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Award Number: DAMD17-03-1-0455

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

PRINCIPAL INVESTIGATOR: Franklin C. Wong, M.D.; Ph.D.

CONTRACTING ORGANIZATION: The University of Texas M. D. Anderson Cancer
Center
Houston, TX 77030

REPORT DATE: July 2007

TYPE OF REPORT: Revised Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 01-07-2007		2. REPORT TYPE Revised Annual		3. DATES COVERED (From - To) 9 JUN 2006 - 8 JUN 2007	
4. TITLE AND SUBTITLE Radiation Dosimetry of Intratumoral Injection of Radionuclides into Human Breast Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER DAMD17-03-1-0455	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Franklin C. Wong, M.D.; Ph.D. E-Mail: fwong@mdanderson.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas M. D. Anderson Cancer Center Houston, TX 77030				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT This study has been designed to evaluate 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). While the refinement of the human protocol is ongoing, we continued on basic studies of in vivo imaging and therapy using other drugs under revised versions of our approved rat tumor protocols. We gained approval of our canine tumor model protocol by the Army Animal Research Committee. Seven canine studies have confirmed the prolonged loculation of tumors after injection of Ga-67 GIMA. However, we also encountered organ- specific technical challenges that may not be relevant to human breast cancer. In preparation of the clinical trial, we gained approval of our human protocol 2005-0219 by our institutional IRB, pending final approval of federal regulatory processes. Initial contact with the FDA officials indicated that RDRC route was not the preferred route. We have therefore engaged our institutional IND committee to assist our application for IND/eIND. We have developed specific QA procedures for Ga-67 GIMA and engaged an outside radiopharmaceutical vendor for manufacturing, quality control as well as toxicology testing. We expect to be able to provide the necessary data for regulatory approval within the next year.					
15. SUBJECT TERMS No subject terms were provided.					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			USAMRMC
			UU	55	19b. TELEPHONE NUMBER (include area code)

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Introduction

In previous report periods, we completed preclinical studies to confirm the sequestration of radiolabeled Iron Macroaggregates (Ga-67, In-111 or Y-90) and their effects on the suppression of tumors. (Task 1, completed). We have also developed quality assurance procedures of GIMA for radiochemical purity, sterility and pyrogen-free conditions. (Task 2, completed). A human protocol using Ga-68 GIMA and Ga-67 GIMA to study radiation dosimetry in human breast cancer (MDACC ID03-0070) was developed and received our IRB approval (Task 3, completed), under the supervision of MDACC Radioactive Drug Research Committee (RDRC), without IND from the FDA. According to DOD Army HSRRB recommendations, the MDACC protocol ID03-0070 was bifurcated for Ga-67 GIMA (MDACC protocol 2005-0219) and Ga-68 GIMA (MDACC 2005-0220), pending HSRRB approval. However, because of the unforeseen closure of MDACC RDRC, efforts are sought to obtain an IND from the FDA to pursue only Ga-67 GIMA study (2005-0219) of 5 patients. In place of the Ga-68 GIMA study, we have sought and gained approval from the DOD to perform intratumoral injection in dog experiments (Appendix I, approved by DOD Animal Use Committee).

Our laboratory has continued to proceed in a two-prong approach. We have refined our quality assurance procedures of GIMA for IND application (Appendix II). We continue to refine the human protocol (the IRB approved version of 12/2006 is enclosed in the Appendix III) and to conform to the US Army HSRRB requirements. We have gained the preliminary approval from our IRB, pending regulatory approval. Initial contact with the FDA indicated that they reversed the position of allowing Ga-67 GIMA to go with the RDRC processes. Therefore, in preparation of application for an IND, we have engaged Isotex, an outside radiopharmaceutical vendor to manufacture and to provide quality control for Ga-67 GIMA in a GMP environment.

In the meantime, we conducted basic research studies to support future clinical applications. The basic research work includes preparation of different radiopharmaceuticals, imaging methods (CT, MRI and PET) for in vivo visualization of these radiopharmaceuticals. 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

According to the Revision of statement of work (6/15/2005):

- 1) For Task 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):** Earlier findings have been included in 2004 Annual Report. A canine tumor model protocol measuring intratumoral Ga-68 GIMA has been approved by the MDACC Animal Care and Utilization committee (Appendix I, approved by DOD Animal Utilization Committee). Using our approved canine tumor models (lung and/or prostate implantation of canine venereal tumors or cTVT), Ga-68 GIMA was injected under MRI and/or CT guidance to tumors in the prostate and lungs. Tumors were established in 7/7 dogs. However, one animal expired during anesthesia. Another showed spontaneous tumor regression. Nevertheless, inoculation of Ga-68 GIMA were accomplished in multiple sites in 6/6 dogs and confirmed prolonged retention for several hours after intratumoral injection. Although the retention Ga-67 GIMA over several days cannot be directly examined, selected MRI experiment indeed show persistent paramagnetic signal for days. For instance, as demonstrated in Figure 1 below, the baseline F-18 FDG PET scan of a 25 kg dog showed hypermetabolism in the implanted left and right lung tumors (1A). Under MRI guidance, 0.27 mCi of Ga-68 GIMA was injected into the right tumor. The degree of loculation of Ga-68 was measured by standard uptake value (SUV, even distribution has a SUV of 1.0) and was found to be >1600 indicating very high degree of loculation (1B). It also persisted through the PET imaging session of several hours. Follow-up studies 4 days later confirmed persistent loculation of iron (from GIMA) by MRI and suppression of right lung tumor metabolic activities as revealed by F-18 FDG-PET (1C). These are encouraging findings in a large animal model to confirm our rat tumor model findings in Task 1. Since both our earlier rat tumor model and the canine tumor models have confirmed prolonged loculation of Ga-68 GIMA for at least several hours, we have decided not to pursue the evaluation of Ga-68 GIMA in this human study because the goal of confirmation has been accomplished. In stead, we have decided to only pursue Ga-67 GIMA which is identical to Ga-68 GIMA except for half-life. Because of its longer half-life of 3 days, Ga-67 GIMA will allow GMP production and will be able to irradiate tumors over a longer duration of cell cycling to make use of the benefits of lower dose rates. These experiments and other intratumoral and locoregional therapy experiments will be presented in the upcoming Second International Conference of Radionuclide Therapy. Publication is progress.

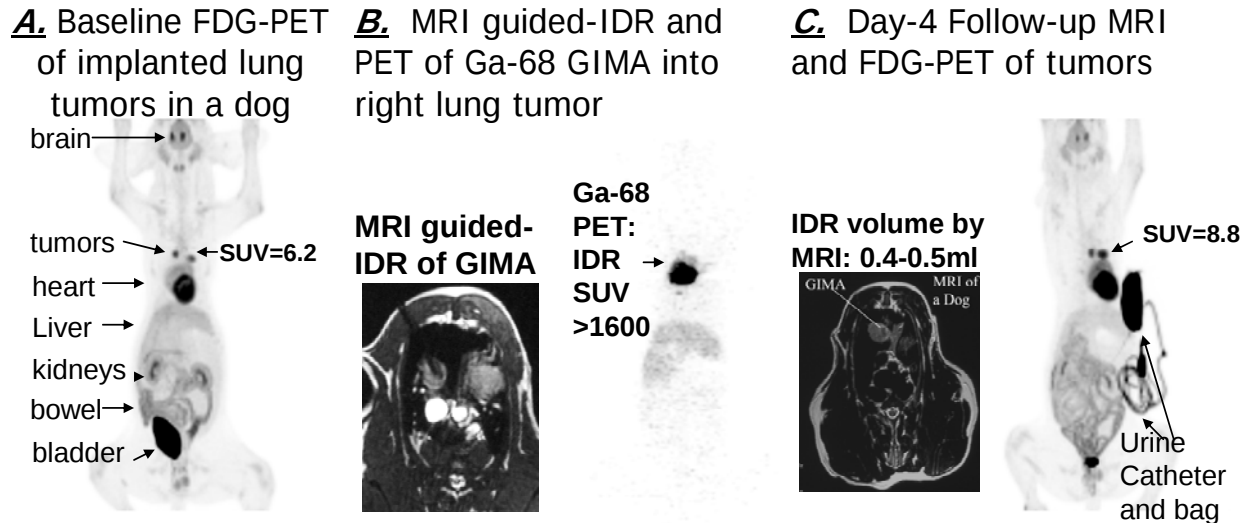


Figure 1. **A:** Baseline F-18 FDG-PET of metabolically-active tissues in a canine transmissible venereal tumor model. Hypermetabolic organs (brain, heart, liver and kidneys) are noted along with implanted tumors in the left and right lungs having SUV values of 6.2 and 6.5 respectively. **B:** Upon MRI-guided IDR of 0.27 mCi of Ga-68 GIMA, PET showed retention with maximum SUV>1600. Exaggerated display level was used to show faint Ga-68 in the liver but the IDR appeared much larger. **C:** Day 4 follow-up MRI found IDR volume at 0.4-0.5ml. FDG-PET found increasing SUV in the left tumor (=8.8) but not in the right tumor (=6.6) nor in other organs. The radiation absorbed dose in IDR was 1050 cGy. Necropsy identified necrosis inside right tumor.

