with breast tumor > 2 cm compressed thickness on mammogram but no tumor necrosis by MRI.
- 4.1.9 Patients must have scheduled surgical resection (either mastectomy or conservation surgery) of the breast tumor within 2 weeks after injection.
- 4.1.10 Patients with a F-18 FDG PET within 2 weeks showing a tumor SUV uptake > 2.0

4.2 Ineligible Patients

- 4.2.1 Patients of child-bearing potential (not post-menopausal for 12-24 months or not surgical sterile) who have positive pregnancy test or are lactating.
- 4.2.2 Patients with septicemia, severe infection or acute hepatitis.
- 4.2.3 Patients who had radiation therapy or chemotherapy of the breast cancer prior to the planned surgery.
- 4.2.4 Patients who had residual radiation from previous radionuclide administration, from the day of injection:
 - F-18 agents of more than 10 mCi within 2 days for Ga-67 GIMA group.
 - In-111, Ga-67 or I-131 agents of more than 1 mCi within 14 days for the Ga-67 GIMA groups.
- 4.2.5 Patients who cannot undergo MRI procedures (including nonvisualization of tumor on MRI and implants incompatible with MRI)
- 4.2.6 Patients with claustrophobia cannot be entered for the Ga-67 GIMA groups

because of the requirements of repeated MRI requiring repeated conscious sedation.

- 4.2.7 Patients who have scheduled surgical resection of the breast tumor in less than 7 days are not eligible to enter the Ga-67 GIMA groups
- 4.2.8 Patient who cannot understand the procedures as explained in English or who cannot provide meaningful informed consent.

5.0 Treatment Plan

Select Section Title: 5.0 Investigational Plan

Despite the title, this section is indeed an Investigational Plan.

Breast cancer is predominantly a disease of the adults and only patients above 18 year old will be eligible to enroll. No dose adjustment is made for younger patients because of statistical requirements of uniformity for small sample sizes and because GIMA is expected to stay only within the tumor.

5.0.1 Subject Identification

A unique patient research ID number will be assigned to each individual participating the study. The subject ID will consist of 5 digits in the format of GG-NNN where GG is the group ID for the institution and NNN is the accession number within the institution. The unique patient research ID number will be assigned by the study PI. A password protected secured file will be created to store the cross reference list between the patient research ID number and confidential patient information such as name, birth date, hospital number, and social security number (if available), etc. Patient research ID number will be used throughout the trial and in database for patient identification purpose. Confidential patient information will be used only when it is necessary such as in patient care setting.

5.0.2 Ethical and Legal Considerations

This study will undergo full approval in accordance with the human surveillance requirements of our institution. Blood and urine samples will be obtained for the evaluations as described in the protocol. Measures will be taken to ensure confidentiality of participant information. Data collected on paper forms will be stored in locked file cabinets with restricted access. Data collected on electronic media will be stored in computer files with restricted password access. All staff members in the study will be informed prior to employment and at regular intervals of the necessity for keeping all data confidential. Computers will not be accessible to the public and will be located in locked offices. Subjects will be assigned a separate study number to protect subject identification. No patient identifiers will be used in any publications of this research. Data will be maintained indefinitely and representatives of the United States Army Medical Research and Materiel Command may inspect research records. When the time comes to dispose of the data, all database files will be deleted.

As of 06/10/2003, the following investigators on this study have disclosed an equity or stock option interest in the sponsor of this study: Through the University of Texas M. D. Anderson Cancer Center, Dr. Franklin C. Wong, a collaborator of this protocol has filed a patent application to the U.S. Patent and Trademark Office on radionulcide cancer therapies including the method of producing carrier-free GIMA. For these reasons, there is potential conflict of financial interest (intellectual properties) of this study involving Dr. Franklin C. Wong, The University of Texas, and UTMDACC. Dr. Franklin C. Wong is also the principal investigator of a U.S. Army Breast Cancer Research Grant supporting this study. Dr. Gary Whitman is the principal investigator who will supervise this study in UTMDACC. Either Dr. Gary Whitman or Dr. Mark Dryden will perform the injection of Ga-67 GIMA.

5.0.3 Division of Responsibilities

5.0.3.0 Study Personnel

A description with roles and responsibilities of the study personnel, as well as , contact information can be found in appendix K.

5.0.3.1 Medical Monitor

For this protocol Dr. Richard Theriault has been designated medical monitor. Dr. Theriault is a qualified physician, other than the principal investigator, not associated with the protocol, able to provide medical care to research volunteers for conditions that may arise during the conduct of the study, and who will monitor the volunteers during the conduct of the study. Dr. Theriault will review serious adverse events and unanticipated problems.

Dr. Theriault will review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor should and will comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor should and will indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the Human Subjects Research Review Board (HSRRB).

5.1 Human Study:

Patients will be recruited from female breast cancer patients scheduled for surgery at least one-week from the planned day of injection. One of the inclusion criteria will be a tumor size of > 2cm in diameter but no tumor necrosis as determined by MRI. No spillage outside of the tumor is expected from an injection of 1 cc. The MRI and nuclear imaging studies will follow routine clinical procedure.

This group will receive 0.2 mCi intratumoral injections of Ga-67 GIMA (in 1cc saline) under MRI guidance and then undergo whole-body scintigraphy, MRI and

ultrasound at 2, 4, 24 hours and one of the 2nd, 3rd or 4th day after injection. Blood sample (2-5 cc each) collection will take place during each of the scintigraphy session. Although the exact radiation dosimetry has yet to be determined, the radiation doses to the tumor can be estimated from the published biological half-life of 30 hours [20]. This group of 5 patients will receive estimated doses of 262 cGy in the tumor, with a 10% isodose range of 0.02 cm from the injection site edge.

5.1.1 Patient Entry Requirements

Patients entered into this patient treatment study must meet eligibility requirements and sign the informed consent form. Each patient will be given a standard medical examination including a breast MRI with a medical history and laboratory work to determine eligibility. Patients will be accrued from the breast oncology clinics. When a patient with a breast cancer larger than 2 cm and who needs to undergo surgery is identified, the patient will be interviewed and provided information about this study. Signed informed consent will be obtained by Dr. Gary Whitman, Dr. Mark Dryden or other physician investigators (except Dr. Franklin Wong because of potential conflict of interest) no earlier than the next day after the initial interview to provide adequate time for the patient to consider participation.

5.1.2 Informed Consent Process

Referring physicians and clinic staff will be provided with flashcards (Appendix B) including eligibility criteria and contact information. After a patient is considered a candidate, the referring physician will explain the study and give the patient a Recruitment Information Brochure (Appendix C). Upon patient's contact with our research coordinator or protocol chairman, the eligibility criteria will be reviewed. If found eligible, a physician investigator (Dr. Gary Whitman, Dr. Mark Dryden or others) will interview the patient and provide further information of the study and answer relevant questions before obtaining informed consent as evidenced by patient's signing on the current version of IRB-approved informed consent form. Then the patient will be given a Study Procedure Instructions Flyer (Appendix E) which further explain the details of the procedures.

A statement reading : " There is no direct benefit to the patient from this investigational study" will be included in the informed consent form.

5.2 MRI and Injection

The patients will be scanned using a GE Signa Lx 1.5 Tesla MRI scanner equipped with a high performance gradient system (amplitude = 22mT/m; slew rate = 120 T/m/s). A phased-array bilateral breast RF coil will be used to maximize the signal-to-noise ratio. A breast positioning system with two compression plates and Vitamin E markers will be used to hold the breast in a reproducible position. Image slice thickness will be approximately 6mm and the scan will be positioned to also include the axilla as much as possible.

Before entry into the study, patients will have been asked about metallic implants

such as pacemakers and other devices. Patient with these conditions will have been excluded as determined by the MRI radiologist collaborators. Specific and easily accessible tumors will be preferred over other locations. An MR-compatible 20-gauge needle of 7 cm in length will be used. If requested, ear plugs will be provided to decrease noise during MRI.

The breast tumor will first be localized using a fast gradient echo T1-weighted 3D pulse sequence in the sagittal plane. An MR-compatible disposable sterile needle will be placed intra-tumorally. Areas of necrosis, if any (developed after the pre-injection MRI), will be avoided. An MR scan will be performed to ensure the proper location of the needle. Prior to injection, a high-resolution baseline image will be obtained using a gradient echo (GRE) pulse sequence with parameters selected to be sensitive to T2*. Injection may be preceded with surface or subcutaneous local anesthesia (1cc of 4% lidocaine, if the patient has no history of allergy or adverse reactions to lidocaine; otherwise, an alternative local anesthetic will be used) at the needle entrance through the surface. The GIMA will be injected in one single intratumoral injection into the tumor over 1 minute. The needles will then be slowly removed. This procedure is similar to routine breast lymphoscintigraphy. Immediately after injection, images (without breast compression) from multi-phase T2*-weighted MRI will be acquired using the same pulse sequence in quick successions up to 1 hour. All subsequent MRI images will be without breast compression. The volume of the injectate will be determined from manual segmentation.

5.3 Nuclear imaging using High Resolution Scintigraphy

After MR guided injection and imaging, the patient will be sent to the Nuclear Medicine Clinic. The radioactivity residence time in the tumor and lymph nodes will be derived from serial scintigrams. Scintigrams will be acquired in a Siemens dual-head ECAM gamma camera equipped with ultra-high resolution collimators. This combination will be able to achieve a system resolution of 7mm FWHM (tested with Tc-99m at a distance of 10 cm). One transmission scan will be performed before injection. Then, whole-body and planar imaging will continue at 2, 4, 24 hours and one of the 2nd, 3rd or 4th day after injection. Geometric-mean images will be used to derive the Ga-67 concentration in the tumor and in the lymph node and be correlated with volumes from anatomic imaging including MRI.

5.3.1 Urine and Blood Collection.

For the Ga-67 GIMA group, patients will be asked to provide urine samples at the following time intervals: before injection and during scintigraphy sessions at 2, 4, 24 hours and one of the 2nd, 3rd or 4th day after injection. Patients will be instructed and provided containers to collect all urine output up to the last day of imaging and urine samples will be collected during the scintigraphy sessions. The patients will be provided with urinals and urine containers that are marked with time information and are encouraged to adhere to the time marking by using the correct container and to

return the containers during the nuclear imaging sessions. Urine samples will have volume, time of excretion, and radioiodine content measured. After measurement, urine samples will be stored behind lead bricks for decay for at least a week. It will be disposed of after the radiation level falls to background level- this will occur within a few days because of the relatively low activity as a small fraction excreted from the 0.2 mCi total dose. All these patients will be asked to provide blood samples (2-5 cc each) at nuclear imaging sessions to characterize the systemic Ga-67 clearance and whole-body and organ radiation dosimetry. Blood samples will be collected by a physician, nurse or nuclear medicine technologist. Aseptic technique will be used and care taken to avoid infection and discomfort. If occurs, fainting spells will be monitored through observation and vital sign measurement until they resolves..

5.3.2 Ultrasonography

The injectate volumes will be monitored using ultrasonography using parameters for routine breast imaging, following each MRI session. Our animal experiments indicate that using the routine clinical ultrasound instrument, GIMA was detectable with the implanted tumors of 3 cm in diameter. Therefore, the patients will be studied with routine clinical parameters (10-12 MHz or 5 MHz with harmonics in a Seimens Anteres clinical ultrasound imaging devices) after MRI. Harmonics will be applied also to decrease potential artifacts. Since ultrasound measurements are relatively operator-dependent, to avoid biases ultrasound images will not be acquired or interpreted by the radiologist who performs the injection under MRI or interprets the MRI.

5.3.3 F-18 FDG PET

The patient will have had a baseline F-18 FDG PET scan as part of the clinical work-up to evaluate the tumor glucose metabolism before entering this protocol. Immediately after the last nuclear follow-up scan of Ga-67 GIMA, the patient will undergo another F-18 FDG PET scan. About 15 mCi F-18 FDG will be injected intravenously followed by a period of 45 minutes of uptake and then scanning for about 30 minutes for the whole body. The F-18 PET scan will be evaluated qualitatively for uptake in the breast tumor and semi-quantative using the established standard uptake values (SUV's).

5.4 Dosimetry modeling of beta and gamma emissions from radionuclides

There are three components necessary for the estimation of absorbed doses to tissues surrounding the injected activity: 1) The energy deposited in the surrounding tissues will be determined using radiation transport analysis [21], 2) the geometry of the activity distribution (source region) will be determined using MR image data, and 3) the total number of radioactive transitions that occur in the region will be determined using data from the scintigram. Both beta and gamma emissions will be evaluated. The total radiation absorbed doses will be derived for the tumor and surrounding

tissues.

The volumetric data measured from MRI will be used to derive the S-values of the tumors using voxel-based simulation [22] to calculate the radiation absorbed doses to the injection sites and the surrounding tissues. Radiation dose rates, or S-values, will be compared with those from the sphere [18] and shell models [19] to evaluate the effects of potentially heterogeneously distributed injectate in the tumor. Such comparison will establish the boundaries of the models and aid choices of dosimetric methods in future studies.

5.5 Pathologic and Autoradiographic Evaluation

Histopathologic data will be collected from the surgical specimen obtained during the scheduled tumor resection (about 7-14 days from injection). If present, the histologic changes from radiation effects [23, 24] in and around the tumor/lymph nodes will be correlated with predicted and measured dosimetry. Selected sections of the tumor with GIMA, as evidence by the light brownish color of deposits, will be frozen and placed on autoradiographic films to develop overnight. Then these tissue sections will be returned to histopathology service to continue tissue processing. No tissue will be retained by our group.

5.6 Potential Radiation Effects and Radiation Safety to the personnel

Although we believe that the radiation involve is low, it should be noted that any amounts of radiation may increase the chance of getting new tumors and radiation from the GIMA may affect the tumor cells. With a biologic half-life of 30 hours and effective half-life of 21 hours, the residual Ga-67 GIMA will be less than 2% of the original dose after the 5 days of imaging (or 6 effective lives). At 7 days after injection, there will be essentially negligible residual amount (2 micro Ci, in total) of Ga-67 and health risk is minimal to health personnel, as long as general body-fluid precaution is followed including washing hands.

6.0 Pretreatment evaluation

Select Section Title: 6.0 Pretreatment evaluation

Prior to the imaging procedures, subjects will be questioned to obtain a medical history, and given a complete physical examination including a mammogram, breast MRI and laboratory tests including CBC to determine eligibility. Patients who have not previously received a breast MRI (with contrast if necessary) and F-18 FDG PET will have one of each performed (at the cost of the study) prior to entry into the study to evaluate the tumor size and assure absence of tumor necrosis. Women with child-bearing potential will receive a pregnancy test.

7.0 Evaluation During Study

Select Section Title: 7.0 Evaluation During Study

Immediately before and up to the 4th day after injection with Ga-67 GIMA, blood samples and urine collection will be taken from the patients at each of the nuclear imaging sessions (2, 4, 24 hours and one of the 2nd, 3rd or 4th day) to measure radiogallium clearance and retention. A F-18 FDG PET will be performed on the same day after the last nuclear imaging and PET.

8.0 Evaluation of Toxicity

Select Section Title: 8.0 Evaluation of Toxicity

8.1 Toxicity

The radiation absorbed doses to the body and organs are low for both the low doses (0.2 mCi, maximum) of Ga-67, compared with the routine dose of 8 mCi of Ga-67 citrate for tumor localization studies. The residual of 1 mg of Fe in the tissue is also not expected to present significant toxicity, considering the routine intramuscular injection of up to 1000 mg of iron sulfate for the treatment of anemia.

8.2 Stopping Rule

End-point will be defined as grade III/IV toxicity (NCI criteria, Appendix L) in 2 or more of the 5 patients in a particular dose level.

9.0 Criteria for Removal from the Study

Select Section Title: 9.0 Criteria for Removal from the Study

- 9.1 All patients will be followed with reasonable efforts until 2 months after injection. Any patient initially accepted into the study, but who subsequently is determined to be ineligible for radionuclide evaluation will be removed from the study. The reason and time of removal will be documented.
- 9.2 The development of unacceptable toxicity is defined as unexpected, irreversible or grade 4 toxicity.
- 9.3 Non-compliance by patient with protocol requirements.
- 9.4 Patients have the right to withdraw from the study at any time without consequence. If a patient withdraws from the study, reasonable attempts will be made to document the reason for withdrawal.
- 9.5 Any patient can be removed at the discretion of the investigator or sponsor.

10.0 Number of Patients

Select Section Title: 10.0 Number of Patients

This is a study of biodistribution. One group of 5 patients will be studied with 0.2 mCi Ga-67 GIMA.

10.1 Only descriptive statistics (mean, variance, ratios and diagrams) will be applied to analyze the results of dispersed volumes by MRI and ultrasound, tumor and organ percentage of injected doses, dosimetry, potential toxicity and histologic changes.