- 2) **For Task 2- Development of quality assurance of GIMA for radiochemical purity, sterility and pyrogen-free conditions, in preparation for human studies (months 12-20, accomplished);**
Earlier findings have been included in 2004 Annual Report. Additionally, in preparation of application for an IND from the FDA, we have also refined our quality assurance procedures to develop ITLC procedures with saline at pH2-3 to differential Ga-67 citrate from Ga-67 GIMA as illustrated in Appendix II. This additional procedure is used for quality assurance of GIMA.
- 3) **For Task 3- Preparation of human protocol (ID03-0070) in compliance to M. D. Anderson rules for IRB approval (months 6-12, accomplished);** A copy of the approved protocol (ID03-0070) has been included in the 2005 Annual Report.
- 4) **For Task 4- Modification of human protocol in compliance of human protection rules and regulations of the US Army HSRRB (month 8-24; preliminary approval obtained; final approval pending);** - A copy of the MDACC IRB- approved 2005-0219 is enclosed in Appendix III, pending further revision and additional toxicity data to submit for IND application

- 5) **For task 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);** MDACC Protocol 2005-0219, initial approval by MDACC IRB in 9/2006; revision approved in 12/2006 (Appendix III). In 12/2006, an FDA official advised our team to pursue an IND on the Ga-67 GIMA protocol, additional animal toxicity and biodistribution are underway to support an IND application.
- 6) **Task 6- Initiation of human study-** pending IND approval and securing further funding

Key Research Accomplishments

- ❑ Development of Institutional ACUF approved animal protocol (Appendix I)
- ❑ Confirmation of the prolonged retention of GIMA after intratumoral injection in large animals (canine up to 30 kg).
- ❑ Development of additional quality assurance procedures for GIMA (Appendix II)
- ❑ Gaining MDACC IRB-approval of protocol 2005-0219, pending regulatory approval.

Reportable Outcomes

Presentation:

Wong, FC. Locoregional Radionuclide Treatment of Primary and Metastatic Tumors. During the Second International Conference of Radiopharmaceutical Therapy organized by World Radiopharmaceutical Therapy Council in Ulaanbator, Mongolia, Sept. 3, 2007.

Publication of the Canine tumor model experiments is progress.

Conclusions

During this report period (7/2006-6/2007), we have gained MDACC IRB approval of our bifurcated protocol to procedure with Ga-67 GIMA, pending regulatory approval. Upon consulting with the FDA on the regulatory approval, our team was advised of an alternative route to pursue an IND. We are continuing our 2-prong approach with different degree of success. The modification of human protocol (2005-0219) of Ga-67 GIMA for the conformance with vacillating FDA requirements is still ongoing. We have developed more quality assurance procedures for Ga-67 GIMA and are collecting more toxicity and biodistribution data to proceed with an IND approval process.

Our large animal canine tumor model confirm prolonged intratumoral retention of Ga-68 GIMA. Together, the rat and dog tumor models have provided sufficient scientific evidence to establish the short-term (hours) retention of GIMA. Therefore, the Ga-68 GIMA human trial appears redundant in humans and is canceled for better human protection. Since we have obtained another period of no cost extension to complete this work, we will pursue with full rigor to apply for an IND on the use of Ga-67 GIMA.

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References

Wong, FC. Locoregional Radionuclide Treatment of Primary and Metastatic Tumors. During the Second International Conference of Radiopharmaceutical Therapy, organized by World Pharmaceutical Therapy Council, in Ulaanbator, Mongolia, Sept. 3, 2007.

MDACC Dog Protocol 06-04-05871

Image-Monitoring of Locoregional Radionuclide Therapy

P.I. : Franklin C. Wong

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DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:

Office of Laboratory Animal Welfare
Division of Assurances
6705 Rockledge Drive
RKL1, Suite 360, MSC 7982
Bethesda, Maryland 20892-7982

FOR EXPRESS MAIL:

Office of Laboratory Animal Welfare
Division of Assurances
6705 Rockledge Drive-Suite 360
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Fax: (301) 402-2803

<http://grants.nih.gov/grants/olaw/olaw.htm>

September 2, 2005

Re: Animal Welfare Assurance #A3343-01

Leonard A. Zwelling, MD, MBA
Vice President for Research Administration
University of Texas M.D. Anderson Cancer Center
Unit 705
P.O. Box 301499
Houston, Texas 77035

Dear Dr. Zwelling:

The Office of Laboratory Animal Welfare (OLAW) has reviewed and approved the revised Animal Welfare Assurance (Assurance) which was submitted in compliance with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy) revised August 2002.

Your Assurance renewal bearing the identification number A3343-01 became effective on 8/31/05. This Assurance supersedes all previously issued Assurances. The approval period is for four years, and will expire on 8/31/09. Please be sure to reference your Assurance number in all correspondence to this Office.

The Assurance is a key document in defining the relationship of your Institution to the PHS since it sets forth the responsibilities and procedures of your Institution regarding the care and use of laboratory animals. Among the important elements of the Assurance, I would especially call your attention to the reporting requirements which are essential for continued compliance with the PHS Policy.

This letter also acknowledges acceptance and receipt of the 2004 Annual Report for the University of Texas M.D. Anderson Cancer Center.

A copy of the approved Assurance signature page is enclosed. If I may be of any further assistance, please contact me.

Sincerely,

Venita B. Thornton, D.V.M., M.P.H.
Senior Assurance Officer
Office of Laboratory Animal Welfare

Enclosure

cc:
David Mitchell



Office of Research Administration

Office of Research Administration
Unit 176
phone 713-563-3888
Fax 713-794-4535

12/19/2006

To: Franklin Wong/RAD
Copy: Rosie M. Handy/MDACC

From: Lydia G. Jackson/MDACC

ACUF ID #: 06-04-05871
Version: 10
Subject: e-ACUF Generic Memo from IACUC, Re: 06-04-05871

01 Wong 01 ver 10

MD ANDERSON CANCER CENTER
MODIFICATION REQUEST FOR ANIMAL CARE AND USE COMMITTEE
HOUSTON CAMPUS

Date Received 12/12/2006

To: IACUC
From: Franklin Wong/RAD

ACUF ID #: 06-04-05871
PI Name: Franklin Wong
Version: 10
Subject: ACUF Resubmission Cover Memo for '06-04-05871'
Title: Image-Monitoring of Locoregional Radionuclide Therapy

Did the IACUC Analyst request this modification for grant compliance? No

THIS BLOCK FOR THE ANIMAL CARE AND USE COMMITTEE ONLY

Meeting Date: January 16, 2007	Pre-Reviewer: Tinkey
Recommendation:	Pre-Review Date: 12/18/06
Administrative approval	
Modification Requests:	
Use previously approved radioisotopes for IM injxns as described in previous modification.	
Comments/Questions/Contingencies:	
ACUF is approved to use tumor-bearing dog model to assess anti-tumor efficacy of locoregional radioisotope agents. One of the areas of study is to assess the ability of these agents to remain in the area of the injection. A previously approved modification added intramuscular injections of Ga-67 and In-111 chloride followed by	

imaging. The current modification seeks approval for to use other radioisotope conjugated agents (which are already approved for use in the ACUF intratumorally) IM. No changes are requested in the injection technique, and no additional compounds are requested. Fundamentally, this modification is to add an additional route of administration for previously approved compounds.

Date Approved: December 18, 2006

Modification Requests

Revision # 1

Section and Item: VI Flow chart, after the last paragraph (Modification 4-11-06)

Change Made: Add new paragraph

Modification 12-12-2006: Intramuscular injections as described by the 4-11-06 Modification may also be performed with Ga-68 labeled compounds at less than 1 mCi levels (including GIMA, Ga-68 Gallium gadolinium macroaggregates, Ga-68 chloride with 1 mg Fe chloride or Gd chloride). Because of the short Ga68 half-life of 1 hour. The radiation protection measures will be more readily carried out and last less duration than in that modification of 4-11-06.

Rationale: Use of Ga-68 which has a half-life of 1 hour will simplify some of the radiation protection measures. Use of gadolinium will allow better visualization.

Revision # 2

Section and Item: Section VI Flow sheet entire section

Change Made: Change margination to 10% Left and 90% right.

Rationale: Aesthetics

Sincerely,

Lydia G. Jackson

12/19/2006 02:14:52 PM

Animal Care and Use Form
University of Texas M. D. Anderson Cancer Center

ACUF Protocol # 06-04-05871
(Temporary Identification Number: FW-20EV-0420P)

Have you had a previously IACUC approved ACUF on this work? ☐ Yes ☒ No

I. Investigator and Proposal

A. Principal Investigator:	Franklin Wong
PI Title:	Associate Professor
PI Phone:	713-794-4649
PI Department:	Nuclear Medicine
PI Fax:	713-563-3693
PI Unit:	1264
B. Contact Person:	Farrah Chickerneo
Contact Title:	Research coordinator
Contact Phone:	713-794-5349
C. Study Location:	Houston
D. Document Details	
Version:	10
Version Status:	Administratively Approved and Activated 12/18/2006
Save Status:	Saved as "Final"
Re-Submitted By:	Resubmitted by: Franklin Wong -- 12/12/2006 3:49:32 PM
ACUF Admin Action:	Accepted By: Lydia G. Jackson -- 12/19/2006 2:13:26 PM
E. Proposal Title:	Image-Monitoring of Locoregional Radionuclide Therapy
F. Describe the goal(s) of this research project in <u>lay terms</u>:	
1.	To evaluate the distribution of particulate and soluble radiopharmaceuticals in lung and prostate tumors in a large animal model using MRI.
2.	To characterize the rate of loculation or spread of radiopharmaceutical after injection in tumors and normal tissues using nuclear imaging instruments including gamma-camera or PET measurement.
3.	To evaluate the effects on tumor and other organs after injection with radiopharmaceuticals by measuring the metabolic rates using PET.
4.	To compare the distribution and loculation of radiopharmaceuticals after injecting into prostate cancers and lung cancers.
5.	To derive the radiation doses to the tumor and to different organs by combining the information from the MRI and nuclear imaging, so as to project to future human doses.
6.	To evaluate the histologic changes in tumors after intratumoral injection of radionuclides

G. Who will perform experimental manipulations on the animals?

1. Kamran Ahrar; MD; Assistant Professor; YEARS EXPERIENCE WITH SPECIES: 3 Training form on file; Will perform surgery
2. Dvms Personnel; Various; Various; YEARS EXPERIENCE WITH SPECIES: Various; Will administer anesthesia

3. Sacrif Veterinary Staff; Various; Various; YEARS EXPERIENCE WITH SPECIES: Various;
Will administer anesthesia

4. Farrah Chickerneo; MD; Research Coordinator, will perform radiation monitoring after isotope injection; YEARS EXPERIENCE WITH SPECIES: 1/Training form on file

5. Latoya Crayton; BS; Research Assistant, will perform radiation monitoring after isotope injection; YEARS EXPERIENCE WITH SPECIES: 1/Training form on file

Will Any personnel need training or assistance in surgical procedures, aseptic technique or postsurgical care? ☐ Yes ☒ No

H. List All Collaborators (include all individuals other than those directly involved with the animal Manipulations)

1. Kenneth Wright; Diagnostic Radiology; CONTRIBUTION TO PROJECT: Provide facility, guidance and mentoring

2. Jason Stafford; Physics; CONTRIBUTION TO PROJECT: Provide support for MRI scanning of the animals

3. Dawn Cavanaugh; Physics; CONTRIBUTION TO PROJECT: CT image Quality Analysis

4. Osama R Mawlawi; Imaging Physics; CONTRIBUTION TO PROJECT: PET imaging

I. Do the studies proposed within this ACUF involve the use of industry-sponsored research?
(e.g. sponsored research agreements with pharmaceutical or biotechnology companies)

☐ Yes ☒ No

II. Animal Model

Description of Animals

A. Species: Dog

B. Stock/Strain: Mongrel
Beagle

Trans/KO Gene:

Do any of these strains develop unique pathological conditions?

☐ Yes ☒ No ☐ Unknown

C. Sex: Both

D. Age: Adult

E. Weight: 15-60kg

F. Why is it necessary to use animals in this project?

Preclinical information from animal models is necessary before proceeding to clinical trials

Cell culture or mathematical models cannot simulate host-tumor interactions involved in metastasis (or angiogenesis)

We have developed a tumor model in the lungs and in the prostate in dogs.

G. Why is this species used?

Large tumors comparable to human tumors can be developed in the canine model, suitable for development and refinement of imaging techniques and/or targeted therapy

FDA recommended model

H. Total number of animals requested over a three year period.

Note: ACUFs are approved for a 3 year period.

Number to be purchased: 20

Number to be bred on site: 0

Current Inventory of
animals from previous
protocol: 0

Total Number Request: 20

I. Why is this number of animals required?

We are investigating the distribution of radiopharmaceuticals in tumor grafts in 2 different locations- each study will take 9 animals to reach statistical significance. Small allowance will be given to the small rate (10%) with which the tumor will not grow.

J. Can in vitro systems or other approaches, e.g. mathematical models, be used to reduce the number of animals in this project? ☐ Yes ☒ No

Why can other methods not be used to minimize the number of animals used?

In vitro studies do not take into account the dynamic physiological events that take place in a live animal. Blood circulation has significant impact on the extent of radiopharmaceutical distribution in the lungs or prostate and on the radioaction dosimetry. The only effective method of evaluating these devices, is to simulate a patient with a live animal model. Gross mathematical models have been performed but need to be refined and cannot substitute for various effects of blood flow and geometry. In vitro studies would not simulate a lung tumor or prostate in a live human being.

III. Animal Housing and Nutrition

A. Type of Animal Facility:

Conventional:

large animals, rabbits, guinea pigs, hamsters, chickens, frogs, and some rodents

Radioactive:

animals which are to receive radioisotopes

B. Animals will be housed:

Clinical Research Building:

dogs, swine, monkeys, rabbits

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

Conventional:

large animals, rabbits, guinea pigs, hamsters, chickens, frogs, rodents

D. Animal Feed

Conventional:

large animals, rabbits, guinea pigs, hamsters, chickens, frogs, and some rats and mice

E. Drinking Water

Conventional:

large animals, all non-SPF animals, unless otherwise specified.

IV. Agents Used In Animal

HAZARDOUS AGENTS: include carcinogenic chemicals, antineoplastic drugs, infectious microbial agents, viral agents, toxins, recombinant DNA. Do not include anesthetics or routine antibiotics

Note: Grants, programs, projects, etc., involving the use of hazardous agents are reviewed by the Institutional Biosafety Committee. Contact the Office of Research Administration (713-563-3879) to determine the appropriate method of approval for pilot projects involving hazardous agents' use in animals.

Note: In your flow chart(s), please include an informational description (e.g. dosage, routes of administration, frequency, durations of exposure, etc.) of agents to be used in this research protocol.

A. Will Hazardous Agents Be Used? ☒ Yes ☐ No

1. Indicate Hazardous Agents to be used below:

Hazardous Agents must be reviewed by the Institutional Biosafety Committee (IBC).