10.2 The volumes by MRI and ultrasound, and percentage of injected radiation in the tumor and organs as determined by PET/CT will be collected and sent to our outside consultant Dr. Rick Sparks (creative Developments, Inc, Dosimetry Service, Knoxville, TN) for computation of the dosimetry profiles. Because of the limited number of 5 patients, only descriptive statistics will be applied to analyze the data. The raw data will be stored until both this and the companion Ga-68 GIMA study are completed and then will be disposed of. The raw data will be kept under lock and key in our files for at least 5 years from the onset of this study.

11.0 Reporting Requirements

11.0 Reporting Requirements

Select Section Title: 11.0 Reporting Requirements

11.1 Adverse Event Reporting to USAMRMC

Unanticipated problems involving risk to volunteers or others, serious adverse events related to participation in the study, and all volunteer deaths should be promptly reported by telephone (301-619-2165), by email (hsrrb@amedd.army.mil), or by facsimile (301-619-7803) to the Army Surgeon General's Human Subjects Research Review Board. A complete written report should follow the initial telephone call. In addition to the methods above, the complete report can be sent to the U.S Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

11.2 Procedures for Reporting Serious Adverse Events

Serious and/or unexpected adverse events are submitted in writing to the M.D. Anderson Cancer Center Institutional Office of Protocol Research (OPR) within 10 working days of the adverse experience. Unexpected fatal or life-threatening experiences are phoned immediately to the Office of Protocol Research (713-792-2933). A follow up written report is submitted to OPR within 10 working days. The form for communication to OPR of all serious adverse events is appended to this plan.

For all protocols conducted at M.D. Anderson Cancer Center, the Principal Investigator is responsible for submitting adverse event reports to the Institutional IRB and the HSRRB and/or USAMRMC, Office of Research Protections on an ongoing basis. Adverse event reports are submitted to the Institutional Office of Protocol Research (OPR), where they are entered into

PDMS and forwarded to the designated IRB vice chairperson for review. Attached to each adverse event report is a listing of all prior adverse events submitted for that protocol. Any comments, questions or changes the IRB requests to the protocol as a result of this review are conveyed to the principal investigator. The investigator response and protocol modification process is monitored by the IRB vice-chairperson and OPR support staff. The vice chairperson presents the report on adverse event review to the full committee at the next IRB meeting.

An adverse event report compilation is provided once annually to the M.D. Anderson Cancer Center IRB. Comments, questions or other considerations from the IRB are conveyed to the principal investigator for evaluation, discussion and implementation.

11.3 Reporting of Subject Death

The death of any subject during the study or within 30 days of study completion (as defined above), regardless of the cause, must be reported within 24 hours by telephone, to the principal investigator and/or study coordinator and the HSRRB and/or USAMRMC, Office of Research Protections. A full written report must follow as soon as possible. If an autopsy is performed, the report must be provided to the Sponsor (MD Anderson Cancer Center).

Reports of all **serious adverse events, including deaths**, must be communicated to the appropriate Institutional Review Board or ethical review committee and/or reported in accordance with local law and regulations.

11.4 Other reports to DOD HSRRB.

- All amendments will be approved by the MD Anderson Cancer Center Clinical Research Compliance and the HSRRB prior to implementation. If there are deviation from the protocol, the deviation will be reported to the medical monitor and MDACC IRB for approval. Deviation that effects patient safety or integrity of the study will be reported to the DOD HSRRB.

12.0 References

Select Section Title: 12.0 References

1. Vaidya JS, Baum M., Clinical and biological implications of the Milan breast conservation trials. Eur J Cancer Jul;34(8):1143-4, 1998.
2. Edmundson GK. Vicini FA. Chen PY. Mitchell C. Martinez AA. Dosimetric characteristics of the MammoSite RTS, a new breast brachytherapy applicator. Int. J. of Radiat. Oncol. Biol. Physics. 52(4):1132-9, 2002.
3. Nag S, Kuske RR, Cicini FA, Arthur DW and Wwicker RD. Brachytherapy in the

treatment of breast cancer. *Oncology (Huntington)*. 15(2): 195-202, 2001.

4. King TA, Bolston JS, Kuske RR, Fuhrman GM, Scroggins TG and Jiang XZ Long-term results of wide-field brachytherapy as the sole method of radiation therapy after segmental mastectomy for T(is, 1,2) breast cancer. *Amer. J. Surg.* 180(4):299-304, 2000.
5. Vicini FA, Baglan KL, Kestin LL, Mitchell C, Chen PY, Frazier RC et al. Accelerated treatment of breast cancer. *J. Clin. Oncol.* 19(7):1993, 2001.
6. deGuzman AF, Karvelis SKC, Shaw EG, et al. Brachytherapy Of Re-Resected Malignant Glioma Cavity Margins Using An Inflatable Balloon Catheter And Liquid 125I Source: A Phase I Study. *J.Nucl.Med.* 41(5 Suppl): 274P, 2000.
7. Dempsey JF, Williams JA, Stubbs JB, Patrick TJ and Williamson JF. Dosimetric properties of a novel brachytherapy balloon applicator for the treatment of malignant brain-tumor resection-cavity margins. *Int. J. Radiat. Oncol. Biol. Phys.* 42(2): 421-429, 1998.
8. Johnson N. Soot L. Nelson J. Franzini MD. Vea H. Gruner S. Kulawiak L. Young K. Sentinel node biopsy and internal mammary lymphatic mapping in breast cancer. *American Journal of Surgery.* 179(5):386-8, 2000.
9. Doting MH. Jansen L. Nieweg OE. Piers DA. Tiebosch AT. Koops HS. Rutgers EJ. Kroon BB. Peterse JL. Olmos RA. de Vries J. Lymphatic mapping with intralesional tracer administration in breast carcinoma patients. *Cancer.* 88(11):2546-52, 2000.
10. Bergqvist L, Strand SE, Persson B, et al. Dosimetry in lymphoscintigraphy of Tc-99m antimony sulfide colloid. *J Nucl Med* 1982; 23:698-705.
11. Glass EC, Basinski JE, Krasne DL, et al. Radiation safety considerations for sentinel node techniques. *Ann Surg Oncol* 1999; 6:10-11.
12. Hung JC, Wiseman GA, Wahner HW, et al. Filtered technetium-99m-sulfur colloid evaluated for lymphoscintigraphy. *J Nucl Med* 1995; 356:1895-1901.
13. Kim, E. E., Wong, F. C. Radiation Dosimetry and Safety in Lymphoscintigraphy. *In: K. Hunt, M. Ross (eds), The Mastery of Lymphatic Mapping.* New York. Springer-Verlag, (in press).
14. Loevinger R, Budinger TF, Watson EE. *MIRD Primer.* Society of Nuclear Medicine, 1991.
15. Evans JF. Stabin MG. Stubbs JB. Specific absorbed fractions of energy from internal photon sources in brain tumor and cerebrospinal fluid. *Medical Physics.* 22(3):331-40, 1995.

16. Ferrari M, Cremonesi M, Bartolomei M, Stabin M, Agosteo S, Chinol M, et al. Dosimetric Modeling for locoregional treatment of brain tumors with 99-Y-Biotin. *Eur. J. Nucl. Med.* 27(8):925, 2000.
17. Sparks, R.B., Crowe, E. A., Wong, F. C., Toohey, R. E., Siegel, J.A. Radiation dose distributions in normal tissue due to adjacent tumors containing I-131 or 90Y: *J Nucl Med.* 43(5):91P, 2002, Suppl.
18. Wong, F.C., Kim, E. E., Sparks, R., Wong, W-H, Yang, D.J. and Podoloff, D.A. Three Sphere Dosimetry Models (0.4 cc, 2cc and 10cc) of 12 Commonly Available Radionuclides for Locoregional Therapy or Extravasation. *J. Nucl. Med.* 42:243P, 2001.
19. Wong, F.C., Sparks, R., Kim, E. E., Yang, D. J., Podoloff, D. A. Simulation of radiation dosimetry in intra-cavitary injection of eight radionuclides in five shell models. *J Nucl Med*, 43(5):90P, 2002, Suppl.
20. Colombetti LG, Goodwin DA, Togami E. Ga-68-labeled macroaggregates for lung studies. *J. Nucl. Med.* 11(12): 704-707, 1970
21. MCNP manual, Monte Carlo N-Particle Transport Code System. RSIC 1994.
22. Yoriyaz H, Stabin MG, dos Santos A. Monte Carlo. MCNP-4B-based absorbed dose distribution estimates for patient-specific dosimetry. *J Nucl Med.* 42(4):662-9, 2001
23. McCormick B, Strom E., Craighead PS, et al, Radiation Therapy Oncology Group. Research Plan 2002-2006. Breast Cancer Working Group. *Int. J. of Radiation Oncol, Biol, Physics.* 51(3Suppl 2):56-7, 2001.
24. Mirza AN, Fornage BD, Sneige N, Unger HM, Newman LA, Ames FC and Singletary SE. Radiofrequency ablation of solid tumors. *Cancer J.* 7(2):95-102, 2001.

PDOL Draft

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Informed Consent Printer Database**

Radiation Dosimetry from Intratumoral Injection of Radionuclides into
Human Breast Cancer Ga 67 GIMA
2005-0219

Subtitle: Intratumoral Injection of Gallium 67 GIMA for Gamma Camera Imaging and
Magnetic Resonance Imaging

1. _____
Participant's Name I.D. Number

You are being asked to take part in this clinical research study at The University of Texas M.D. Anderson Cancer Center (hereinafter referred to as "UTMDACC" or "the institution"). This research study is strictly voluntary. This consent form explains why we are performing this research study and what your role will be if you choose to participate. This form also describes the possible risks connected with being in this study. After reviewing this information with the person responsible for your enrollment, you should know enough to be able to make an informed decision on whether you want to participate in the study. This study complies with all laws and regulations that apply.

You are being asked to take part in this study because you have breast cancer and you have a surgery scheduled to remove the cancer.

DESCRIPTION OF RESEARCH

2. PURPOSE OF STUDY

The goal of this clinical research study is to learn how special radioactive molecules called gallium-iron macroaggregates (GIMA) distribute (travel and spread) in the body after they are injected into breast cancer tissue. This is not a therapeutic trial and you will not benefit from your participation.

3. DESCRIPTION OF RESEARCH

For the common diagnostic procedure called lymphoscintigraphy, small radioactive particles (called colloids) are injected into a lymph node. Then, a special nuclear medicine scanner is used to watch these particles slowly distribute in the body. Researchers noticed the larger the molecule, the slower the particles move in the body. In fact, some of the larger colloid molecules seem to stay at the site of injection with very little movement. Since the amount of radiation on these colloids is very low, they cannot be used to treat cancer. New molecules have been developed called GIMA. These GIMA molecules have been designed to act like the colloid molecules (very slow-moving). However, the GIMA molecules were made to carry larger radioactive particles. Since these molecules are very slow-moving, they can be injected directly into a tumor without spreading out very far from the site of injection. In this way, radiation can be delivered directly to the tumor tissue without spreading to normal tissue.

For this study, small particles of radiation (Ga-67) will be attached to GIMA molecules. These molecules will then be injected into the breast cancer tumor. Special scanners will be used (gamma camera and MRI) to "see" how far the molecules move away from the injection site. If it is found that the molecules do not move very far, in the future, more radiation particles can be attached to the GIMA molecules. These GIMA molecules can then be used to treat cancer with radiation. The radiation level in GIMA (but not its size) can be measured from outside of the body by a gamma camera. The amount of iron in GIMA can be measured from outside of the body by a MRI scanner. The distribution of iron in GIMA can be used to determine the size of the GIMA collection by the appearance of darkening in the images around the injection site. It will be injected by a MRI-compatible needle to avoid potential injury during MRI. The small amounts of iron (one thousandth of a gram) in the GIMA injection will not be sufficient to cause physical movement of the particles because the particles are firmly dispersed within the tumor tissues. Ultrasound measurement is non-invasive and may provide similar size information and may in fact prove to be a more convenient method to monitor the size of GIMA in the future. Combining the radiation level and size measurements will allow researchers to determine how much radiation is delivered to the tumor and to other organs (if any).

A total of 5 people with breast cancer will be recruited in this protocol. Participants will receive a GIMA with a particle of radiation attached (Ga-67) that gives off a lower amount of radiation (0.2 mCi). The amount of radiation you will receive from the GIMA molecules is very low, about the same as a routine nuclear medicine scan (or

2-3 Chest X-rays).

The GIMA will be injected directly into the tumor tissue with the help of a MRI scanner. If you prefer to have local anesthesia, you will receive about 1cc of lidocaine at the needle entry site above the tumor. If you have allergy or previous adverse reaction to lidocaine, another local anesthetic will be applied. A 20-gauge 7cm long needle that is compatible with MRI facilities will be used to inject the GIMA. The MRI scanner will be used to make sure the needle is inserted directly into the tumor. Also, MRI scans will be done within one hour after the injection. An ultrasound examination of the injection site will also be performed to confirm the location of the GIMA particles

You will undergo nuclear imaging scans in the nuclear medicine clinic at 2, and 4 hours after the injection as well as 24 hours, and one of the following 3 days. You will also have MRI scans and ultrasound following each of these nuclear imaging sessions. You will be asked to have blood samples (1 teaspoon) and urine samples collected during each nuclear imaging session. Blood samples will be obtained by our nurses or physician or nuclear medicine technologist. This study also requires collection of all your urine output at different time points from before injection to the last imaging session. Urinals as well as time-marked containers will be provided to you from our clinic staff. It is important to use the correctly marked containers and return them during the nuclear imaging sessions. The physicians, nurses and technologist will assure of your comfort during the imaging sessions and you may freely express your wishes to avoid discomfort during the imaging sessions. If you require ear plugs during MRI's, they will be provided to you.

After the last nuclear imaging procedure, you will have a F-18 FDG PET scan to measure the use of glucose in your breast tumor. This is a procedure you already had before entering this study and involve injection of a small amount of radioactive sugar.

There has been no known toxicity in human use for the FDG PET and the additional radiation is in the range of 1 to 2 chest X-rays.

Participants will have their regularly scheduled surgery. The tissue that is collected during the surgical procedure will also be studied by the pathologist doctor to see whether there is effect of this investigation on the tumor tissue. Part of the tissue collected will be retained temporarily (1 to 2 days) for analysis of the small amount of radioactivity left before the tissue is returned to the pathologist for routine analyses of tumor tissues and subsequent disposal. No tumor tissue will be retained specifically for this investigational study. Participation into this study will not delay your surgical schedule which is scheduled between 7-14 days from the injection of GIMA.

This is an investigational study. The research human use of radioactive Ga-67 GIMA has been approved by the UTMDACC Radiation Drug Research Committee

which has been authorized by the FDA. The GIMA, nuclear imaging scans, ultrasound examinations and MRI scans performed for this study will be provided free of charge. Because participation in the Ga-67 GIMA group requires additional trips to UTMDACC over 5 days, you may be partially reimbursed for lodging expenses. If you live more than 50 miles from UTMDACC, and choose to stay in a local hotel during the study, you may be partially reimbursed up to 4 nights (up to \$70 per night). Up to 5 participants will take part in this Ga-67 GIMA portion of the research and up to a total of 5 participants will take part in this study. All will be enrolled at UTMDACC.

This protocol is partially funded by a research grant from United States Army Medical Research and Materiel Command. It should be noted that representatives of the U.S. Army Medical Research and Materiel Command and the Food and Drug Administration (FDA) are eligible to review research records as a part of their responsibility to protect human subjects in research. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this study.

4. RISKS, SIDE EFFECTS, AND DISCOMFORTS TO PARTICIPANTS

While on this study, you are at risk for the side effects listed in this form. You should discuss these with the study doctor or your regular doctor. The known side effects are listed in this form, but they will vary from person to person. Many side effects go away shortly after the study drug is stopped, but in some cases, side effects may be serious, long lasting, and/or permanent and may even cause death.

Giving GIMA through the needle into the breast cancer tumor may result in pain at the injection site and or infection. GIMA have radiation particles attached to them. Radiation may increase the chance of developing new cancer. The radiation may also alter the cells in the tumor causing changes for the future planning of further treatment.

MRI (Magnetic Resonance Imaging) uses a large magnet instead of x-rays to take pictures of the inside of your body. People who have metal in their bodies (pacemakers, neurostimulators, certain clips, or staples from prior surgery) may not receive a MRI. The magnetic field used in MRI scanning may harm such people or cause problems with devices such as pacemakers. Part or all of the body will be passed into a long, narrow tube (scanner) which is open at both ends. The scanner has an intercom, which will allow you to speak to the doctors and staff during the procedure. The machine will produce a loud knocking noise. This is normal. You will be given earplugs to protect your ears. In addition, you may feel light vibrations throughout your body. Some people, especially those who have a tendency to feel uncomfortable in small or closed spaces, may feel "closed in" and become anxious while in the scanner. If you feel ill or anxious during scanning, doctors and the MRI staff will give comfort or the scanning will be stopped.