Submit a copy of your approval letter to the IACUC Office (required for approval)

Please update your IBC to include this protocol Number

Chemical Agents:

1. Cyclosporine -- IBC Approval #: 0904-103-HA-1

B. Will Human or Animal tissues or cells be injected or transplanted as part of this study?

☒ Yes ☐ No

Will Cells or Tissue be genetically modified before use in animals? ☐ Yes ☒ No

Animal Tissues or Cells Types:

1. Type: cTVT; Species of origin: Dog; Source: SCID Mice

C. Will Radioactive Agents Be Used? ☒ Yes ☐ No

Submit a copy of your approval letter to the IACUC Office (required for approval)

Please update your RSC to include this protocol Number

Radioactive Agents:

1. Ga-68 GIMA -- RSC Approval #: 1062

2. F-18 FDG -- RSC Approval #: 1062

3. Ga-67 citrate or chloride -- RSC Approval #: 1062

4. In-111 Chloride -- RSC Approval #: 1062

5. Y-90 Chloride or Zevalin (FDA approved monoclonal antibodies for human lymphoma), 2 mCi intratumoral -- RSC Approval #: 1062

D. Will External Radiation be administered to animals? ☐ Yes ☒ No

E. Will Non-Hazardous Experimental Agents be used? ● Yes ○ No

List all non-hazardous agents that will be used. Provide information on flowsheet

Non-Hazardous Agents:

1. Radiographic contrasts for CT and for MRI
2. Sodium Iodide solution (0.5 - 5.0 iodide mg/ ml in saline) in 0.5-2 ml for interstitial injection.
This is a nonradioactive drug used for protection of thyroid from radioactive iodine.
3. Iron (ferric) chloride (1mg Fe in 1ml saline or water) for IM injection to provide MRI/CT signal
4. Gadolinium (Gd) chloride (1mg Gd in 1ml of saline or water) for IM injection to provide MRI/CT signal
5. Calcium DTPA 500 mg IV (FDA approved human drug to increase excretion of radiometals)

F. During administration of any of the above agents, animals will be:

- ☒ Anesthetized/Unconscious
☐ Unanesthetized/Conscious
-

V. Experimental Procedures

A. Type of Restraint

1. Will restraint of animal be necessary? ☒ Yes ☐ No

Answer "Yes" if using any degree of restraint. The housing of animals in standard cages is not deemed restraint.

Indicate type of restraint, and the maximum time any one animal would be restrained within a 24 hour period.

Restraint Type:	Duration:	Dosage Information:
☒ Physical	Less than 1 minute	
☒ Chemical	Less than 90 minutes	buprenex - 0.01mg pentothal - to effect isoflurane - 1.5-3.0%

2. Will paralytic drugs be used without associated general anesthetic? ☐ Yes ☒ No

B. Anesthesia

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.

1. Will anesthesia be used for any reason? ☒ Yes ☐ No

Anesthetic	Dose	Route
1) buprenex	0.01mg/kg	i.m.
2) pentothal	to effect	i.v.
3) isoflurane	1.5-3%	Endotracheal tube
4)		
5)		

2. Indicate what methods will be used to monitor anesthetic depth

Measure Respiratory Rate
Measure Body Temperature
Measure Heart Rate

3. Building and Room Number where animal(s) will be anesthetized: Clinical Research Building
TB.4262

C. Analgesia

Note: For information about the regulations/policies concerning the use of analgesia, please consult the [IACUC's Analgesia Standard Operating Procedure](#).

1a. Moribund animals must be euthanized-In the event that an animal associated with this protocol experiences pain or suffering (e.g. after major survival surgery), analgesics will be given.

Euthanized

Treated with appropriate anesthetics/analgesics/tranquilizers after consultation with veterinary staff

2. Will you use other techniques to minimize experimental pain or distress? ☒ Yes ☐ No

Techniques:

- Other:

Observation to ensure uneventful recovery from anesthesia. Post-procedural analgesics (buprenex): will be for 3 days, then as determined is necessary by DVMS vet.

Building and Room Number: Research Clinical Building

D. Surgery

1. Will there be any surgical manipulations of these animals? ☒ Yes ☐ No

Include in the Flow Sheet, Section VII, a detailed 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. Surgery will be performed in:

Other

Building and Room Number:

Tan Zone Basement, Computed Tomography, TB.3848

3. The surgical manipulations will result in animal: ☒ Survival ☐ Nonsurvival

Survival Surgery Categories (**Aseptic technique and appropriate analgesia is required for all survival surgeries**)

Major Survival Surgery - surgical interventions that penetrate a body cavity or have the potential for producing a permanent handicap in a recovering animal

[IACUC's Analgesia Standard Operating Procedure](#)

4. Will there be multiple survival surgery required? ☐ Yes ☒ No

5. What post surgical analgesic care or therapy will be used?

Routine observation post anesthesia. The surgery required is CT guided needle placement in lung and is considered minimally invasive. Recovery from it is quick.

Analgesic	Dose	Route
1)		
2)		
3)		

6. What other postsurgical care or therapy will be used?

(e.g. heat lamps, etc.)

E. Sample collection from living animals

1. Will you be collecting tissues from animals? ☐ Yes ☒ No

F. Other Information

1. Will adjuvant be used? ☐ Yes ☒ No
<http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual>
2. Will food and/or water be restricted for reasons other than a normal fast ($\leq$ to 12 hrs) associated with surgery/anesthesia? If Yes, please provide the reason and length of time food and /or water will be restricted. ☐ Yes ☒ No
<http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual>
3. Will the mouse ascites method be used for monoclonal antibody (MAB) production?
☐ Yes ☒ No
<http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual>
4. Will any animal manipulations not previously mentioned be performed? ☐ Yes ☒ No
5. Are there any postmortem procedures? ☒ Yes ☐ No

Please describe the procedures:

Necropsy and harvest of tissues from the chest including lungs and mediastinum and pleura and/or prostate glands and pelvic tissues. Tissues will be evaluated by gross and histopathology

6. Will animals be removed from the DVMS/DVS facilities for any experimental procedure?
☒ Yes ☐ No

Indicate building and room number where the procedure will be performed.

Research MRI Facility, RB.2625

LightSpeed-16 scanner is in the Green Zone on the third floor (G3.3585a)

G. Monitoring of Animals

1. Describe any physical or physiological impairment of animals resulting from experimental manipulations (e.g., MTD50, neoplasia). If tumor(s) exist, 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.
The IACUC policy on tumor burdens in animals is available on the IACUC Website at:
<http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual>

All animals will be inoculated with cTVT for development of lung tumors and or prostate to a maximum size of 4 cm. A desirable tumor size for treatment is 2 to 3 cm. Based on our previous experience with this model, it will take 6 to 8 weeks following inoculation to achieve this goal. All treatment and follow up should be completed in 1 week. The maximum length of time anticipated to complete the project in each animal is 10 weeks.

2. Describe monitoring procedure/schedule, including weekends and holidays, for morbid and moribund animals.*

Animals will be monitored daily.

3. Describe criteria to determine morbidity, and the point at which moribund animals will receive euthanasia.*

The following criteria will be assessed for detection of moribund animals. Lack or loss of appetite will be investigated and correctable causes will be addressed. Those animals that continue to have marked diminished appetite leading to emaciation and dehydration will be considered moribund. Lack or decreased activity level will be evaluated, and those animals with persistent and prolonged decreased mobility will be considered moribund. Respiratory status will be assessed, and those animals that show signs of respiratory distress despite adequate measures to correct the underlying problem will be considered moribund. Persistent urinary or bowel obstruction (from prostate tumor) will be considered moribund

***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.

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 carcinogenic hazards)

Note: The use of hypothermia to induce anesthesia in rodent pups < 6 days old requires the use of an acceptable method of euthanasia and must be scientifically justified.
If applicable, please include justification below:

1. Indicate the method(s) to be used:

Exsanguination with Anesthesia

2. Will death be used as an endpoint? ☐ Yes ☒ No

<http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual>

VI. Flow Sheet

Immunosuppression: Immunosuppression therapy will begin 1-4 days prior to tumor inoculation. Each dog will be immunosuppressed with 10 mg/kg cyclosporin b.i.d. for 2 weeks and then s.i.d. until the end of the study. Cyclosporin will be administered by mouth (po).

Anesthesia: For all imaging and interventional procedures, sedation is induced by an i.m. injection of buprenex (0.3 mg) and a s.c. of atropine (0.04 mg/kg). Pentothal will then be given i.v. to effect and an endotracheal tube will be inserted. Anesthesia will be maintained with Isoflurane (1.5-3%) and oxygen.

Tumor Inoculation: With lung tumor grafts, the animal is placed in a CT scanner, and the right and left lateral chest walls are shaved and prepared for aseptic surgery. CT scans of the lungs are obtained without and with intravenous injection of contrast medium (meeglumine-diatrizoate; 35 ml). At least 2 sites will be selected for a single inoculation each. The site will be shaved, the skin will be prepared in sterile fashion using betadine. Under direct CT guidance, a 22 gauge Chiba needle is placed in a preselected location and 0.5 ml of freshly harvested and prepared canine sarcoma tumor fragments are injected into the lung parenchyma. Following inoculation, CT scans of the lungs is repeated without contrast injection. The animal is then allowed to recover from anesthesia.

For prostate tumor grafting, a ventral midline laparotomy will be performed to expose the prostate, followed by injection of the tumors (0.5 ml total) in the anteroventral aspects of both lobes and the prostate will be surgically fixated under the anterior wall of the bladder for future image-guided injection of radionuclides. The animal is then allowed to recover from anesthesia. The tumor will be monitored with MRI and/or CT and/or ultrasounds without contrast.

Additional CT images are obtained using a clinical CT scanner located on the 3rd floor of the green zone as well as a volumetric CT scanner located in the basement of Tan zone. These CT scans will be performed on different days and will require anesthesia on separate occasions. These images will be obtained once a week with and without contrast starting at 2 weeks after inoculation to monitor the growth of the tumor. The transport route to the diagnostic CT scanner in the green zone is included in the attached file.

Transport route:

T1 to B1, via the north end lifts & overhead door
B1 Gimbel corridor to Y1 elevators (8, 9, 10) to 3rd floor.
Y3 corridor through patient waiting room to G3 corridor
G3.corridor across bridge to G3.CT facility
See the attached map of the route along the 3rd floor corridor.



Transport route to G3 CT&PET.

When tumors reach approximately 2 cm in size, the animals are treated as follows.

Pre-injection Imaging: At least one day prior to radionuclide injection, each animal undergoes non-contrast CT, MRI and F-18 FDG (1-5mCi) baseline PET scan to determine the metabolic status of the tumor. The MRI will include diffusion weighted images that are particularly sensitive to acute and subacute ischemic changes.

The dogs are transported from TB animal facility along the 1st floor corridor hallway (Blue Zone)

to the Yellow Zone as indicated in the enclosed Transport route.



Route Map to Signa 1.5T.p

Radionuclide injection, MRI and PET/CT: Approximately 1 mCi of Ga-68 GIMA (half-life of 1 hour) or 2 mCi of F-18 FDG (with 3 mg FeCl₃ or 5 mg of Magnevist, a MRI contrast agent, and/or 10mg iodine equivalent in Omnipaque, a CT contrast agent) will be injected intratumorally, under MRI and/or ultrasound (for the prostate group) guidance. MRI will be then used to determine the initial physical distribution of the particulate drug Ga-68 GIMA (from the Iron) or soluble drug F-18 FDG (from the co-injected Fe Cl₃ or Magnevist or CT contrast). PET/CT scanning will be used to determine the radioactivity distribution and decay of Ga-68 GIMA or F-18 FDG in the injectate over the next 1-2 hours. A follow-up MRI session will be performed to evaluate the potential dispersion of the injectate

An alternative to intratumoral injection of Ga-68 GIMA or F-18 FDG will be for intratumoral injection of 1-2 mCi of Y-90 Chloride or Y-90 Zevalin (an FDA-approved human monoclonal antibody drug against lymphoma). For injection with Y-90 chloride, calcium DTPA (500 mg) will be administered intravenously one time each day from the day after treatment to accelerate excretion of Y-90. For both Y-90 Chloride and Y-90 zevalin, because of the 3-day physical half-life of Y-90, established radiation protection procedures will be carried out beyond necropsy, until radiation exposure levels of all specimens falls to background levels.

Follow-up FDG-PET: Whole-body FDG PET scan using 1-5 mCi of F-18 FDG intravenously will be performed to compare the metabolic status of the tumor.

Following completion of the follow-up FDG-PET scan, an optional procedure may be performed to study movement of solutes after interstitial injection. Nonradioactive sodium iodide (0.5- 5mg iodide /ml in saline) in 0.5- 2ml volumes with or without 0.2-1 mCi of F-18 FDG will be injected under CT guidance into the lungs in the PET/CT suite. Serial PET/CT will be performed over 1-1.5 hours to obtain the spatial and temporal clearance patterns of F-18 FDG using sodium iodide as the CT contrast for better spatial resolution.

PET scanning will be performed during the weekend or afterhours in the clinical PET/CT scanners when no patient is on schedule.

No animal will undergo more than 3 consecutive days of imaging. This is not likely to occur because of the short half-lives of the radiopharmaceuticals.

Necropsy and Pathology: Following the last MRI scan, each animal is sacrificed by exsanguination under deep anesthesia with isoflurane. The lung and/or prostate tissues are harvested for gross and histopathology evaluation. The effectiveness of treatment is assessed by light microscopic evaluation of the treated lung tumors.



dog flow chart-FW-2.p

Aug 17, 2005: This ACUF is approved to perform survival surgery to implant TVT tumors in the lungs and prostate of dogs in up to 4 sites; to perform multiple MR and PET/CT imaging procedures using radioisotopes and standard contrast agents; and to perform percutaneous intratumor injections of radioisotopes + various carrier agents. We currently have a dog that has

undergone tumor implantation, imaging, and intratumoral treatment. The tumors were treated under MR and CT guidance on Mon, Aug 15. The dog is scheduled for another imaging session (MR and PET/CT) on Friday. Due to technical difficulties on Monday, the therapy injections missed the target (the tumors) in both sides of the lungs - the prostate injections went OK. We are now requesting to repeat the pulmonary tumor injections on Friday, Aug 19, 2005 during the regularly scheduled imaging session. We will perform the procedures exactly as already approved in the protocol; no additional anesthesia or imaging is required; basically, the only additional procedure is to perform the percutaneous lung injection. We are requesting this addition on this one dog, one time only.

Modification 4-11-06: Shortly before or after the CT/MRI evaluation, a nuclear imaging procedure will be undertaken on selected groups of animals (2-3, based on logistics) for confirmatory studies of interstitial location of radionuclides after intramuscular (IM) injection. This will be conducted while the animal is already under anesthesia for a previously approved procedure, to eliminate the need for additional anesthesia procedures. The animal will then be transported to YB.5774 to receive intramuscular injection in the leg muscle of 0.5 mCi of Ga-67 chloride or In-111 chloride (with or without 1mg of Fe chloride or Gd chloride for MRI signals). Nuclear imaging will be performed using an animal gamma camera Siemes M-CAM to obtain static 5-10 minute images. Subsequently, the animal will undergo serial similar gamma imaging during subsequent anesthesia sessions for other planned CT, MR or PET. Radiation protection procedures including daily swipe test and radiation exposure measurement will be conducted until after necrosy during which the injected muscule(s) will be preserved for at least 3 more weeks before histopathologic evaluation.

Please note that these procedures will only be conducted in connection with a previously approved imaging study, and the extent of the additional manipulation will be an intramuscular injection followed by approximately 15 minutes of imaging. There are no procedures will represent significant additional pain or distress to the animal.

Radiation safety approval for use of these agents in the dog has been received. Existing transport routes are appropriate for these additional procedures and isotopes.

Modification 12-12-2006: Intramuscular injections as described by the 4-11-06 Modification may also be performed with Ga-68 labeled compounds at less than 1 mCi levels (including GIMA, Ga-68 Gallium gadolinium macroaggregates, Ga-68 chloride with 1 mg Fe chloride or Gd chloride). Because of the short Ga68 half-life of 1 hour. The radiation protection measures will be more readily carried out and last less duration than in that modification of 4-11-06.

VII. Addenda

USDA Addendum:

USDA Category "C"

- ☐ This project will not involve pain or distress to animals, and therefore, no pain relieving drugs are needed.

USDA Category "D"

- ☒ This study involves accompanying pain or distress to animals. However, appropriate anesthetic, analgesic or tranquilizing drugs will be used*

* 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"

- ☐ This study involves accompanying pain or distress to animals without the use of an appropriate anesthetic, analgesic, or tranquilizing drugs. **

** 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.

USDA Responses to Animal Act Regulations

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.

Part [ii]

Response to part [ii]: Select the appropriate choice below

- ☐ No pain, discomfort, or suffering is involved.
☒ Alternatives to procedures that may cause more than momentary or slight pain or distress to animals were not found.
☐ Alternatives to procedures that may cause more than momentary or slight pain or distress to animals were found.