Ultrasound examination involves no ionizing radiation and is a safe routine procedure to evaluate breast tissues. In this study, it is used to confirm and follow the gross distribution of GIMA particles; while MRI is used to follow the refined distribution of GIMA.

Gamma camera scan is a medical technique that externally monitors the radioactivity in body and will be used to track the movement of the Ga-67 GIMA through the body. The gamma camera can take pictures of Ga-67 GIMA and "see" where it is in the body. By watching how Ga-67 GIMA travels through the body and studying where Ga-67 GIMA collects, researchers can learn if any radiation is deposited in certain organs in the body. Some people may feel "closed in" while lying in the scanner. However, the scanner is open at both ends and an intercom allows you to talk with doctors and staff. If you feel ill or anxious during scanning, doctors and/or technicians will give comfort or the scanning will be stopped.

For this study, the Ga-67 GIMA is radioactive substances of low radiation levels. The total amount of radiation you receive from this study is about the same as 2-3 chest x-rays.

You may experience pain, bleeding, and/or bruising from the blood draws. You may faint and/or develop an infection with redness and irritation of the vein at the site where blood is drawn. These infections (if present) may delay your scheduled surgical procedure.

This research study may involve unpredictable risks to the participants.

If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study (Dr. Franklin C. Wong, 713-794-4649) or the Protocol chairman (Dr. Gary Whitman, 713-745-3520). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at 301-610-7663/2211.

5. POTENTIAL BENEFITS

If this research study shows that GIMA stays at the injection site, future cancer therapies may be developed. This information may be of benefit to future patients. There [are](#) no benefits for you in this study.

6. ALTERNATE PROCEDURES OR TREATMENTS

You may choose not to take part in this study, participate in a different research study or go directly to surgery without participating in any other study.

I understand that the following statements about this study are true:

7. According to the institutional conflict of interest policy, the principal investigator of this study and my primary physician cannot have a financial interest in any aspect of this research. However, in instances of medical emergency, it is possible that I may be cared for by a physician and/or administrator who has some form of financial interest in the sponsor of this study.

As of 06/10/2003, the following investigators on this study have disclosed an equity or stock option interest in the sponsor of this study: Through the University of Texas M. D. Anderson Cancer Center, Dr. Franklin C. Wong, a collaborator of this protocol has filed a patent application to the U.S. Patent and Trademark Office on radionulcide cancer therapies including the method of producing carrier-free GIMA. For these reasons, there is potential conflict of financial interest (intellectual properties) in this study involving Dr. Franklin C. Wong, The University of Texas, and UTMDACC. Dr. Franklin C. Wong is also the principal investigator of a U.S. Army Breast Cancer Research Grant supporting this study. Dr. Gary Whitman is the principal investigator who will supervise this study in UTMDACC. Either Dr. Gary Whitman or Dr. Mark Dryden will perform the injection of Ga-67 GIMA while you are in the MRI scanner.

The University of Texas M.D. Anderson Cancer Center has a financial interest in the sponsor of this study.

The University of Texas System has a financial interest in the sponsor of this study.

8. If I want to receive updated information regarding the financial interests of any physician and/or administrator at UTMDACC who has cared for me, I may call the Conflict of Interest Coordinator at (713) 792-3220. Upon request, I will be given access to information disclosing the identity of all physicians and/or administrators who have a financial interest in the sponsor of this study.
9. My participation is voluntary.
10. I may ask any questions I have about this study, including financial considerations, of my treating physician. I may contact the principal investigator for this study Dr. Gary Whitman at 713-745-3520 or the Chairman of the institution's Surveillance Committee at 713-792-2933 with any questions that have to do with this study.
11. I may withdraw at any time without any penalty or loss of benefits. I should first

discuss leaving the study with my physician. Should I withdraw from this study, I may still be treated at UTMDACC.

12. I understand that the study may be changed or stopped at any time by my doctor, the principal investigator, the study sponsor, or the Surveillance Committee of UTMDACC.
13. I will be informed of any new findings that might affect my willingness to continue participating in the study.
14. The institution will take appropriate steps to keep my personal information private. However, there is no guarantee of absolute privacy. The Food and Drug Administration ("FDA"), and/or United States Army Medical Research and Materiel Command might review my record to collect data or to see that the research is being done safely and correctly. Under certain circumstances, the FDA could be required to review the names of participants.
15. You will not be responsible for research-related costs. If I suffer injury as a direct result of participation in this study, the institution will provide reasonable medical care. I understand that I will not receive reimbursement of expenses or financial compensation from the institution, the sponsor, the investigators or the United States Army Medical Research and Materiel Command for this injury. If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, (insert name and telephone number of principal investigator). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U. S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221. for this injury. I may also contact the Chairman of UTMDACC's Surveillance Committee at 713-792-2933 with questions about study related injuries.
16. Unless otherwise stated in this consent form, all of the costs linked with this study, which are not covered by other payers (HMO, Health Insurance company, etc.), will be my responsibility.
17. I recognize that there are no plans to provide any compensation to me for any patents or discoveries that may result from my participation in this research.

Authorization for Use and Disclosure of Protected Health Information

- A. During the course of this study, the research team at UTMDACC will be collecting information about you that they may share with the FDA and/or United States Army Medical Research and Materiel Command. This information may include your treatment schedule and the results of any tests, therapies, or procedures that you undergo for this study. The purpose of collecting and sharing this information is to learn about how the treatment affects your disease and any side effects you experience as a result of your treatment.

Your doctor and the research team may share study information with certain individuals. These individuals may include representatives of the FDA and/or the above listed sponsor, clinical study monitors who verify the accuracy of the information, individuals with medical backgrounds who determine the effect that the treatment has on your disease, and/or individuals who put all the study information together in report form. The UTMDACC research team may provide this information to the FDA and/or the above listed sponsor at any time.

- B. There is no expiration date for the use of this information as stated in this authorization. You may withdraw your authorization to share this information at any time in writing. More information on how to do this can be found in the UTMDACC Notice of Privacy Practices (NPP). You may contact the Office of Protocol Research at 713-792-2933 with questions about how to find the NPP.
- C. If you refuse to provide your authorization to disclose this protected health information, you will not be able to participate in the research project.
- D. I understand that my personal health information will be protected according to state and federal law. However, there is no guarantee that my information will remain confidential, and may be re-disclosed at some point.

CONSENT/AUTHORIZATION

Having read and understood the above, and having had the chance to ask questions about this study and reflect and consult with others, I give _____ permission to enroll me on this study. I have been given a copy of this consent.

SIGNATURE OF PARTICIPANT

DATE

TYPED OR PRINTED NAME OF PARTICIPANT

ADDRESS OF PARTICIPANT

WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR

DATE

SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP

DATE

TYPED OR PRINTED NAME OF PERSON RESPONSIBLE

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language that is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks and that the participant understood this explanation.

SIGNATURE OF STUDY DOCTOR OR PERSON OBTAINING
CONSENT

DATE

PDOL Draft

Radiation Dosimetry from Intratumoral Injection of Radionuclides into
Human Breast Cancer- Ga 68 GIMA
2005-0220

Core Protocol Information

Short Title	Intratumoral Injection of Radionuclides (Ga 68 GIMA) into Human Breast Cancer
Study Chair:	Gary Whitman
Additional Contact:	Franklin Wong
Department:	Nuclear Medicine
Phone:	713-794-4649
Unit:	59
Full Title:	Radiation Dosimetry from Intratumoral Injection of Radionuclides into Human Breast Cancer- Ga 68 GIMA
Protocol Type:	Standard Protocol
Protocol Phase:	N/A
Version Status:	Draft
Version:	00

Protocol Body

1.0 Objectives

Select Section Title: 1.0 Objectives

The Objectives are:

1. Use MRI to measure the spatial and temporal profiles of GIMA after intratumoral injection into breast cancer
2. Use Positron Emission Tomography (PET) for Ga-68 GIMA to measure the spatial and temporal profiles of the radioactivity of GIMA after intratumoral injection into breast cancer;
3. Use the imaging data from MRI and nuclear imaging to calculate whole-body, organ, and locoregional radiation dosimetry to evaluate safety and efficacy factors for intratumoral GIMA.

The Hypotheses are:

1. After intratumoral injection, GIMA will be dispersed but remain contained in the tumor.
2. The radiation absorbed doses will be high within the tumor but low in the body and surrounding organs.

2.0 Background

Select Section Title: 2.0 Background

Locoregional Radiation Therapy of Breast Cancer - a beginning

Multiple trials of breast conservation in patients treated with and without whole breast radiation have found that the majority (> 90%) of local recurrences occur at the site of surgical resection [1]. Clinical trials have confirmed the usefulness of sealed radionuclides as internal radiation sources for locoregional adjuvant treatment of breast cancer, as demonstrated by the recent FDA approval of MammoSite using Iridium-192 [2]. Therefore, conventional radiation treatment to the whole breast following breast conserving surgery may not be a necessary approach for the majority of women. More directed local treatment with radiotherapy appears to be safe and effective treatment. Conventional brachytherapy involves the implanting of sealed radiation sources implanted into the post-surgical field for several weeks [3, 4]. Recent clinical trials have reported favorable outcomes treating brain and breast cancer patients using a single implanted catheter filled with Iodine-125 Iotrex and Iridium-192 seeds irradiating the tissues around the post-surgical cavity (by Proxima Therapeutics, Inc.). This approach has recently gained FDA approval (GlialSite for brain tumor and MammoSite for breast cancer [1, 5, 6, 7]). Locoregional radionuclide therapy offers several desirable features: predictable dosimetry, the capability of being monitored, and short duration. Ablating breast tumors using intratumoral injection of radionuclides without sealing (e.g. by a catheter) has not been explored. This is due to the lack of requisite information on radionuclide dispersion and on radiation dosimetry in the tumor and surrounding tissues to establish efficacy and safety. This proposed study aims to explore the feasibility of using intratumoral injection of unsealed radionuclides as internal

radiation sources.

Breast Lymphoscintigraphy - an opportunity to study radionuclides in human tissues

Breast lymphoscintigraphy is a nuclear medicine procedure that is increasingly important in the identification of sentinel lymph node(s). Typically, aliquot(s) of about 1cc containing 0.5 mCi of Technetium-99m (Tc-99m) labeled sulfur colloid (SC) is injected percutaneously into the tumor or breast tissues around the tumor. Smaller sizes (<0.22 micron) of SC allow better lymphatic drainage and therefore better visualization of the sentinel lymph node(s). Only a small fraction (<1%) [8, 9] of the SC injected ever drains via the lymphatics to allow visualization of the sentinel lymph node(s). Conversely, particles of larger sizes (>0.22 micron) or direct intratumoral (IT) injection of SC into the breast tumor reveals even less lymphatic drainage. Although unsealed, radionuclides injected into the tumor or surrounding tissues are indeed subject to spatial sequestration. The injection site appears spherical and unchanged (for days) on scintigrams. Although difficult to quantify, ultrasound guidance during selected breast lymphoscintigraphy shows that injections of SC into the breast tissue result in a larger dispersed volume which has not been adequately assessed. Radiation dosimetry of breast lymphoscintigraphy have shown variations up to ten-fold [10, 11, 12], partly because of the imprecision in determining the volume of the dispersed injectate. An injection of 0.5 mCi Tc99m SC delivers about 40 cGy to the injection site and 4 cGy to the sentinel lymph node. When standard guidelines are observed, there is good margin for radiation safety and the radiation absorbed dose to the sentinel lymph node is about one tenth that of the injection site [13]. The Medical Internal Radionuclide Dosimetry (MIRD) schemes require accurate determination of volume and residence time of dispersed radionuclides [14]. A recent report directly measured the injectate volume using the full-width half maximum (FWHM) of the injection site from the scintigram. The accuracy of this volume estimate was limited by the system resolution of 2 cm [12]. The search for an accurate measurement of the dispersed injectate volume for dosimetry has been futile because, besides the radioactivity, there is no other physical signal from the injected radionuclide for external imaging.

A paramagnetic radiopharmaceutical Gallium-Iron Macroaggregate (GIMA) has been identified to provide both radioactive and paramagnetic signals for external measurement. This study is designed to evaluate the volume of dispersion and radiation dosimetry of GIMA after intratumoral injection into untreated human breast tumor.

Radionuclide Dosimetry of Unsealed Sources- Simulated Radiation doses to tumor and surrounding tissues

Earlier general internal dosimetry schemes including MIRDose3 (an established Medical Internal Radiation Dosimetry program) do not provide depth dosimetry to account for surrounding tissues. Earlier reports of simulation are limited to specific radionuclides in specific configurations [15, 16]. In our study, Monte Carlo simulation for Y-90 Zevalin was applied and found helpful in defining regions of toxicity [17]. A simulation project using sphere and shell models with common core volumes of 0.4, 2, 10, 50 and 250 cc is continuing and we reported radiation dosimetry in the core and 30 concentric layers from 19 radionuclides [18]. As predicted before, the radiation absorbed doses to the sentinel lymph nodes will be about one tenth of those to the injection sites in the tumor. The extremes of heterogeneous distribution of

radionuclides in the lesion were reported using shell models assuming that all the radionuclide was confined to the first layer around the central cavity. There was little dosimetry difference from the sphere models (<10%) in tissues beyond 1 cm. These sphere [18] and shell models [19] provide estimates of dosimetry ranges. Although the exact radiation dosimetry has yet to be determined, the radiation doses to the tumor can be estimated from the published biological half-life of 30 hours [20]. This group (0.5 mCi Ga-68 GIMA) of patients will receive estimated dose of 463cGy in the injection site, with a 10% isodose range of 0.20 cm from the injection site edge respectively. Based on preclinical studies suggesting a total of 2% leakage of radiogallium in the form of free Ga(+3), the MIRDose3 models predict low radiation absorbed doses to the vital organs in units of cGy/mCi:

SIMULATED DOSIMETRY	Ga-68 GIMA	0.5 Mcl Ga-68 GIMA for this study
ORGAN	Rad/mCi	Total rads
Adrenals	0.0130	0.0065
Brain	0.0025	0.0013
Breast w/ Injectate	1.0000	0.5000
Breast wo/Injectate	0.0550	0.0275
Gallbladder Wall	0.0100	0.0050
LLI Wall	0.0030	0.0015
Small Intestine	0.0091	0.0046
Stomach	0.0140	0.0070
ULI Wall	0.0084	0.0042
Heart Wall	0.0560	0.0280
Kidneys	0.0072	0.0036
Liver	0.0170	0.0085
Lungs	0.0400	0.0200
Muscle	0.0110	0.0055
Ovaries	0.0026	0.0013
Pancreas	0.0140	0.0070
Red Marrow	0.0140	0.0070
Bone Surfaces	0.0120	0.0060
Skin	0.0170	0.0085
Spleen	0.0130	0.0065
Testes	0.0011	0.0006
Thymus	0.0530	0.0265
Thyroid	0.0090	0.0045
Urine Bladder Wall	0.0026	0.0013
Uterus	0.0028	0.0014
Total Body	0.0600	0.0300
EFF DOSE EQUIV	1.2000	0.6000
EFF DOSE	0.4200	0.2100

Using human breast tumor as a model system, dosimetric measurement will be achieved

by acquiring the spatial and temporal distribution of injected GIMA, measured from MRI and nuclear imaging. Ga-68 GIMA (with a physical half-life of 1.1 hours) is used to take advantage of the higher resolution and sensitivity of PET to measure the short-term spatial distribution of GIMA; while Ga-67 GIMA (physical half-life of 78 hours) is used in a separate protocol to measure the prolonged distribution of radioactivity using a gamma camera. Confirmation of the sequestration and derivation of radiation dosimetry will permit variations to achieve high radiation dose for therapeutic effects. For instance, larger amounts of radioactivities may be achieved by using larger volumes of GIMA while maintaining the Ga/Fe ratio; alternatively, larger radioactivities may be delivered by increasing the Ga/Fe ratio while maintaining the volume of the injectate. Results from this dosimetric study will provide bases for the design of future phase I and II clinical trials to use this class of radiopharmaceuticals to treat selected subgroups of patients with breast cancers and to correlate with biologic markers.

3.0 Background Drug Information

Select Section Title: 3.0 Background Drug Information

A known radiopharmaceutical Ga-68 /Fe macroaggregates (GIMA) [20] that may provide paramagnetic signals for volume measurement by MR imaging and simultaneously emit gamma rays for nuclear imaging was identified . It has a biologic half-life of 30 hours, a physical half-life of 1.1 hours and measures 10-30 micron in size. It was used in human lung perfusion imaging in the 1970's until the advent of the current imaging agent of Tc-99m -macroalbumin aggregates. It was produced in a carrier-added (additional nonradioactive gallium) preparation (0.12 Ci/mole) containing large amounts of additional nonradioactive gallium which in turn caused dose-limiting toxicity [20]. Following similar steps while deleting the toxic nonradioactive gallium (carrier), our laboratory has managed to produce carrier-free GIMA with Ga-68 of good stability (>98% after incubation in PBS for 24 hours) and confirmed the large sizes (99%>0.5 microns). Additionally, we have demonstrated decreases in Gradient Echo (GRE) signals on MRI with increasing Fe contents in the concentration range intended for intratumoral injection. Ga-68 GIMA is a positron emitter with a physical half-life of 1.1 hours with which the short-term organ distribution will be monitored using PET to exploit the high sensitivity and good spatial resolution (advantages over gamma-camera).

3.1 Supplier/How Supplied

- 3.1.1 Carrier-free Gallium-68 GIMA will be prepared according to the method of Colombetti [20] with the exception that no non-radioactive gallium (carrier) will be added. The 1-hour short-lived isotope Ga-68 will be obtained from a Ga-68 generator (Du Pont Radiopharmaceuticals, N, Billerica, MA). The Ga-68 generator provides Ga-68 in aqueous solution by flushing the Ge-68/Ga-68 source inside the generator which has a useful shelf-life of up to one year. In addition to the routine testing of radiochemical purity by observation decay over 10 half-lives, the radioactivity purity of Ga-68 from the generator will also be tested 2 days before injection. Only when the radioactivity purity is greater than 99% will the Ga-68 be used for preparation of Ga-68-GIMA. Since this is a no-carrier-added preparation, there is only a nominal specific activity of over

30Ci/mmmole of Ga. The starting material also involved ultra-high grade of Iron Chloride (Iron (III) Chloride, anhydrous, powder, 99.99+% LOT # 04134TB SIGMA-ALDRICH, Inc, 3050 Spruce St. St Louis, MO 63103 USA.) and 0.22um-filtered sterilized PBS buffer and ammonia (Ammonium hydroxide, 28% NH₃ in water, 99.99% LOT# 07923LA SIGMA-ALDRICH, Inc, 3050 Spruce St Louis, MO 63103 USA). The final product (Ga-68 GIMA, synthesized according to Appendix A) is a colloid suspended in saline. Our previous experiments have consistently produced Ga-68 GIMA with >90% radiochemical yields. Only batches of >90% radiochemical yield will be used. Aseptic procedures will be followed and pyrogenicity test will be performed and negativity will be confirmed before injection. Waterproof gloves will be worn by the personnel during preparation procedures. Ga-68 GIMA will be stored in sterile vials behind lead bricks. All vials will be brought to room temperature immediately prior to use. Part of the contents in the vial to be injected will be tested for the evaluation of pyrogenicity using the LAL assay (Whittaker Bioproducts, Walkersville, MD) which will last approximately 30 minutes. Unused vials or portions of Ga-68 products will be eliminated by nuclear decay in storage behind lead bricks for at least one day. The synthesis and testing procedures typically last 80 minutes. Therefore, about three-fold more radioactivity will be prepared for each vial. The suspended colloid is available in screw-cap vials with radioactivity ranging from 0.1 to 2 mCi per vial. The total iron content is approximately 2 milligrams. The sterilized products will be tested and monitored for 10 days for aerobic and anaerobic pathogens using BD Bactec Plus/F and Thioglycolate cultures (Becton and Dickinson, Sparks, MD), as a standard testing procedure of radiopharmaceuticals of short half-lives.

The dry density of Iron Macroaggregates is about 2.66 gm/cc. However, with only 1 mg in the 1 cc solution, the density of the solution is only slightly higher than 1.0 gm/cc. With periodic shaking before injection, our team had no difficulty during injection in animal into small size tumors; we do not expect difficulty injecting into humans.

3.2 Determination of radioactivity of GIMA.

The radioactivity of Ga-68 GIMA total products and individual patient dose will be measured by a Capintec dose calibrator in the MDACC Nuclear Medicine Nuclear Pharmacy (with daily quality assurance check) and the volume will be noted along with the time of measurement.

3.3 Storage and Disposal

Ga-68 GIMA will only be handled by our clinical and scientific staff (physician, nurse, chemist or nuclear medicine technologist). Unopened vials of Ga-68 GIMA will be stored for decay at room temperature and shielded from sunlight behind lead blocks in the nuclear pharmacy storage vault. After their radiation level fell to background level, they will be disposed. It is expected the Ga-68 GIMA will take 1 day to decay to background level.

3.4 Toxicity.

From the published results of human lung scanning, no adverse effects have been attributed to GIMA . Published toxicity of gallium compound has been correlated with the nonradioactive free gallium (carrier) with a limiting dose of 1mg, corresponding to the about 0.1 mCi of the low-specificity GIMA. At multi-milligram levels of systemic administration, the typical symptoms include gastrointestinal discomfort and hepatic failure. The high specific activity GIMA prepared by our method contains no nonradioactive gallium and the physical amount of gallium (Ga-68) is one-billionth that of the earlier preparation [20] and is therefore well below the toxicity threshold. In fact, cancer patients injected with larger systemic doses up to 10 mCi carrier-free Ga-67 (as used in routine tumor localization imaging) do not have signs of toxicity.

4.0 Patient Eligibility

Select Section Title: 4.0 Patient Eligibility

All Study patients must meet the eligibility criteria:

4.1 Eligible Patients

- 4.1.1 Patients must understand the procedures and the explanations in English and must provide informed consent by signing the informed consent form.
- 4.1.2 Patients must be 18 years of age or older
- 4.1.3 Patients must have breast cancer diagnosed by histopathology but no surgical resection of the tumor.
- 4.1.4 Patients should have received no previous focal external beam radiation therapy to the thorax.
- 4.1.5 Patients who have not received systemic or cytotoxic chemotherapy for the breast cancer under study. Patient under hormonal therapy alone will be eligible.
- 4.1.6 Patients with adequate platelets to avoid excessive bleeding and adequate white cells to avoid infection.
 - Granulocytes ≥ 1000 cells/mcl
 - Platelets $\geq 40,000$ /mcl
- 4.1.7 Patients with Zubrod performance scale of 2 or below.
- 4.1.8 Patients with breast tumor > 2 cm compressed thickness on mammogram but no tumor necrosis by MRI.
- 4.1.9 Patients must have scheduled surgical resection (either mastectomy or conservation surgery) of the breast tumor within 2 weeks after injection.
- 4.1.10 Patients with a F-18 FDG PET within 2 weeks showing a tumor SUV uptake > 2.0

4.2 Ineligible Patients

- 4.2.1 Patients of child-bearing potential (not post-menopausal for 12-24 months or not surgical sterile) who have positive pregnancy test or are lactating.
- 4.2.2 Patients with septicemia, severe infection or acute hepatitis.
- 4.2.3 Patients who had radiation therapy or chemotherapy of the breast cancer prior to

- the planned surgery.
- 4.2.4 Patients who had residual radiation from previous radionuclide administration, from the day of injection:
 - F-18 agents of more than 10 mCi within 2 days for the Ga-68 GIMA.
 - 4.2.5 Patients who cannot undergo MRI procedures (including nonvisualization of tumor on MRI and implants incompatible with MRI)
 - 4.2.6 Patients with claustrophobia that may preclude MRI still may be accepted with only one session of conscious sedation for the MRI.
 - 4.2.7 Patient who cannot understand the procedures as explained in English or who cannot provide meaningful informed consent.

5.0 Treatment Plan

Select Section Title: 5.0 Investigational Plan

Despite the title, this section is indeed an Investigational Plan.

Breast cancer is predominantly a disease of the adults and only patients above 18 year old will be eligible to enroll. No dose adjustment is made for younger patients because of statistical requirements of uniformity for small sample sizes and because GIMA is expected to stay only within the tumor.

5.0.1 Subject Identification

A unique master subject ID will be assigned to each individual participating the study. The subject ID will consist of 5 digits in the format of GG-NNN where GG is the group ID for the institution and NNN is the accession number within the institution. The unique master ID will be assigned by the study PI. A password protected secured file will be created to store the cross reference list between the master ID and confidential patient information such as name, birth date, hospital number, and social security number (if available), etc. Master ID will be used throughout the trial and in database for patient identification purpose. Confidential patient information will be used only when it is necessary such as in patient care setting.

5.0.2 Ethical and Legal Considerations

This study will undergo full approval in accordance with the Institutional Review Board (IRB) requirements of MD Anderson Cancer Center. Measures will be taken to ensure confidentiality of participant information. Data collected on paper forms will be stored in locked file cabinets with restricted access. Data collected on electronic media will be stored in computer files with restricted password access. All staff members in the study will be informed prior to employment and at regular intervals of the necessity for keeping all data confidential. Computers will not be accessible to the public and will be located in locked offices. Subjects will be assigned a separate study number to protect subject identification. No patient identifiers will be used in any publications of this research. Data will be maintained indefinitely and representatives of the United States Army Medical Research and Materiel Command may inspect research records. When the time comes to dispose of the data, all database files will be deleted.

As of 06/10/2003, the following investigators on this study have disclosed an equity or stock option interest in the sponsor of this study: Through the University of Texas M. D. Anderson Cancer Center, Dr. Franklin C. Wong, a collaborator of this protocol has filed a patent application to the U.S. Patent and Trademark Office on radionulcide cancer therapies including the method of producing carrier-free GIMA. For these reasons, there is potential conflict of financial interest (intellectual properties) of this study involving Dr. Franklin C. Wong, The University of Texas, and UTMDACC. Dr. Franklin C. Wong is also the principal investigator of a U.S. Army Breast Cancer Research Grant supporting this study. Dr. Gary Whitman is the principal investigator who will supervise this study in UTMDACC. Either Dr. Gary Whitman or Dr. Mark Dryden will perform the injection of Ga-68 GIMA.

5.0.3 Division of Responsibilities

5.0.3.0 Study Personnel

A description with roles and responsibilities of the study personnel, as well as , contact information can be found in appendix K.

5.0.3.1 Medical Monitor

For this protocol Dr. Richard Theriault has been designated medical monitor. Dr. Theriault is a qualified physician, other than the principal investigator, not associated with the protocol, able to provide medical care to research volunteers for conditions that may arise during the conduct of the study, and who will monitor the volunteers during the conduct of the study. Dr. Theriault will review serious adverse events and unanticipated problems.

Dr. Theriault will review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor should and will comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor should and will indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the Human Subjects Research Review Board (HSRRB).

5.1 Human Study:

Patients will be recruited from female breast cancer patients scheduled for surgery at least one-week from the planned day of injection. One of the inclusion criteria will be a tumor size of > 2cm in diameter but no tumor necrosis as determined by MRI. No spillage outside of the tumor is expected from an injection of 1 cc. A total of 5 patients will be studied. The MRI and nuclear imaging studies will follow routine

clinical procedure.

This group of 5 patients will receive 0.5 mCi of Ga-68 GIMA (in 1 cc saline) intratumorally under MRI guidance and imaged with MRI and then undergo PET/CT studies at 1 hour after injection. Although the exact radiation dosimetry has yet to be determined, the radiation doses to the tumor can be estimated from the published biological half-life of 30 hours [20]. This group of patients will receive estimated doses of 463 cGy in the tumor, with a 10% isodose range of 0.20 cm from the injection site edge.

5.1.1 Patient Entry Requirements

Patients entered into this investigational study must have signed the informed consent form and meet eligibility requirements. Each patient will be given a standard medical examination including a breast MRI with a medical history and laboratory work to determine eligibility. Patients will be accrued from the breast oncology clinics. When a patient with a breast cancer larger than 2 cm and who needs to undergo surgery is identified, the patient will be interviewed and provided information about this study. Signed informed consent will be obtained by Dr. Gary Whitman, Dr. Mark Dryden or other physician investigators (except Dr. Franklin Wong because of potential conflict of interest) no earlier than the next day after the initial interview to provide adequate time for the patient to consider participation

5.1.2 Informed Cosent Process

Referring physicians and clinic staff will be provided with flashcards (Appendix B) including eligibility criteria and contact information. After a patient is considered a candidate, the referring physician will explain the study and give the patient a Recruitment Information Brochure (Appendix C). Upon patient's contact with our research coordinator or protocol chairman, the eligibility criteria will be reviewed. If found eligible, a physician investigator (Dr. Gary Whitman, Dr. Mark Dryden or others) will interview the patient and provide further information of the study and answer relevant questions before obtaining informed consent as evidenced by patient's signing on the current version of IRB-approved informed consent form. Then the patient will be given a Study Procedure Instructions Flyer (Appendix E) which further explain the details of the procedures.

A statement reading : " There is no direct benefit to the patient from this investigational study" will be included in the informed consent form.

5.2 MRI and Injection

The patients will be scanned using a GE Signa Lx 1.5 Tesla MRI scanner equipped with a high performance gradient system (amplitude = 22mT/m; slew rate = 120 T/m/s). A phased-array bilateral breast RF coil will be used to maximize the signal-to-noise ratio. A breast positioning system with two compression plates and Vitamin E markers will be used to hold the breast in a reproducible position. Image

slice thickness will be approximately 6mm and the scan will be positioned to also include the axilla as much as possible.

Before entry into the study, patients will have been asked about metallic implants such as pacemakers and other devices. Patient with these conditions will have been excluded as determined by the MRI radiologist collaborators. Specific and easily accessible tumors will be preferred over other locations. An MR-compatible 20-gauge needle of 7 cm in length will be used. Patients with claustrophobia will be assured by the radiologist and MRI staff and if sedation is required, oral dose of 1 mg of Ativan or 5 mg of valium will be administered as per established procedures in the MRI suite. If requested, ear plugs will be provided to decrease noise during MRI.

The breast tumor will first be localized using a fast gradient echo T1-weighted 3D pulse sequence in the sagittal plane. An MR-compatible disposable sterile needle will be placed intra-tumorally. Areas of necrosis, if any (developed after the pre-injection MRI), will be avoided. An MR scan will be performed to ensure the proper location of the needle. Prior to injection, a high-resolution baseline image will be obtained using a gradient echo (GRE) pulse sequence with parameters selected to be sensitive to T2*. Injection may be preceded with surface or subcutaneous local anesthesia (1cc of 4% lidocaine, if the patient has no history of allergy or adverse reactions to lidocaine; otherwise, an alternative local anesthetic will be used) at the needle entrance through the surface. The GIMA will be injected in one single intratumoral injection into the tumor over 1 minute. The needles will then be slowly removed. This procedure is similar to routine breast lymphoscintigraphy. Immediately after injection, images (without breast compression) from multi-phase T2*-weighted MRI will be acquired using the same pulse sequence in quick successions up to 1 hour. All subsequent MRI images will be without breast compression. The volume of the injectate will be determined from manual segmentation.

5.3 Nuclear imaging using PET/CT

The earlier biodistribution of GIMA sequestration in the tumor and lymph nodes will be studied with Ga-68 GIMA using PET. Accurate localization and quantitation of radioactivity will be derived from the superior accuracy and resolution of PET. However, delayed PET studies will not be useful because Ga-68 decays rapidly (1.1 hour half-life).

After MR guided injection and imaging, the patient will be sent to the PET Suite. The radioactivity residence time in the tumor and lymph nodes will be derived from serial PET scans. PET scans will be performed with attenuation correction using a high resolution GE DST PET/CT scanner with spatial resolution of 6 mm covering the thorax. The patient's comfort will be assured by our attendant nurses, physicians and technologists. Images will be reconstructed in 2D/3D mode and the Ga-68 voxel concentration will be measured in the tumor and in the lymph node (if any) and be correlated with volumes from anatomic imaging including MRI and CT (as part of the PET/CT procedure).

5.3.1 Ultrasonography

The injectate volumes will be monitored using ultrasonography using parameters for routine breast imaging, following each MRI session. Our animal experiments indicate that using the routine clinical ultrasound instrument, GIMA was detectable with the implanted tumors of 3 cm in diameter. Therefore, the patients will be studied with routine clinical parameters (10-12 MHz or 5 MHz with harmonics in a Siemens Antares clinical ultrasound imaging devices) after MRI. Harmonics will be applied also to decrease potential artifacts. Since ultrasound measurements are relatively operator-dependent, to avoid biases ultrasound images will not be acquired or interpreted by the radiologist who performs the injection under MRI or interprets the MRI.

5.3.2 F-18 FDG PET

The patient will have had a baseline F-18 FDG PET scan as part of the clinical work-up to evaluate the tumor glucose metabolism before entering this protocol. About 5-7 days after Ga-68 GIMA injection, the patient will undergo another F-18 FDG PET scan. About 15 mCi F-18 FDG will be injected intravenously followed by a period of 45 minutes of uptake and then scanning for about 30 minutes for the whole body. The F-18 PET scan will be evaluated qualitatively for uptake in the breast tumor and semi-quantitatively using the established standard uptake values (SUV's).