Part [iii]

Literature Search Details:

20 relevant references were found for the last 9 years. The UTMDACC Research Library resource personnel and Medline and Pubmed computer-assisted literature reviews (e.g. AWIC, Agricola, Medline) were used to search for information about alternatives.

List the top three searches:

Date:	Search terms included
07/19/2004	Intratumoral and radionuclide and prostate and animal model
07/19/2004	Intratumoral and radionuclide and lung and animal mode
07/19/2004	dosimetry and intratumoral and animal model

The Alternative(s) found did not satisfy my research requirements for the following reasons:

Intratumoral unsealed radionuclide injection is a novel treatment for lung tumors and prostate cancer. There is very little data on safety, effectiveness and complications of the intervention. Despite some antedoctal clinical reports in humans, to date there is no studies done to evaluate the distribution and dosimetry of radionuclides after intratumoral injection in large animals.

Response to Part [iii]:

- ☒ There are no activities involving animals that duplicate previous experiments.
 - ☐ Replications of some activities involving animals are necessary.
-

VIII. Funding Sources

Source
NIH/NCI

Status
Pending

Investigators are responsible for budgeting sufficient funds for animal purchase and maintenance. Information on the current maintenance charges is available on the DVMS Intranet Website.

IX. Investigator's Assurance Statement

Principal Investigator:

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.

Principal Investigator
Signature: _____

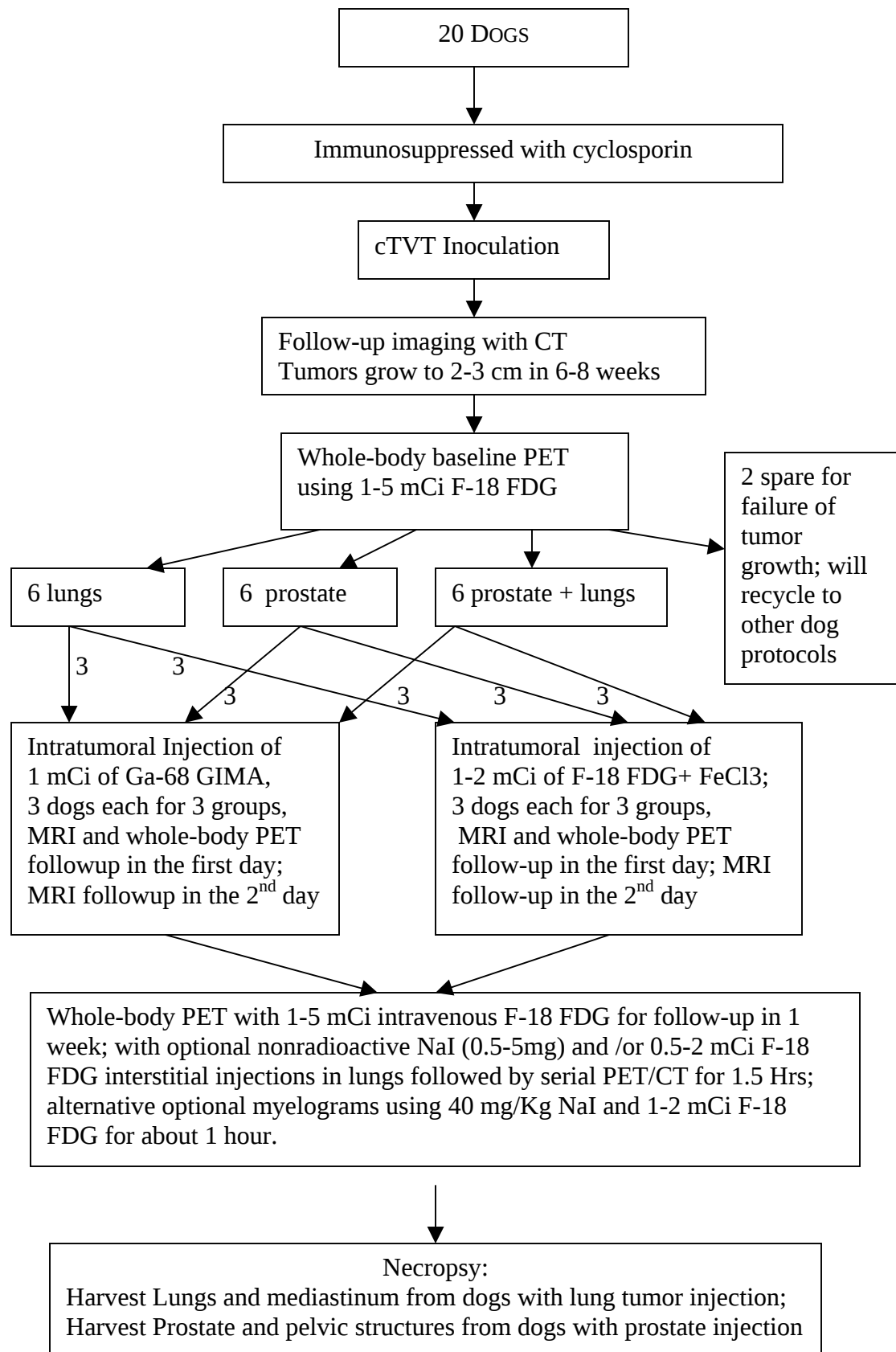
Principal Investigator Name: Franklin Wong Date: _____

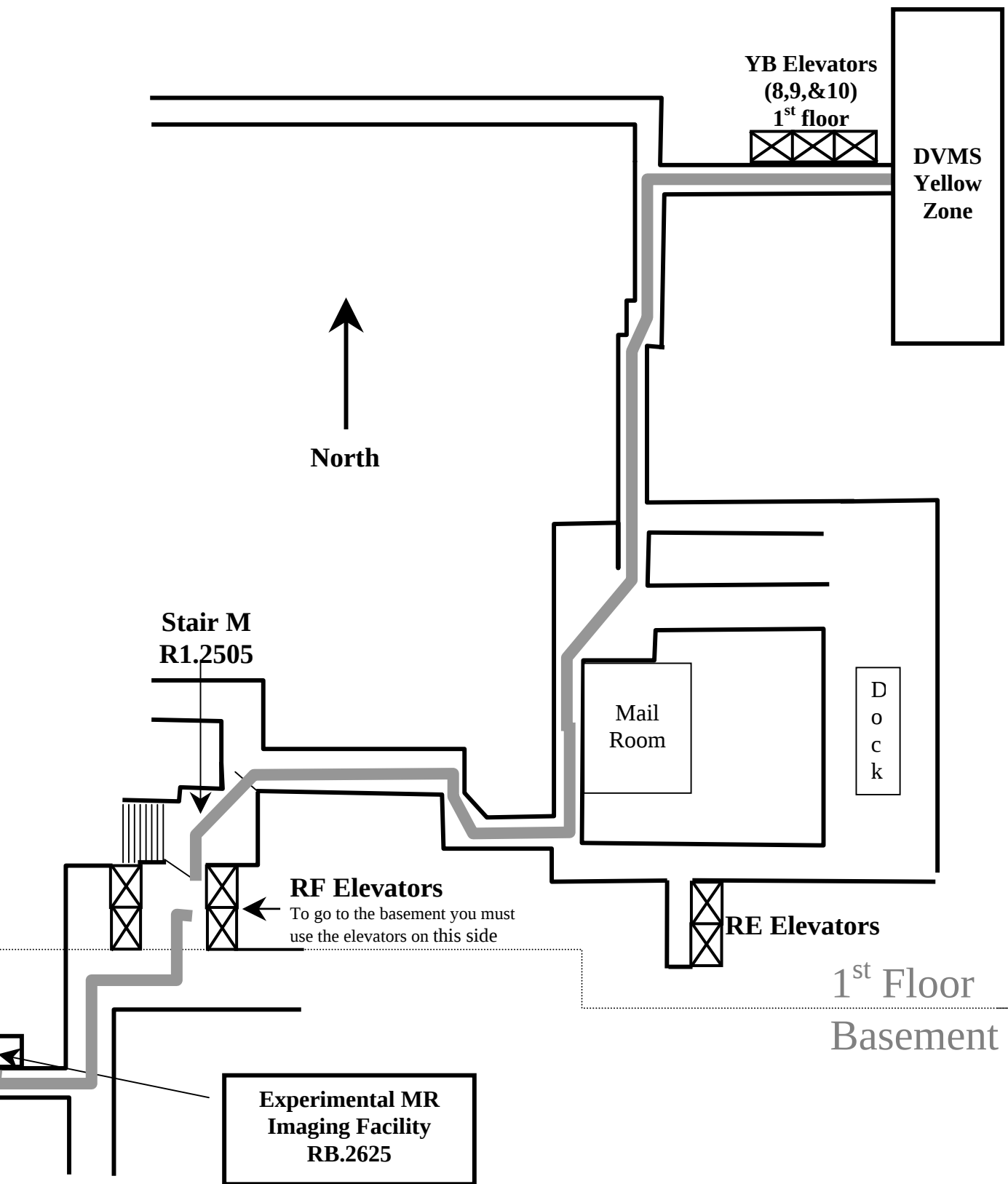
Chairman/Division Head:

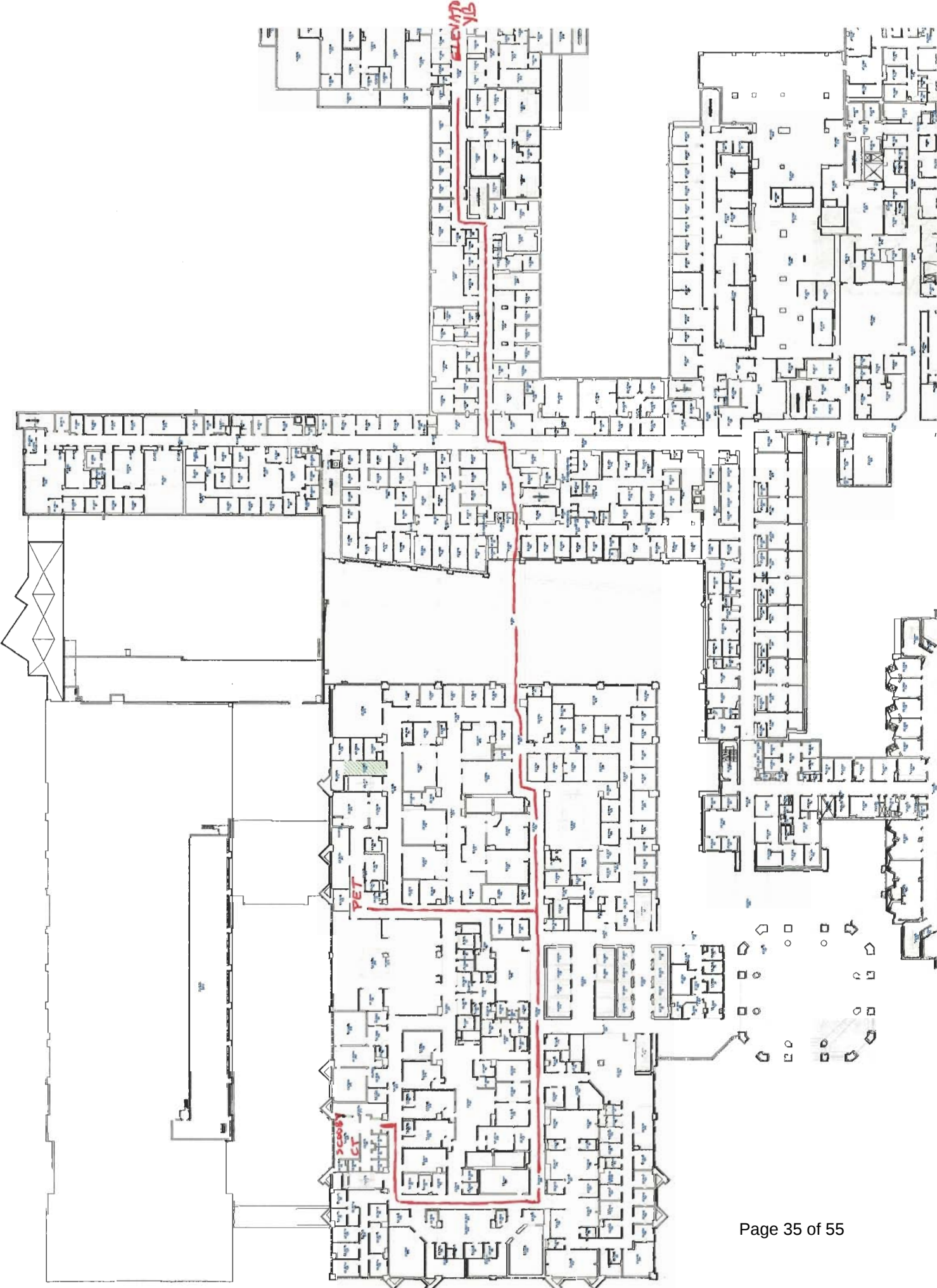
I have reviewed this request for animal care and use and have found the proposed research to be scientifically meritorious.

Chairman/Division Head
Signature: _____

Chairman/Division Head Name: Donald Podoloff Date: _____







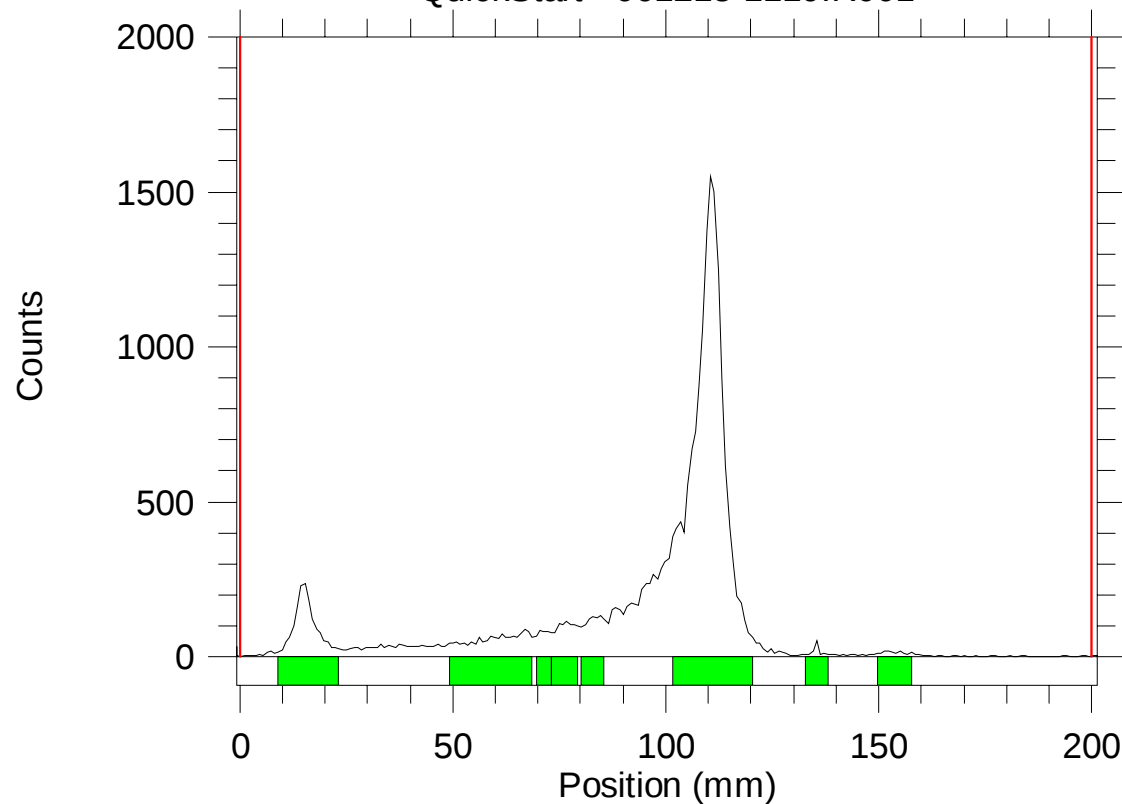
^{67}Ga -Citrate in Saline (pH=2-3)