5.4 Dosimetry modeling of beta and gamma emissions from radionuclides

There are three components necessary for the estimation of absorbed doses to tissues surrounding the injected activity: 1) The energy deposited in the surrounding tissues will be determined using radiation transport analysis [21], 2) the geometry of the activity distribution (source region) will be determined using MR image data, and 3) the total number of radioactive transitions that occur in the region will be determined using data from the scintigram. Both beta and gamma emissions will be evaluated. The total radiation absorbed doses will be derived for the tumor and surrounding tissues.

The volumetric data measured from MRI will be used to derive the S-values of the tumors using voxel-based simulation [22] to calculate the radiation absorbed doses to the injection sites and the surrounding tissues. Radiation dose rates, or S-values, will be compared with those from the sphere [18] and shell models [19] to evaluate the effects of potentially heterogeneously distributed injectate in the tumor. Such comparison will establish the boundaries of the models and aid choices of dosimetric methods in future studies.

5.5 Pathologic Evaluation

Histopathologic data will be collected from the surgical specimen obtained during the scheduled tumor resection (about 1-14 days from injection). If present, the histologic changes from radiation effects [23, 24] in and around the tumor/lymph nodes will be

correlated with predicted and measured dosimetry.

5.6 Potential Radiation Effects and Radiation Safety to the personnel

Although we believe that the radiation involve is low, it should be noted that any amounts of radiation may increase the chance of getting new tumors and radiation from the GIMA may affect the tumor cells. Because of the rapid decay of Ga-68 GIMA with half-life of 1.1 hours, negligible residual radioactivity (0.2 nano Ci) is expected 1 day after injection. Therefore, the planned surgery can be performed without health risk to the personnel. The surgical specimens will not undergo autoradiography although histopathologic correlations will be performed.

6.0 Pretreatment evaluation

Select Section Title: 6.0 Pretreatment evaluation

Prior to the imaging procedures, subjects will be questioned to obtain a medical history, and given a complete physical examination including a mammogram, breast MRI and laboratory tests including CBC to determine eligibility. Patients who have not previously received a breast MRI (with contrast if necessary) and F-18 FDG PET will have one of each performed (at the cost of the study) prior to entry into the study to evaluate the tumor size and assure absence of tumor necrosis. Women with child-bearing potential will receive a pregnancy test.

7.0 Evaluation During Study

Select Section Title: 7.0 Evaluation During Study

The patient will be accompanied by our team staff during the imaging sessions and encouraged to express feeling of discomfort and wishes to alleviate discomforts. Our physicians, nurses and nuclear medicine technologist and research staff will accommodate as much as possible while monitoring their objective parameters including vitals signs if necessary. A final F-18 FDG PET will be performed on the same day after the last nuclear imaging and PET.

8.0 Evaluation of Toxicity

Select Section Title: 8.0 Evaluation of Toxicity

8.1 Toxicity

The radiation absorbed doses to the body and organs are low for 0.5 mCi of Ga-68, compared with the routine dose of 8 mCi of Ga-67 citrate for tumor localization studies. The residual of 1 mg of Fe in the tissue is also not expect to present significant toxicity, considering the routine intramuscular injection of up to 1000 mg of iron sulfate for the treatment of anemia.

8.2 Stopping Rule of Study

Stopping rule will be defined as grade III/IV toxicity (NCI criteria as in Appendix L) in 2 or more of the 5 patients in a particular dose level.

9.0 Criteria for Removal from the Study

Select Section Title: 9.0 Criteria for Removal from the Study

- 9.1 All patients will be followed with reasonable efforts(routine clinical follow-up with surgeon) until 1 month after injection. Any patient initially accepted into the study, but who subsequently is determined to be ineligible for radionuclide evaluation will be removed from the study. The reason and time of removal will be documented.
- 9.2 The development of unacceptable toxicity is defined as un-expected, irreversible or grade 4 toxicity.
- 9.3 Non-compliance by patient with protocol requirements.
- 9.4 Patients have the right to withdraw from the study at any time without consequence. If a patient withdraws from the study, reasonable attempts will be made to document the reason for withdrawal.
- 9.5 Any patient can be removed at the discretion of the investigator or sponsor.

10.0 Number of Patients

Select Section Title: 10.0 Number of Patients

This is a study of biodistribution. A group of 5 patients will be studied with 0.5 mCi Ga-68 GIMA.

- 10.1 Only descriptive statistics (mean, variance, ratios and diagrams) will be applied to analyze the results of dispersed volumes by MRI and ultrasound, tumor and organ percentage of injected doses, dosimetry, potential toxicity and histologic changes.
- 10.2 The volumes by MRI and ultrasound, and percentage of injected radiation in the tumor and organs as determined by PET/CT will be collected and sent to our outside consultant Dr. Rick Sparks (Creative Developments, Inc, Dosimetry Service, Knoxville, TN) for computation of the dosimetry profiles. Because of the limited number of 5 patients, only descriptive statistics will be applied to analyze the data. The raw data will be stored until both this and the companion Ga-67 GIMA study are completed and then will be disposed of. The raw data will be kept under lock and key in our files for at least 5 years from the onset of this study.

11.0 Reporting Requirements

Select Section Title: 11.0 Reporting Requirements

11.1 Adverse Event Reporting to U.S Army Medical Research and Materiel Command (USAMRMC).

Unanticipated problems involving risk to volunteers or others, serious adverse events related to participation in the study, and all volunteer deaths should be promptly reported by telephone (301-619-2165), by email (hsrrb@amedd.army.mil), or by facsimile (301-619-7803) to the Army Surgeon General's Human Subjects Research Review Board. A complete written report should follow the initial telephone call. In addition to the methods above, the complete report can be sent to the U.S Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

11.2 Procedures for Reporting Serious Adverse Events

Serious and/or unexpected adverse events are submitted in writing to the M.D. Anderson Cancer Center Institutional Office of Protocol Research (OPR) within 10 working days of the adverse experience. Unexpected fatal or life-threatening experiences are phoned immediately to the Office of Protocol Research (713-792-2933). A follow up written report is submitted to OPR within 10 working days. The form for communication to OPR of all serious adverse events is appended to this plan.

For all protocols conducted at M.D. Anderson Cancer Center, the Principal Investigator is responsible for submitting adverse event reports to the Institutional IRB and the HSRRB and/or USAMRMC, Office of Research Protections on an ongoing basis. Adverse event reports are submitted to the Institutional Office of Protocol Research (OPR), where they are entered into PDMS and forwarded to the designated IRB vice chairperson for review. Attached to each adverse event report is a listing of all prior adverse events submitted for that protocol. Any comments, questions or changes the IRB requests to the protocol as a result of this review are conveyed to the principal investigator. The investigator response and protocol modification process is monitored by the IRB vice-chairperson and OPR support staff. The vice chairperson presents the report on adverse event review to the full committee at the next IRB meeting.

An adverse event report compilation is provided once annually to the M.D. Anderson Cancer Center IRB. Comments, questions or other considerations from the IRB are conveyed to the principal investigator for evaluation, discussion and implementation.

11.3 Reporting of Subject Death

The death of any subject during the study or within 30 days of study completion (as defined above), regardless of the cause, must be reported within 24 hours by telephone, to the principal investigator and/or study coordinator and the HSRRB and/or USAMRMC, Office of Research Protections. A full written report must follow as soon as possible. If an autopsy is performed, the report must be provided to the Sponsor (MDACC) and Supporter (USAMRMC).

Reports of all **serious adverse events, including deaths**, must be communicated to the appropriate Institutional Review Board or ethical review committee and/or reported in accordance with local law and regulations.

11.4 Other reports to MDACC and DOD HSRRB.

- All amendments will be approved by: MDACC Clinical Research Compliance, MD Anderson Cancer Center IRB and the HSRRB prior to implementation. If there are deviation from the protocol, the deviation will be reported to the medical monitor and MDACC IRB for approval. Deviation that effects patient safety or integrity of the study will be reported to the DOD HSRRB.

12.0 References

Select Section Title: 12.0 References

1. Vaidya JS, Baum M., Clinical and biological implications of the Milan breast conservation trials. Eur J Cancer Jul;34(8):1143-4, 1998.
2. Edmundson GK. Vicini FA. Chen PY. Mitchell C. Martinez AA. Dosimetric characteristics of the MammoSite RTS, a new breast brachytherapy applicator. Int. J. of Radiat. Oncol. Biol. Physics. 52(4):1132-9, 2002.
3. Nag S, Kuske RR, Cicini FA, Arthur DW and Wwicker RD. Brachytherapy in the treatment of breast cancer. Oncology (Huntington). 15(2): 195-202, 2001.
4. King TA, Bolston JS, Kuske RR, Fuhrman GM, Scroggins TG and Jiang XZ Long-term results of wide-field brachytherapy as the sole method of radiation therapy after segmental mastectomy for T(is, 1,2) breast cancer. Amer. J. Surg. 180(4):299-304, 2000.
5. Vicini FA, Baglan KL, Kestin LL, Mitchell C, Chen PY, Frazier RC et al. Accelerated treatment of breast cancer. J. Clin. Oncol. 19(7):1993, 2001.
6. deGuzman AF, Karvelis SKC, Shaw EG, et al. Brachytherapy Of Re-Resected Malignant Glioma Cavity Margins Using An Inflatable Balloon Catheter And Liquid 125I Source: A Phase I Study. J.Nucl.Med. 41(5 Suppl): 274P, 2000.
7. Dempsey JF, Williams JA, Stubbs JB, Patrick TJ and Williamson JF. Dosimetric properties of a novel brachytherapy ballon applicator for the treatment of malignant brain-tumor resection-cavity margins. Int. J. Radiat. Oncol. Biol. Phys. 42(2): 421-429, 1998.
8. Johnson N. Soot L. Nelson J. Franzini MD. Veal H. Gruner S. Kulawiak L. Young K. Sentinel node biopsy and internal mammary lymphatic mapping in breast cancer. American Journal of Surgery. 179(5):386-8, 2000.

9. Doting MH, Jansen L, Nieweg OE, Piers DA, Tiebosch AT, Koops HS, Rutgers EJ, Kroon BB, Peterse JL, Olmos RA, de Vries J. Lymphatic mapping with intralesional tracer administration in breast carcinoma patients. *Cancer*. 88(11):2546-52, 2000.
10. Bergqvist L, Strand SE, Persson B, et al. Dosimetry in lymphoscintigraphy of Tc-99m antimony sulfide colloid. *J Nucl Med* 1982; 23:698-705.
11. Glass EC, Basinski JE, Krasne DL, et al. Radiation safety considerations for sentinel node techniques. *Ann Surg Oncol* 1999; 6:10-11.
12. Hung JC, Wiseman GA, Wahner HW, et al. Filtered technetium-99m-sulfur colloid evaluated for lymphoscintigraphy. *J Nucl Med* 1995; 356:1895-1901.
13. Kim, E. E., Wong, F. C. Radiation Dosimetry and Safety in Lymphoscintigraphy. *In*: K. Hunt, M. Ross (eds), *The Mastery of Lymphatic Mapping*. New York. Springer-Verlag, (in press).
14. Loevinger R, Budinger TF, Watson EE. *MIRD Primer*. Society of Nuclear Medicine, 1991.
15. Evans JF, Stabin MG, Stubbs JB. Specific absorbed fractions of energy from internal photon sources in brain tumor and cerebrospinal fluid. *Medical Physics*. 22(3):331-40, 1995.
16. Ferrari M, Cremonesi M, Bartolomei M, Stabin M, Agosteo S, Chinol M, et al. Dosimetric Modeling for locoregional treatment of brain tumors with 99-Y-Biotin. *Eur. J. Nucl. Med.* 27(8):925, 2000.
17. Sparks, R.B., Crowe, E. A., Wong, F. C., Toohey, R. E., Siegel, J.A. Radiation dose distributions in normal tissue due to adjacent tumors containing I-131 or 90Y: *J Nucl Med*. 43(5):91P, 2002, Suppl.
18. Wong, F.C., Kim, E. E., Sparks, R., Wong, W-H, Yang, D.J. and Podoloff, D.A. Three Sphere Dosimetry Models (0.4 cc, 2cc and 10cc) of 12 Commonly Available Radionuclides for Locoregional Therapy or Extravasation. *J. Nucl. Med*. 42:243P, 2001.
19. Wong, F.C., Sparks, R., Kim, E. E., Yang, D. J., Podoloff, D. A. Simulation of radiation dosimetry in intra-cavitary injection of eight radionuclides in five shell models. *J Nucl Med*, 43(5):90P, 2002, Suppl.
20. Colombetti LG, Goodwin DA, Togami E. Ga-68-labeled macroaggregates for lung studies. *J. Nucl. Med*. 11(12): 704-707, 1970
21. MCNP manual, Monte Carlo N-Particle Transport Code System. RSIC 1994.
22. Yoriyaz H, Stabin MG, dos Santos A. Monte Carlo. MCNP-4B-based absorbed dose

distribution estimates for patient-specific dosimetry. J Nucl Med. 42(4):662-9, 2001

23. McCormick B, Strom E., Craighead PS, et al, Radiation Therapy Oncology Group. Research Plan 2002-2006. Breast Cancer Working Group. Int. J. of Radiation Concol, Bilol, Physics. 51(3Suppl 2):56-7, 2001.
24. Mirza AN, Fornage BD, Sneige N, uerer HM, Newman LA, Ames FC and Singletary SE. Radiofrequency ablation of solid tumors. Cancer J. 7(2):95-102, 2001.

PDOL Draft

Please Do Not Use for Patient Consent

**Go to the PDOL Homepage to access the
Informed Consent Printer Database**

Radiation Dosimetry from Intratumoral Injection of Radionuclides into
Human Breast Cancer- Ga 68 GIMA
2005-0220

Subtitle: Intratumoral Injection of Gallium 68 GIMA for Imaging by Positron Emission
Tomography and Magnetic Resonance Imaging

1. _____
Participant's Name I.D. Number

You are being asked to take part in this clinical research study at The University of Texas M.D. Anderson Cancer Center (hereinafter referred to as "UTMDACC" or "the institution"). This research study is strictly voluntary. This consent form explains why we are performing this research study and what your role will be if you choose to participate. This form also describes the possible risks connected with being in this study. After reviewing this information with the person responsible for your enrollment, you should know enough to be able to make an informed decision on whether you want to participate in the study. This study complies with all laws and regulations that apply.

You are being asked to take part in this study because you have breast cancer and you have a surgery scheduled to remove the cancer.

DESCRIPTION OF RESEARCH

2. PURPOSE OF STUDY:

The goal of this clinical research study is to learn how special radioactive molecules called gallium-iron macroaggregates (GIMA) distribute (travel and spread) in the body after they are injected into breast cancer tissue. This is not a therapeutic trial and you will not benefit from your participation.

3. DESCRIPTION OF RESEARCH:

For the common diagnostic procedure called lymphoscintigraphy, small radioactive particles (called colloids) are injected into a lymph node. Then, a special nuclear medicine scanner is used to watch these particles slowly distribute in the body. Researchers noticed the larger the molecule, the slower the particles move in the body. In fact, some of the larger colloid molecules seem to stay at the site of injection with very little movement. Since the amount of radiation on these colloids is very low, they cannot be used to treat cancer. New molecules have been developed called GIMA. These GIMA molecules have been designed to act like the colloid molecules (very slow moving). However, the GIMA molecules were made to carry larger radioactive particles. Since these molecules are very slow moving, they can be injected directly into a tumor without spreading out very far from the site of injection. In this way, radiation can be delivered directly to the tumor tissue without spreading to normal tissue.

For this study, small particles of radiation (Ga-68) will be attached to GIMA molecules. These molecules will then be injected into the breast cancer tumor. Special scanners will be used (PET and MRI) to "see" how far the molecules move away from the injection site. If it is found that the molecules do not move very far, in the future, more radiation particles can be attached to the GIMA molecules. These GIMA molecules can then be used to treat cancer with radiation. The radiation level in GIMA (but not its size) can be measured from outside of the body by scanners such as a PET scanner. The amount of iron in GIMA can be measured from outside of the body by a MRI scanner. The distribution of iron in GIMA can be used to determine the size of the GIMA collection by the appearance of darkening in the images around the injection site. It will be injected by a MRI-compatible needle to avoid potential injury during MRI. The small amounts of iron (one thousandth of a gram) in the GIMA injection will not be sufficient to cause physical movement of the particles because the particles are firmly dispersed within the tumor tissues. Ultrasound measurement is non-invasive and may provide similar size information and may in fact prove to be a more convenient method to monitor the size of GIMA in the future. Combining the radiation level and size measurements will allow researchers to determine how much radiation is delivered to the tumor and to other organs (if any).

A total of 5 people with breast cancer will be recruited in this protocol. Participants in this group will receive a GIMA with Ga-68 attached at a radiation dose of 0.50 mCi. The amount of radiation you will receive from the GIMA molecules is very low, about the same as a routine nuclear medicine scan. Even with the added radiation from the CT portion of the PET/CT measurement procedures, the total radiation will be

about 3-4 chest X-rays.

The GIMA will be injected directly into the tumor tissue with the help of a MRI scanner. If you prefer to have local anesthesia, you will receive about 1cc of lidocaine at the needle entry site above the tumor. If you have allergy or previous adverse reaction to lidocaine, another local anesthetic will be applied. A 20-gauge 7cm long needle that is compatible with MRI facilities will be used to inject the GIMA. The MRI scanner will be used to make sure the needle is inserted directly into the tumor. Also, MRI scans and ultrasound examination will be done within one hour after the injection and also after the 1-hour PET scanning which will take place after the initial MRI and ultrasound imaging. In other words, the sequence of event will be: an initial MRI for the injection of Ga-68 GIMA, a MRI and ultrasound to measure the particle size distribution, PET scanning to measure Ga-68 radioactivity distribution followed by repeated MRI and ultrasound to measure the particles. These procedures are expected to last about 3-4 hours totally. The physicians, nurses and technologist will assure of your comfort during the imaging sessions and you may freely express your wishes to avoid discomfort during the imaging sessions. If you have fear about being in a closed space (claustrophobia), you may still be able to participate in this study if you can tolerate it after taking oral doses of ativan (1mg) or valium (5mg).

After the last nuclear imaging procedure, you will have a F-18 FDG PET scan to measure the use of glucose in your breast tumor. This is a procedure you already had before entering this study and involve injection of a small amount of radioactive sugar.

There has been no known toxicity in human use for the FDG PET and the additional radiation is in the range of 1 to 2 chest X-rays.

Participants in all groups will have their regularly scheduled surgery. The tissue that is collected during the surgical procedure will also be studied to see the effect of this investigation on the tissue. No tissue will be retained specifically for this investigational study. Participation into this study will not delay your surgical schedule which is scheduled between 1-14 days from the injection of GIMA.

This is an investigational study. The human research use of radioactive Ga-68 GIMA has been approved by the UTM DACC Radiation Drug Research Committee which has been authorized by the FDA. The Ga-68 GIMA, PET scans, ultrasound examinations and MRI scans performed for this study will be provided free of charge. Five participants will take part in this group. All will be enrolled at UTM DACC.

This protocol is partially funded by a research grant from United States Army Medical Research and Materiel Command. It should be noted that representatives of the U.S. Army Medical Research and Materiel Command are eligible to review

research records as a part of their responsibility to protect human subjects in research. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this study..

4. RISKS, SIDE EFFECTS, AND DISCOMFORTS TO PARTICIPANTS:

While on this study, you are at risk for the side effects listed in this form. You should discuss these with the study doctor or your regular doctor. The known side effects are listed in this form, but they will vary from person to person. Many side effects go away shortly after the study drug is stopped, but in some cases, side effects may be serious, long lasting, and/or permanent and may even cause death.

Giving Ga-68 GIMA through the needle into the breast cancer tumor may result in pain at the injection site and or infection. Ga-68 GIMA have radiation particles attached to them. Radiation may increase the chance of developing new cancer. The radiation may also alter the cells in the tumor causing changes for the future planning of further treatment.

MRI (Magnetic Resonance Imaging) uses a large magnet instead of x-rays to take pictures of the inside of your body. People who have metal in their bodies (pacemakers, neurostimulators, certain clips, or staples from prior surgery) may not receive a MRI. The magnetic field used in MRI scanning may harm such people or cause problems with devices such as pacemakers. Part or all of the body will be passed into a long, narrow tube (scanner) which is open at both ends. The scanner has an intercom, which will allow you to speak to the doctors and staff during the procedure. The machine will produce a loud knocking noise. This is normal. You will be given earplugs to protect your ears. In addition, you may feel light vibrations throughout your body. Some people, especially those who have a tendency to feel uncomfortable in small or closed spaces, may feel "closed in" and become anxious while in the scanner. If you feel ill or anxious during scanning, doctors and the MRI staff will give comfort or the scanning will be stopped.

Ultrasound examination involves no ionizing radiation and is a safe routine procedure to evaluate breast tissues. In this study, it is used to confirm and follow the gross distribution of GIMA particles; while MRI is used to follow the refined disibution of GIMA.

A PET (Positron Emission Tomography) scan is a medical technique that monitors the activity in the brain and other organs and will be used to track the movement of the Ga-68 GIMA through the body. The PET scanner can take pictures of Ga-68 GIMA and "see" where it is in the body. By watching how Ga-68 GIMA travels through the body and studying where Ga-68 GIMA collects, researchers can learn if any radiation is deposited in certain organs in the body. Some people may feel "closed in" while lying in the scanner. However, the scanner is open at both ends and an intercom allows you to talk with doctors and staff. If you feel ill or anxious

during scanning, doctors and/or technicians will give comfort or the scanning will be stopped.

For this study, the Ga-68 GIMA is radioactive substance of low radiation levels. The Ga-68 GIMA PET scan procedure also exposes your body to low-level of radiation. The total radiation you receive from these procedures is about the same as 1-2 chest x-rays.

This research study may involve unpredictable risks to the participants.

If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study (Dr. Franklin C. Wong, 713-794-4649) or the Protocol chairman (Dr. Gary Whitman, 713-745-3520). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at 301-610-7663/2211.

5. POTENTIAL BENEFITS:

If this research study shows that GIMA stays at the injection site, future cancer therapies may be developed. This information may be of benefit to future patients. There are no benefits for you in this study.

6. ALTERNATE PROCEDURES OR TREATMENTS:

You may choose not to take part in this study, participate in a different research study or go directly to surgery without participating in any other study.

I understand that the following statements about this study are true:

7. According to the institutional conflict of interest policy, the principal investigator of this study and my primary physician cannot have a financial interest in any aspect of this research. However, in instances of medical emergency, it is possible that I may be cared for by a physician and/or administrator who has some form of financial interest in the sponsor of this study.

As of 06/10/2003, the following investigators on this study have disclosed an equity or stock option interest in the sponsor of this study: Through the University of Texas M. D. Anderson Cancer Center, Dr. Franklin C. Wong, a collaborator of this protocol has filed a patent application to the U.S. Patent and Trademark Office on radionulcide cancer therapies including the method of producing carrier-free GIMA. For these reasons, there is potential conflict of financial interest (intellectual properties) of this study involving Dr. Franklin C. Wong, The University of Texas, and UTMDACC. Dr. Franklin C. Wong is also the principal investigator of a U.S. Army Breast Cancer Research Grant supporting this study. Dr. Gary Whitman is the principal investigator who will supervise this study in UTMDACC. Either Dr. Gary Whitman or Dr. Mark Dryden will perform the injection of Ga-68 GIMA while you are in the MRI scanner.

The University of Texas M.D. Anderson Cancer Center has a financial interest in the sponsor of this study.

The University of Texas System has a financial interest in the sponsor of this study.

8. If I want to receive updated information regarding the financial interests of any physician and/or administrator at UTMDACC who has cared for me, I may call the Conflict of Interest Coordinator at (713) 792-3220. Upon request, I will be given access to information disclosing the identity of all physicians and/or administrators who have a financial interest in the sponsor of this study.
9. My participation is voluntary.
10. I may ask any questions I have about this study, including financial considerations, of my treating physician. I may contact the principal investigator for this study Dr. Gary Whitman at 713-745-3520 or the Chairman of the institution's Surveillance Committee at 713-792-2933 with any questions that have to do with this study.
11. I may withdraw at any time without any penalty or loss of benefits. I should first discuss leaving the study with my physician. Should I withdraw from this study, I may still be treated at UTMDACC.
12. I understand that the study may be changed or stopped at any time by my doctor, the principal investigator, the study sponsor, or the Surveillance Committee of UTMDACC.
13. I will be informed of any new findings that might affect my willingness to continue participating in the study.

14. The institution will take appropriate steps to keep my personal information private. However, there is no guarantee of absolute privacy. The Food and Drug Administration ("FDA"), and/or United States Army Medical Research and Materiel Command might review my record to collect data or to see that the research is being done safely and correctly. Under certain circumstances, the FDA could be required to reveal the names of participants.
15. You will not be responsible for research-related costs. If I suffer injury as a direct result of participation in this study, the institution will provide reasonable medical care. I understand that I will not receive reimbursement of expenses or financial compensation from the institution, the sponsor, the investigators or the United States Army Medical Research and Materiel Command for this injury. If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, (insert name and telephone number of principal investigator). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U. S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221. for this injury. I may also contact the Chairman of UTMDACC's Surveillance Committee at 713-792-2933 with questions about study related injuries.
16. Unless otherwise stated in this consent form, all of the costs linked with this study, which are not covered by other payers (HMO, Health Insurance company, etc.), will be my responsibility.
17. I recognize that there are no plans to provide any compensation to me for any patents or discoveries that may result from my participation in this research.

Authorization for Use and Disclosure of Protected Health Information

- A. During the course of this study, the research team at UTMDACC will be collecting information about you that they may share with the FDA and/or United States Army Medical Research and Materiel Command. This information may include your treatment schedule and the results of any tests, therapies, or procedures that you undergo for this study. The purpose of collecting and sharing this information is to learn about how the treatment affects your disease and any side effects you experience as a result of your treatment.

Your doctor and the research team may share study information with certain individuals. These individuals may include representatives of the FDA and/or the above listed sponsor, clinical study monitors who verify the accuracy of the information, individuals with medical backgrounds who determine the effect that the treatment has on your disease, and/or individuals who put all the study information together in report form. The UTMDACC research team may provide this information to the FDA and/or the above listed sponsor at any time. There is no expiration date for the use of this information as stated in this authorization.

- B. You may withdraw your authorization to share this information at any time in writing. More information on how to do this can be found in the UTMDACC Notice of Privacy Practices (NPP). You may contact the Office of Protocol Research at 713-792-2933 with questions about how to find the NPP.
- C. If you refuse to provide your authorization to disclose this protected health information, you will not be able to participate in the research project.
- D. I understand that my personal health information will be protected according to state and federal law. However, there is no guarantee that my information will remain confidential, and may be re-disclosed at some point.

CONSENT/AUTHORIZATION

Having read and understood the above, and having had the chance to ask questions about this study and reflect and consult with others, I give _____ permission to enroll me on this study. I have been given a copy of this consent.

SIGNATURE OF PARTICIPANT

DATE

TYPED OR PRINTED NAME OF PARTICIPANT

ADDRESS OF PARTICIPANT

WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR

DATE

SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP

DATE

TYPED OR PRINTED NAME OF PERSON RESPONSIBLE

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language that is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks and that the participant understood this explanation.

SIGNATURE OF STUDY DOCTOR OR PERSON OBTAINING
CONSENT

DATE

Statement of Work (revised 11/25/03)

- 1) Recruitment of patients from the medical and surgical breast oncology clinics (Months 1-18)
- 2) Establishing MR imaging scheme for measurement of Iron content of the injectate for volume and shape-
 - i) T2-weighted (GRE) MR imaging (Months 2-18)
 - ii) Determination of initial volume and subsequent changes (Months 3-24)
- 3) Establishing ultrasound scheme and measurement of gross distribution of the injectate macroaggregates over the study period (Months 2-24)
- 4) Establishing PET/ scintigraphy for spatial and temporal changes in radioactivity-
 - i) Determination of effective half-life and residence times in the tumor and other organs (months 1-18)
 - ii) Determination of distribution of radioactivity (Months 2-24)
- 5) Construction of radiation absorbed doses for tumor and visualized organs from Monte Carlo Simulation and established biokinetic models
 - i) Apply the models to determine the radiation absorbed doses for GIMA injection in breast tumor and in other visualized organs (Months 1-18)
 - ii) Extend the models to evaluate isotopes with therapeutic potentials to find the optimal candidate(s) for intra-parenchymal radionuclide therapy (Months 13-24)

Revision of Statements of Work (6/15/2005):

- 1) Preclinical studies in rats tumor models to confirm the sequestration of Iron Macroaggregates (Ga-67, In-111 and Y-90 labeled) and their effects on the suppression of tumors (months 1-18, accomplished);
- 2) Development of quality assurance of GIMA for radiochemical purity, sterility and pyrogen-free conditions, in preparation for human studies (months 12-20, accomplished);
- 3) Preparation of human protocol (ID03-0070) in compliance to M. D. Anderson rules for IRB approval (months 6-12, accomplished);
- 4) Modification of human protocol in compliance of human protection rules and regulations of the US Army (month 8-24; preliminary approval obtained; final approval pending);
- 5) Modification of the M. D. Anderson IRB-approved human protocol ID03-0070 in response to the unforeseen closure of M. D. Anderson Radioactive Drug Research Committee (months 20-28) and preparation of Investigation of New Drug documentation of the study drugs (Ga-67 GIMA and Ga-68 GIMA) for FDA approval (months 22-30);
- 6) Initiation of human studies of Ga-67 GIMA and Ga-68 GIMA and efforts to secure further funding for the human studies (months 30-36).

ANIMAL CARE AND USE FORM
The University of Texas M.D. Anderson Cancer Center

Answer all questions. Mark responses "N/A" if they do not apply. **DO NOT LEAVE ANY BLANKS. INCOMPLETE ACUFS WILL BE RETURNED WITHOUT REVIEW.** Use separate sheets if necessary.

I. INVESTIGATOR AND PROPOSAL

A. Research Investigator	<i>Dr. Franklin Wong.</i>	
Investigator's Title	Assoc. Prof.	
Investigator's E-mail	<i>fwong@di.mdacc.tmc.edu</i>	
B. Research Assistant	<i>Yuetang Wang, MD</i>	
Assistant's Title	<i>Research Assistant II</i>	
Assistant's E-mail	<i>ytwang@di.mdacc.tmc.edu</i>	
C. Department	<i>Nuclear Medicine</i>	
D. Phone 713/794-4649	Fax 713/794-5456	E. Box Unit 59
F. Proposal Title: <i>Local Injection of Radioisotopes to Ablate Breast Cancer in Rats</i>		
G. Funding Source		Funding agency deadline date for receipt of proposal:
☐ NIH/NCI		or ☐ No deadline
☐ NSF		or ☐ No deadline
☐ ACS		or ☐ No deadline
☐ None		or ☐ No deadline
☐ Private (Specify):		or ☐ No deadline
☒ Other (Specify): <i>DOD Grant Breast Cancer Concept Award</i>		1/21/2004 or ☐ No deadline
H. Describe the goal(s) of the research and role of animals in the project in lay terms: <i>To test the feasibility of using locoregional beta emitter ablative therapy (BEAT) with macroaggregates to treat breast cancer in rats.</i>		

I. WHO WILL PERFORM EXPERIMENTAL MANIPULATIONS ON THE ANIMALS? *Indicate specific persons when known. When only the position is known, provide minimal requirements for that position.*

NAME AND STAFF TITLE	EDUCATION/TRAINING	YRS EXPER. W/SPECIES
1. Franklin Wong, Assoc. Prof	<i>MD PhD</i>	<i>10</i>
2. <i>Yuetang Wang, Research Assistant</i>	<i>MD</i>	<i>2</i>
3. Suzanne Craig, Asst. Prof.	<i>DVM</i>	<i>10</i>
4. <i>Peggy Tinkey, Assoc. Prof</i>	<i>DVM</i>	<i>10</i>
5. <i>Leonard Chow</i>	<i>BA</i>	<i>2</i>

J. LIST ALL COLLABORATORS. Include all individuals other than those directly involved with the animal manipulations.

NAME	DEPARTMENT/INSTITUTION	CONTRIBUTION TO PROJECT
1. Jason Stafford, PhD	Imaging Physics	MRI of Rats
2. Kenneth Wright, PhD	Diagnostic Radiology	Ultrasonography of Rats
3.		
4.		
5.		

II. ANIMAL MODEL

Description of Animals

A. Species:		Rats	
B. Stock/strain/line (include genotype if relevant to project):		F344	
Does this strain develop any unique pathological condition(s)?		☐ Yes ☒ No	If yes, in the flowsheet describe the condition, age of animal at onset, and how the condition will be handled.
C. Sex:	☐ Male ☒ Female	☐ Either ☐ Both	
D. Age:	8 weeks		
E. Weight:	150 grams		
F. Why is it necessary to use animals in this project? The development and treatment of cancer is complex and functions in a coordinated systemic manner. To determine the physiological significance of the the tumor treatment, in vivo studies are crucial.			
G. Why is this species used? Rats are an accepted model of breast cancer in humans..			
H. Number to be purchased:		Total number of animals requested (Total numbers of animals reflected in the Flow Sheet required for Section VII must equal the total number requested). Note:	
Number to be produced in-house:		ACUFS are approved for a 3 year period.	
Total Number Requested:		480	
I. Why is this number of animals required? In our experience, a minimum of 3 animals per treatment group is required to achieve statistically significant results.			
☐ Yes ☒ No	J. Can in vitro systems or other approaches, e.g., mathematical models, be used to reduce the number of animals in this project?		
If No, why not? The development and treatment of cancer is complex and functions in a coordinated systemic manner. To determine the physiological significance of the the tumor treatment, in vivo studies are crucial.			