QuickStart - 061218-1119.R001

Method: QuickStart
 File: 061218-1119.R001
 Created: 18 Dec 2006 11:20:45
 Evaluated: 18 Dec 2006 11:20:45
 Evaluation by: j
 Collimator Type: Hi Efficiency
 Collimator Width (mm): 10
 Elect. Resol: Normal
 Amp. Range: 50 - 2047
 Resolution (chan): 256
 Chan Size (mm): 0.890
 Chan of Zero mm: 9.8
 Hi Voltage (volts): 1500
 Run Time (min): 2.00
 Relative Pos (mm): 0.0

Analysis Parameters
 Bkg Subtraction: none
 Origin (mm): 0.0
 Normalization: none
 Front (mm): 200.0
 Total Counts: 23942.0
 Total CPM: 11971.0
 Total Region (mm): 0.0 - 200.0
 Total File Counts: 24045
 Comments:
 ^{67}Ga -citrate, ITLC, Saline, acetate, 1.5-15cm

Region Analysis
 Definition: Peak Search
 Peak Slope (counts/mm): 2.0
 Min Width (mm): 2.3
 Min percent of Total: 0.0



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	Pct of Total	Pct of ROI
Rgn 1	9.1	23.3	15.6	0.078	1507.0	753.5	6.29	8.06
Rgn 2	49.1	68.7	59.6	0.298	1265.0	632.5	5.28	6.77
Rgn 3	69.6	73.2	71.0	0.355	317.0	158.5	1.32	1.70
Rgn 4	73.2	79.4	76.0	0.380	683.0	341.5	2.85	3.65
Rgn 5	80.3	85.6	82.7	0.413	710.0	355.0	2.97	3.80
Rgn 6	101.6	120.3	109.8	0.549	13988.0	6994.0	58.42	74.81
Rgn 7	132.8	138.1	135.1	0.676	106.0	53.0	0.44	0.57
Rgn 8	149.7	157.7	153.1	0.766	122.0	61.0	0.51	0.65

8 Peaks

18698.0

9349.0

78.10

100.00

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^{67}Ga -GIMA in Saline (pH=2-3)

Method: QuickStart
 File: 061218-1137.R001
 Created: 18 Dec 2006 11:38:43
 Evaluated: 18 Dec 2006 11:38:43
 Evaluation by: j
 Collimator Type: Hi Efficiency
 Collimator Width (mm): 10
 Elect. Resol: Normal
 Amp. Range: 50 - 2047
 Resolution (chan): 256
 Chan Size (mm): 0.890
 Chan of Zero mm: 9.8
 Hi Voltage (volts): 1503
 Run Time (min): 2.00
 Relative Pos (mm): 0.0

Analysis Parameters

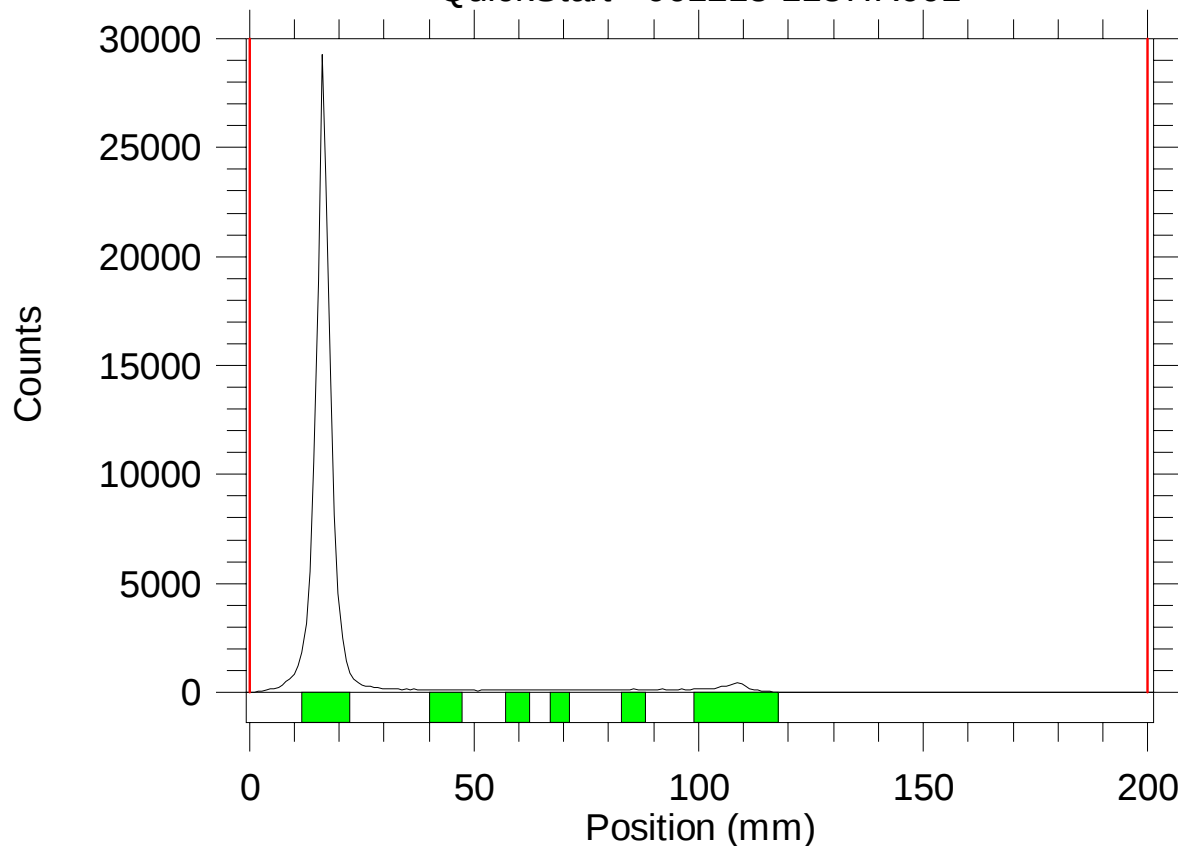
Bkg Subtraction: none
 Origin (mm): 0.0
 Normalization: none
 Front (mm): 200.0
 Total Counts: 144871.0
 Total CPM: 72435.5
 Total Region (mm): 0.0 - 200.0
 Total File Counts: 144912
 Comments:
 ^{67}Ga -Gimma, ITLC, Saline, Acetate, 1.5-15cm

Region Analysis

Definition: Peak Search
 Peak Slope (counts/mm): 2.0
 Min Width (mm): 2.3
 Min percent of Total: 0.0

Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	Pct of Total	Pct of ROI
Rgn 1	11.8	22.4	16.5	0.082	123884.0	61942.0	85.51	94.61
Rgn 2	40.2	47.4	43.2	0.216	909.0	454.5	0.63	0.69
Rgn 3	57.1	62.5	59.4	0.297	638.0	319.0	0.44	0.49
Rgn 4	66.9	71.4	68.7	0.344	554.0	277.0	0.38	0.42
Rgn 5	82.9	88.3	85.2	0.426	735.0	367.5	0.51	0.56
Rgn 6	99.0	117.6	106.8	0.534	4220.0	2110.0	2.91	3.22

QuickStart - 061218-1137.R001



6 Peaks

130940.0

65470.0

90.38

100.00

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Protocol Page

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:	Farrah Chickerneo Franklin Wong
Department:	Diagnostic Radiology
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:	IRB approved with contingencies 04/27/2007
Version:	03
Submitted by:	Farrah Chickerneo--11/1/2006 11:24:15 AM
OPR Action:	Accepted by: LaToya J. Ingram -- 11/13/2006 2:09:01 PM

Protocol Body

1.0 Objectives

Select Section Title: 1.0 Objectives

Objectives:

1. To evaluate the sequestration and biodistribution of radioactive gallium-iron macroaggregates (GIMA) after intratumoral injection into breast tumors
2. Use MRI to measure the spatial and temporal profiles of GIMA after intratumoral injection into breast cancer
3. 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;
4. 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.

Hypotheses:

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 (0.2 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
Gallblader 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.
 - In-111, Ga-67 or I-131 agents of more than 1 mCi within 14 days.
- 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 radionuclide 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 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. 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 anesthetics 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 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.

5.3.3 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 10 to 5 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.

7.1 Each patient will be accrued sequentially. Since they are scheduled for surgery in 7-14 days from entry into the study, a complete evaluation of patient safety will be made (up to the day of surgery) before accrual of another patient.

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 groups 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 kept under lock and key in our files for at least 5 years from the completion 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

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