III. ANIMAL HOUSING AND NUTRITION

A. Animals will be housed:

		DESCRIPTION:
☒	Bates-Freeman Building	floors 8 and 3 (germ free) and floor 1 (conventional, barrier, biohazard)
☐	Basic Research Building	specific pathogen free (SPF) rodents
☐	Clinical Research Building	dogs, swine, monkeys, rabbits
☐	Smith Research Building	specific pathogen free (SPF) rodents
☐	Science Park, Dept. Carcinogenesis	rodents and aquatic animals
☐	Science Park, Dept. Veterinary Science	rodents, rabbits, large animals, non-human primates
☐	Other (Specify)	
☐ No ☐ Yes ☒ N/A	If "other" is checked, are facilities AAALAC accredited?	
☐ No ☐ Yes ☒ N/A	If "other" is checked, does the facility have an assurance statement on file with PHS?	

B. Type of Animal Facility:

		TYPES OF FACILITIES:
☒	Conventional	large animals, rabbits, guinea pigs, hamsters, chickens, frogs, and some rodents
☐	Quarantine	new arrivals, uncertain health status, veterinarian's direction
☒	Radioactive	animals which are to receive radioisotopes
☐	SPF Barrier	SCID, Nude and germ free rodents, and virus-antibody free rodents
☐	Biohazard	animals receiving carcinogens or infectious microbes or are involved in recombinant DNA work
☐	Other (Specify)	

C. Primary animal enclosures housing (cage, run, stall, pasture)

		ANIMAL HOUSING
☒	Conventional	large animals, rabbits, guinea pigs, hamsters, chickens, frogs, rodents
☐	SPF	SCID, nude and germ free rodents, virus anti-body free rodents (micro isolator cages)
☐	Biohazard	Micro isolator & other cages, runs, stalls
☒	Other (Specify)	YB. 5782

D. Animal Feed

		TYPES OF FEED
☒	Conventional	large animals, rabbits, guinea pigs, hamsters, chickens, frogs, and some rats and mice
☐	Autoclavable	rodents in SPF barriers
☐	Irradiated	rodents in SRB facility
☐	Other (Specify):	

E. Drinking Water

		TYPES OF DRINKING WATER
☒	Conventional	large animals, all non-SPF animals, unless otherwise specified.
☐	Reverse Osmosis (RO)	some small animals as required.
☐	RO Autoclaved	SPF rodents, others as required.
☐	Other (Specify):	

IV. HAZARDOUS AND RADIOACTIVE AGENTS USED IN ANIMALS

A. HAZARDOUS AGENTS: <i>include carcinogenic chemicals, antineoplastic drugs, infectious microbial agents, toxins, and recombinant DNA.</i>		C. RADIOACTIVE AGENTS:	
☒ Hazardous agent(s) will <i>not</i> be used.	☐ Hazardous agent(s) will be used.	☐ Radioactive agent(s) will <i>not</i> be used.	☒ Radioactive agent(s) will be used.
If Hazardous agent(s) will be used, identify all hazardous agents to be used: 1. 2. 3. 4. <i>Identify agent(s) used for each experimental group in Section VII, flow sheet.</i>		If Radioactive Agent(s) will be used, identify all radioactive agents to be used: 1. Ga-97 2. In-111 3. Y-90 4. F-18 <i>Identify agent(s) used for each experimental group in Section VII, flow sheet.</i>	
☒ N/A ☐ No ☐ Yes Attach a copy of your approval letter. ☐ Pending *	B. If Hazardous Agents are to be used (as indicated above), has the Hazardous Agent(s) use been approved by the Institutional Biosafety Committee (IBC)? <i>* If pending, no animal manipulation may begin before IBC approval is granted and a copy of your authorization provided to the Office of Research Administration.</i>	☐ N/A ☐ No ☒ Yes Attach a copy of your isotope registration.	D. If Radioactive Agents are to be used (as indicated above), has the Radioactive Agent(s) use been approved by the Institutional Radiation Safety Committee (IRSC)? If yes, provide Isotope Registration number: <u>1062</u> <i>* If pending, no animal manipulation may begin before IRSC approval is granted and a copy of your authorization provided to the Office of Research Administration.</i>
<i>Note: Grants, programs, projects, etc., involving the use of hazardous agents are reviewed by the Institutional Biosafety Committee. Contact the Office of Research Administration (713-794-4581) to determine the appropriate method of approval for pilot projects involving hazardous agents' use in animals.</i>			

V. EXPERIMENTAL PROCEDURES

A. Restraint

☒ Yes ☐ No	1. Will restraint of animal be necessary? Answer "Yes" if using any degree of restraint. The housing of animals in standard cages is not deemed restraint.
List Type - If answer is "Yes," <u>indicate type (manual, restraint device, chemical) and the maximum time any one animal would be restrained over a 24hr period.</u> If anesthetics/analgesics/sedatives are used, include complete dosage information. NOTE: This information should also be provided in Flow Sheet, Section VII, for each experimental group. <i>Manual restraint for tumor cell injection; For surgery, ketamine/xylazine cocktail or Isoflurane, 2-5%, will be used to effect for anesthesia.</i>	
☐ Yes ☒ No	2. Will paralytic drugs be used without associated general anesthetic?
Please Explain. If answer to above question is "YES," provide scientific justification why a general anesthetic cannot be used with the paralytic agent.	

B. ANESTHESIA

☒ Yes ☐ No	1a. Will anesthesia be used for any reason?
1b. If yes, provide complete drug and dose information both here and in the Flow Sheet, Section VII. <i>Ketamine 100mg/kg +Xylazine 10 mg/kg rodent cocktail or Isoflurane, 2-5% to effect</i>	
2. Indicate what methods will be used to monitor anesthetic depth (check all that apply):	
☒ Measure Respiratory Rate ☐ Measure Heart Rate ☐ Measure Blood Pressure ☐ Other (specify):	☐ Measure Body Temperature ☒ Measure Corneal and Pedal Reflexes ☐ Not Applicable
3. Give building and room number where animal(s) will be anesthetized. <i>YB.5774</i>	
4. Who will supervise administration of anesthesia to animals? (<i>Personnel must also be listed in question I.I.</i>) <i>Franklin Wong and Yeutang Wang</i>	
5. What <i>level</i> of personnel will administer anesthesia? <i>MD, PhD, DVM and BS</i>	
6. What other techniques will be used to minimize experimental pain or distress? <i>A heating blanket, giving supportive fluids as needed, close postoperative monitoring until animal is ambulatory.</i> <i>Techniques include hypothermia, periods of conditioning, (e.g. to stimuli or restraint), use of avoidable or escapable stimuli, injection made on non-weight bearing surfaces (e.g. complete Freund's adjuvant).</i>	

C. ANALGESIA

1a. In the event any animal covered by this protocol experiences pain, discomfort, or suffering, that animal will be (select all that apply):	☒ Euthanized ☒ Treated with appropriate anesthetics, analgesics, or tranquilizers after consultation with veterinary staff ☐ Not treated with appropriate anesthetics, analgesics, or tranquilizers (<i>ONLY investigators selecting this option MUST answer item 1b</i>)
1b. It is the policy of The University of Texas M.D. Anderson Cancer Center to relieve pain in research animals by administration of appropriate anesthetics, analgesics, or tranquilizers. If the use of these drugs is not consistent with the protocol, the USDA and PHS require written justification for withholding pain relieving medication. Provide this justification in the space below with attachments if necessary.	

D. SURGERY

☒ Yes ☐ No	1. Will there be any surgical manipulations of these animals? If yes, include in the Flow Sheet, Section VII, a <u>detailed</u> textual description of the surgical technique. Include descriptions of surgical site preparation, incision, all surgical manipulations, and closure technique (e.g., suture material, clips).	
2. The surgery will be performed in:		
☐	DVMS / DVS Surgery Room	
☒	DVMS / DVS Procedure Room (specify building & room number): YB.5774	
☐	Other (specify building & room number):	
3. The surgical manipulations will result in animal:		* SURVIVAL SURGERY CATEGORIES Aseptic technique is required for all survival surgeries. <u>Minor Survival Surgery</u> - wound suturing, peripheral vessel cannulations, and subcutaneous implants are examples. <u>Major Survival Surgery</u> - surgical interventions that penetrate a body cavity or have the potential for producing a permanent handicap in an animal that is expected to recover.
☒	Survival *	
☐	Nonsurvival	
☐ Yes ☒ No	4. Will there be multiple survival surgeries performed on <i>any</i> animal(s)? If "Yes," provide explanation in the Flow Sheet, Section VII.	
☒ Yes ☐ No ☐ N/A	5a. Is rodent survival surgery required? If "Yes," answer question 5b.	
☒ Yes ☐ No ☐ N/A	5b. Will you follow the IACUC Policy on Rodent Survival Surgery? If "No," describe why the policy cannot be used for your experiment and a description of your technique.	
6. If survival surgery is performed, what postsurgical care or therapy will be used? (<u>Postsurgical Care</u> - includes observing the animal to ensure uneventful recovery from anesthesia and surgery; administering of supportive fluids, analgesic and other drugs as indicated; providing adequate care for surgical incision; and maintaining appropriate medical records.) <i>Supplemental heat via heating pad or blanket, supportive fluids as needed, close postoperative monitoring until animal is ambulatory.</i>		
7. Who or what level of personnel will perform the surgery? (Person(s) and/or position must also be listed in I.I.) <i>Shuang Wang</i>		
8a. Who or what level of personnel will perform postsurgical care (if applicable)? (Person(s) and/or position must also be listed in I.I.) <i>Shuang Wang</i>		
☐ Yes ☒ No ☐ N/A	8b. Will they need training or assistance in surgical procedures, aseptic technique or postsurgical care? If "Yes," indicate procedures for which personnel need training or assistance.	

E. Drug, Reagent, Radiation, Material Administration

☒ Yes ☐ No	1. Will drugs, reagents, radiation, or materials (including cells) be administered to animals? Do not include anesthetics/analgesics/sedatives or routine post-operative antibiotics in this section.
If yes, list personnel and/or position (from Section I.I.) who will perform administration. Include substance, dose, route, volume, duration, frequency and potential complications in Section VII, flow sheet for each substance administered to each experimental group. Yeutang Wang	<u>Drug, Reagent, Radiation, Material Administration:</u> F13762 rat mammary tumor cell line in 0.1ml Rdioisotopes, 0.1ml intratumorally or in tumor bed with a 22-25ga. Needle. Example substance administrations: 100,000 tumor cells injected subcutaneously in 0.1 ml, one time using a 22-25 ga. needle. If complete Freund's adjuvant is used (or other agent which induces excessive inflammation, to include certain infectious agents), list as a separate substance. <i>Personnel and/or position should also be listed in I.J.</i>
2. During administration, animals will be ☐ anesthetized/unconscious, ☐ unanesthetized/concious, or ☐ xboth.	

F. SAMPLE COLLECTION FROM LIVING ANIMALS

☐ Yes ☒ No	1. Will you be collecting tissues (e.g. blood, urine, bile, cerebrospinal or ascites fluids, tail snips, ear punches or cuts, or any other tissues) from animals?
If yes, provide the following information for each tissue collection here and in Section VII, flow sheet: amount per sample, frequency of sample collection, and collection method.	
2. During extraction, animals will be ☐ anesthetized/unconscious, ☐ unanesthetized/concious, or ☐ both.	

G. Other Information

☒ No ☐ Yes	1. Will adjuvant be used? If yes, state adjuvant, volume, route of administration, and frequency. The IACUC guidelines for adjuvant use is available in the Office of Research Administration (713-794-4581). If techniques will deviate from this policy, provide scientific justification.
☒ No ☐ Yes	2. Will food and/or water intake be restricted? If yes, describe method of restriction (e.g., withheld completely, reduced ration), duration of restriction, and procedure for monitoring the animals' condition.
☒ No ☐ Yes	3. Will death be used as an endpoint? If yes, provide scientific justification and reason(s) why moribund endpoints cannot be used.
☒ No ☐ Yes	4. Will the mouse ascites method be used for monoclonal antibody (MAB) production? If yes, provide scientific justification why <i>in vitro</i> methods cannot be used. The policy for MAB production is available in the Office of Research Administration (713-794-4581). If techniques will deviate from IACUC guidelines, provide scientific justification.

G. Other Information (Continued)

5. Describe any animal manipulations not mentioned previously which may produce pain or distress. <i>None</i>
6. Describe any physical or physiological impairment of animals resulting from experimental manipulations (e.g., LD50, neoplasia). If tumor(s) is existent, state the maximum size, burden, and length of time the tumor will be present. Scientific justification must be provided if requesting total tumor burdens greater than 1.5cm diameter in mice and 2.0cm diameter in rats. <i>Tumor growth SQ may cause some regional discomfort. There may be some postoperative discomfort following tumor resection. Postsurgical complications such as wound infection or dehiscence can't be ruled out.</i>
7. Describe monitoring procedure/schedule, including weekends and holidays, for morbid and moribund animals. * <i>The rats will be observed on a daily basis, including weekdays and holidays.</i>
8. Describe criteria to determine morbidity, and point at which moribund animals will receive euthanasia.* <i>Any onset of visible distress, inactivity of lack of feeding, tumor size > 2.0cm, wound infection or dehiscence.</i>
9. Describe any postmortem procedures. <i>Tissue harvest of tumor, tumor bed, and all major organs.</i>
<i>*Note: All investigators are expected to continue to monitor animals at least daily, including weekends and holidays. Morbid is defined as affected with disease or illness; moribund is defined as being in the state of dying.</i>

H. Euthanasia - Include age and euthanasia method for unused rodent pups, if applicable. (Ether and chloroform are not approved agents for euthanasia because of potential flammable, toxic, and carcinogen hazards.)

1. Indicate the method(s) to be used:			
☒	Cervical Dislocation (*See #2 Below)	☒	CO ₂
☐	Decapitation (*See #2 Below)	☐	Exsanguination with Anesthesia
☐	Anesthesia Overdose (agent/dose/route):	☐	Other (Specify):
☐ No ☐ Yes		2. * If euthanasia by cervical dislocation or decapitation, will animals be sedated or unconscious during euthanasia? <i>Cervical dislocation after anesthesia for the biodistribution studies.</i>	
2a. If yes, state anesthesia to be used: <i>Ketamine 100mg/kg +Xylazine 10 mg/kg rodent cocktail or isoflurane 2-5%</i>		2b. If no, state scientific justification for this method.	

VI. BUDGET AMOUNTS

Adequate care and efficient use of animals are dependant on sufficient funding to purchase and maintain animals according to the protocol.	
\$8000	Budgeted for animal purchase <u>annually</u> .
\$8000	Budgeted for animal maintenance <u>annually</u> .

VII. FLOW SHEET

NOTE: In previous sections where a "YES" response requires further information to be placed in a flow sheet, please provide the required information for each experimental group or treatment. The IACUC members must be able to follow every manipulation of the animal from experiment initiation to euthanasia and postmortem harvest. Attach additional pages for the flow sheet. Do not attach grants or manuscripts. See attached examples. When completing the flow sheet, please include the following information for EACH drug administered: Dose, Frequency, Route, Volume, and Duration.

VIII. USDA ADDENDUM

If the species requested is laboratory rats or mice, **do not** complete this section.

USDA Category "C"		Comments
☐	This project will not involve pain or distress to animals, and therefore, no pain relieving drugs are needed.	Examples of procedures involving momentary discomfort and pain that do not require anesthesia include administration of anesthetics, analgesics, tranquilizers, fluid and electrolyte therapy, immunization (except where complete Freund's adjuvant is associated), topical and oral medications, blood collection (except intracardiac and periorbital), gastric gavage, certain procedures performed in the normal practice of veterinary medicine, and those involving diagnosis and treatment of diseases (e.g., injections, tattooing, palpation, skin scraping, and radiography), euthanasia, and postmortem procedures. Neoplasia/tumor burdens limited to the skin or subcutaneous region with no metastasis, <u>within approved size limitations</u> , are included in this category.
USDA Category "D"		Comments
☐	This study involves accompanying pain or distress to animals. However, appropriate anesthetic, analgesic, or tranquilizing drugs will be used. *	Examples of procedures involving pain that require anesthesia/analgesia include surgery (including biopsy), burning, freezing, branding, fracturing bones, stimuli including shock reinforcement which produce unavoidable or inescapable persistent pain, injection of any agent which induces excessive inflammation or necrosis (e.g. bradykinin, Freund's complete adjuvant and certain infectious agents), LD50 determinations, neurophysiological preparations, continuous chair or stock restraint of any animal for more than 12 hours, skin or corneal corrosivity testing, drug or radiation toxicity testing, intracerebral inoculations, intracardiac or periorbital blood collections. * List procedures, drugs, volumes, doses, route, and duration of anesthetic, analgesic, or tranquilizing drugs in Section VII, flow sheet, for each experimental group.
USDA Category "E"		Comments
☐	This study involves accompanying pain or distress to animals without the use of an appropriate anesthetic, analgesic, or tranquilizing drugs. **	Examples include euthanasia performed by methods other than approved in the report of the AVMA's Panel of Euthanasia, procedures listed in Question #2 above if they are performed without appropriate drugs or not amenable to relief by a such drugs. All other neoplasia/tumor burdens are included in this category. (USDA "E") ** Attach a complete explanation of the reasons why drugs for relieving pain or distress were not used. For example, explain how and/or why drugs would adversely affect the test/study results.

VIII. USDA ADDENDUM (Continued)

Response to Animal Act Regulations Part 2, Subpart C (Research Facilities), #2.31 IACUC,

[d] Reviews of activities involving animals

[1] [ii] P. I. Has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources (e.g. Animal Welfare Information Center) used to determine that alternatives were not available.

[iii] P.I. has provided written assurance that the activities do not unnecessarily duplicate previous experiments.

Response to [ii]: (use additional sheets if necessary) In each blank, fill in the appropriate number.

☐	No pain, discomfort, or suffering is involved.
☐	Alternatives to procedures that may cause more than momentary or slight pain or distress to animals <u>were not</u> found. _____ relevant references were found for the last _____ years. The UTMDACC Research Library resource personnel and _____ computer-assisted literature reviews (e.g. AWIC, Agricola, Medline) were used to search for information about alternatives. The following searches were conducted (attach additional sheet if necessary): Date: Search terms included:
☐	Alternatives to procedures that may cause more than momentary or slight pain or distress to animals <u>were</u> found. _____ relevant references <u>were</u> found for the last _____ years. The UTMDACC Research Library resource personnel and _____ computer-assisted literature reviews (e.g. AWIC, Agricola, Medline) were used to search for information about alternatives.* (* answer the question in the following box) The following searches were conducted (attach additional sheet if necessary): Date: Search terms included:

*The alternative(s) found do not satisfy my research requirements for the following reasons:

- 1.
- 2.
- 3.

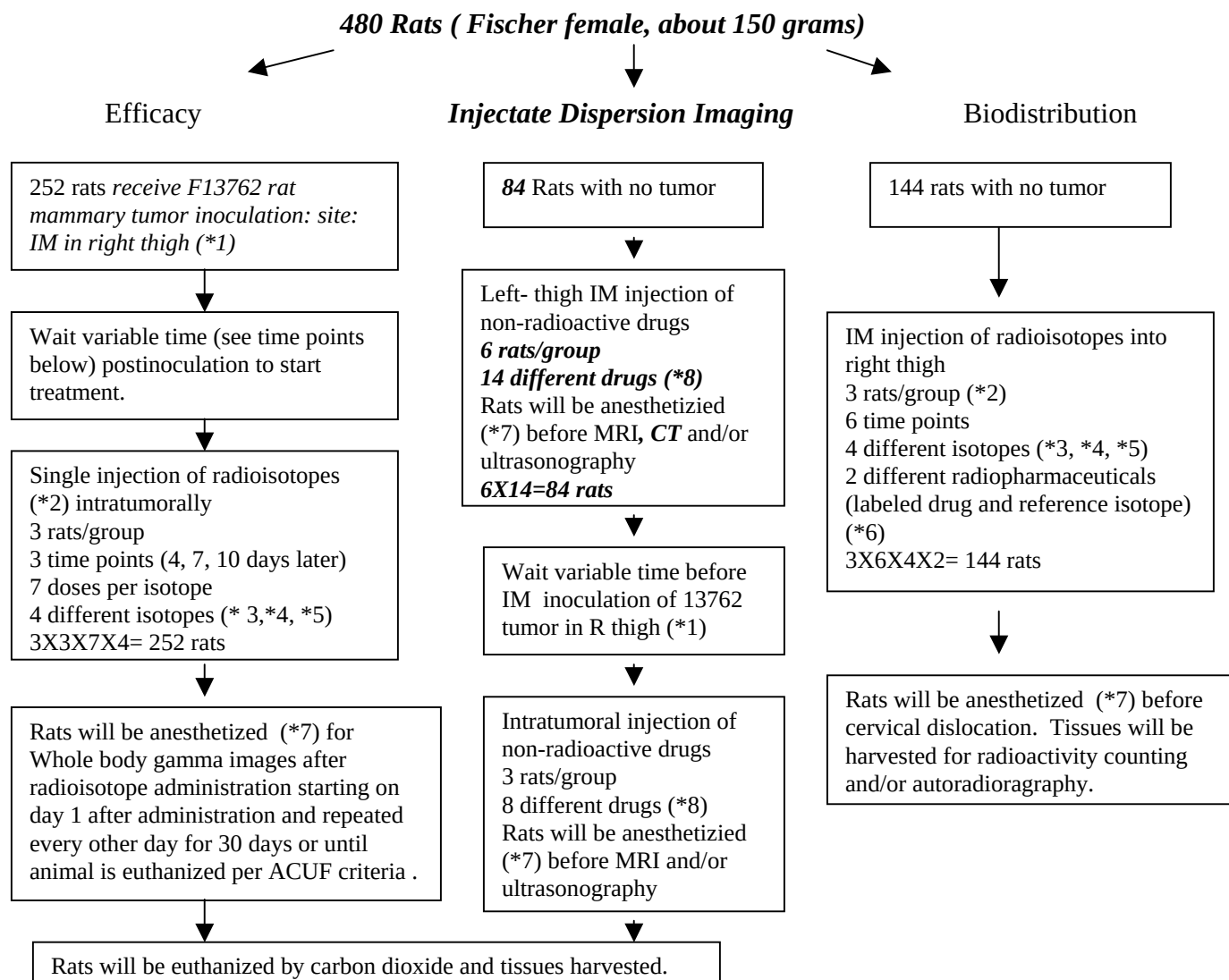
VIII. USDA ADDENDUM (Continued)

Response to [iii]: (use additional sheets if necessary)	
☐	There are not activities involving animals that duplicate previous experiments.
☐	Replications of some activities involving animals are necessary.*
*The activities and the reasons for replications are as follows: <i>(Replicated procedure because reason for replication.)</i> 1. because 2. because 3. because	

IX. INVESTIGATOR'S ASSURANCE STATEMENT

I accept and will conform to all Federal and State laws and guidelines, and all institutional policies and procedures concerning the care and use of animals in research, teaching, or testing. I also assure that I and all persons named on this form will complete the institutional animal care and use training program and submit documentation before working with animals. I understand that I have a responsibility to notify in writing the Institutional Animal Care and Use Committee of any changes in the proposed project or personnel, relative to this application, prior to proceeding with any animal use, and will provide an annual project status report.	<u>Substantive changes generally involve the following:</u> (a) changes in personnel. (b) increase in the number of animals used or the addition of an animal species. (c) change in procedures considered to increase the likelihood that animals will experience pain or distress. Examples include the addition of surgery, elimination of anesthesia, addition of LD50 testing, and addition of complete Freund's adjuvant.
Signature of Principal Investigator: <u>(Signature submitted already)</u>	
Print Principle Investigator's Name: <u>Franklin C. Wong MD PhD</u> Date: <u>2/25/03</u>	

I have reviewed this request for animal care and use and have found the proposed research to be scientifically meritorious.
Signature of Chairman/Division Head: <u>(Signature submitted already)</u>
Print Chairman/Division Head's Name: <u>Donald Podoloff MD</u> Date: _____



- Notes:
1. Injection of tumor cells will be 100,000 13762 tumor cells in 0.1-0.15 ml, injected by 25 gauge needle intramuscularly in the right thigh.
 2. The radiopharmaceuticals involved F-18 fluorodeoxyglucose (FDG), macroaggregates labeled with In-111, Ga-67 or Y-90. F-18 FDG is a tumor seeking agent that also induced apoptosis in breast cancer. FDG in rats may be monitored by micro-PET. Macroaggregates are colloids in aqueous solution with sizes about 18-30 microns. Because of the large sizes compared with capillaries, there is minimal transport after injection into tissues. Such sequestration is the basis for locoregional tumor therapy contemplated in the current study.
 3. Doses of Ga-67 and In-111 iron macroaggregates will be 0.0, 0.2, 1.0 and 2 mCi in 0.1ml colloidal solution containing approximately 1 mg of iron in the form of iron hydroxide. Similar injections of 0.2, 1.0 and 2.0 mCi of free isotopes Ga-67 citrate or In-111 chloridewill be performed as the reference groups for contrast.
 4. Doses of Y-90 iron macroaggregates will 0.0, 0.1, 0.5 and 1.0 mCi in 0.1ml colloidal solution containing 1 mg of iron in the form of iron hydroxide. Reference groups will receive 0.1, 0.5 or 1.0 mCi of Y-90 chloride.
 5. Doses of F-18 will be 0.0, 0.2, 1.0 and 2.0 mCi FDG and 0.2, 1.0 and 2.0 mCi of F-18 floride for contrast
 6. Biodistribution studies will involve 0.05 mCi of FDG and macroaggregates of Ga-67, In-111, Y-90, and reference isotopes F-18 fluoride, Ga-67 citrate, In-111 chloride and Y-90 chloride, respectively.
 7. Rats will be anesthetized using Ketamine 100mg/kg +Xylazine 10 mg/kg rodent cocktail or Isoflurane, 2-5% to effect for 30 minutes for MRI, ultrasonography, gamma/PET imaging. No restraint will be used/needed.
 8. Paramagnetic compounds (8) will be injected for MRI measurement of injectate temporal dispersion using 0.5 mg of Fe or Gd in 0.1 ml in the form of free ion, DTPA chelate, nanocapsules or macroaggregates. Ultrasonography is added to determine nanocapsules and macroaggregates dispersion upon injection. **CT contrast Omnipaque (10, 20, 30 and 100 fold dilutions) and 2 mixtures (30 and 100 fold diluted) with Magnevist wil lalso be studied with CT.**

Franklin C. Wong, M.D. , PhD. , J.D.
Associate Professor
Dept of NUCLEAR MEDICINE
Box 59
(713) 794-4649 FAX: (713) 794-5456
fwong@di.mdacc.tmc.edu

August 26, 2005

MEMORANDUM

TO: Institutional Animal Care and Use Committee (IACUC)
Attention: Lydia Jackson, Coordinator
Box 307

FROM: Franklin C. Wong, MD. , PhD. , J.D.
Associate Professor, Dept of Nuclear Medicine

RE: Addendum to my animal (rat) Protocol entitled 03-03-03641

1. To complement the existing MRI imaging algorithm, I would like to add Animal CT with or without intramuscular or intratumoral injection of contrast agent Omnipaque (an FDA-approved clinically used drug at 10, 20, 30 and 100 fold dilution) along with 2 combinations of Omnipaque and the FDA-approved MRI contrast agent Magnevist as additional imaging modality. These CT procedures will be carried out by the SACRIF staff.

2. Because of the addition of CT procedures and the need to increase the number of each group from 3 to 6, I would also like to add 60 animals to my protocol to study the CT contrast and MR contrast distribution. Enclosed is the modified flow chart for your reference. The modified portion in the flow chart has been highlight for your reference.

Thank you again in advance for considering this request.

Statistical Justification for the sample size of 3 rats per group in Protocol #03-03-03641

The sample size was calculated using a Statistical Software: *PS Power and Sample Size Calculation version 2.1.31* provided by the Vanderbilt University Medical Center
<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>

The assumptions are:

- 1) tests group and control group have independent means
- 2) alpha of 0.05
- 3) power of 0.95
- 4) mean of 8.4 cc on Day 14 or 22.2 cc on Day 17
- 5) standard deviation of 2.4 cc on Day 14 or 6.3 cc on Day 17
- 6) identical number of test group and control group, or, $M=1$
- 7) 4) and 5) are based on a set of 6/2003 experimental results from the control group of rats injected intratumorally with phosphate buffered saline only. This calculation set derives the number of rats required to show statistically (Student's T-test) different tumor growth pattern than those of the control rats at the end of the 2nd week between day 14 and day 17.

The calculation proceed as following:

PS logging enabled 4/7/2005 8:14:02 PM

Type of study: T-test
Requested output: Sample size
Design: Independent
alpha=0.05 power=0.95 DIFF=8.4 SIGMA=2.4 M=1
Sample size=3

Type of study: T-test
Requested output: Sample size
Design: Independent
alpha=0.05 power=0.95 DIFF=22.2 SIGMA=6.3 M=1
Sample size=3

Reference:

Dupont WD, Plummer WD: 'Power and Sample Size Calculations: A Review and Computer Program', Controlled Clinical Trials 1990; 11:116-28.

or

Dupont WD, Plummer WD: 'Power and Sample Size Calculations for Studies Involving Linear Regression', Controlled Clinical Trials 1998; 19:589-601.

This calculation is based on a Control Experiment conducted in June 2003 as below:

Date	PBS (control)	Rat #	25	26	27	Mean	S.D.
		Tumor Size					
	Days						
	post-inoculation						
6/06/03	7	Volume (cc)	1.02	0.86	1.12	1.0	0.1
		Length (mm)	10.60	9.60	11.10		
		Width (mm)	9.60	9.00	10.10		
		Depth (mm)	10.00	10.00	10.00		
6/09/03	10	Volume	0.82	1.60	2.02	1.5	0.6
		Length	11.60	14.40	13.80		
		Width	7.90	10.50	11.80		
		Depth	9.00	10.60	12.40		
6/10/03	11	Volume	2.07	2.71	4.58	3.1	1.3
		Length	14.00	15.60	16.60		
		Width	11.80	11.60	14.30		
		Height	12.50	15.00	19.30		
6/11/03	12	Volume	2.06	2.82	5.90	3.6	2.0
		Length	13.70	15.70	17.90		
		Width	11.20	12.30	18.20		
		Height	13.40	14.60	18.10		
6/12/03	13	Volume	3.41	6.38	7.80	5.9	2.2
		Length	15.70	23.10	23.00		
		Width	12.20	15.70	17.40		
		Depth	17.80	17.60	19.50		
6/13/03	14	Volume	5.90	8.50	10.73	8.4	2.4
		Length	24.90	25.24	26.30		
		Width	15.00	18.11	20.20		
		Depth	15.80	18.60	20.20		
6/16/03	17	Volume	22.39	15.77	28.45	22.2	6.3
		Length	30.50	21.40	33.60		
		Width	26.50	26.60	29.20		
		Depth	27.70	27.70	29.00		
6/17/03	18	Volume	18.99	25.71	34.44	26.4	7.8
		Length	31.70	32.90	37.80		
		Width	26.50	31.90	33.50		
		Depth	22.60	24.50	27.20		
6/18/03	19	Volume	19.74	36.34	47.54	34.5	14.0
		Length	29.10	34.30	37.20		
		Width	25.40	32.50	35.80		
		Depth	26.70	32.60	35.70		
6/19/03	20	Volume	23.89	37.77	47.17	36.3	11.7
		Length	32.00	37.60	38.30		
		Width	28.60	35.50	39.60		
		Depth	26.10	28.30	31.10		
6/20/03	21	Volume	31.39	44.62	57.44	44.5	13.0
			35.40	38.40	40.00		
			30.90	38.10	41.50		
			28.70	30.50	34.60		



United States Department of Agriculture
Animal and Plant Health Inspection Service

EJOHNSON

1476 cust_id
179488 insp_id
4803 site_id

UNIVERSITY OF TEXAS
M.D. ANDERSON CANCER CENTER

1515 HOLCOMBE BLVD
HOUSTON, TX 77030

Customer ID: 1476

Certificate: 74-R-0065

Site: 001

MD ANDERSON CANCER CTR

Inspection

Type: ROUTINE INSPECTION

Date: NOV-09-2004

The last routine inspection was conducted on 11/19/03. This routine inspection and exit review were conducted with Animal Resources Manager II, Tom Rodriguez, Chief of the Laboratory Animal Medicine, Dr. Suzanne Craig and USDA VMO, Earnest Johnson.

NO NON-COMPLIANT ITEM(S) IDENTIFIED THIS INSPECTION.

Prepared By:

Earnest Johnson DVM-VMO
EARNEST JOHNSON, V.M.O., USDA, APHIS, Animal Care

Title: VETERINARY MEDICAL OFFICER, Inspector ID: 4048

Received By:

Suzanne Craig
Dr. Suzanne Craig

Title: CHIEF OF LAM

Date:

NOV-09-2004

Date:

NOV-09-2004