

AD _____

Award Number: DAMD17-97-1-7279

TITLE: Correlative Study of Tumor Hypoxia and Metastatic Potential in Breast Cancer

PRINCIPAL INVESTIGATOR: Mahesh A. Varia, M.D.

CONTRACTING ORGANIZATION: University of North Carolina
Chapel Hill, North Carolina 27599-1350

REPORT DATE: September 2001

TYPE OF REPORT: Final

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20020904 022

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE September 2001	3. REPORT TYPE AND DATES COVERED Final (1 Sep 97 - 31 Aug 01)
---	---	---

4. TITLE AND SUBTITLE Correlative Study of Tumor Hypoxia and Metastatic Potential in Breast Cancer	5. FUNDING NUMBERS DAMD17-97-1-7279
--	---

6. AUTHOR(S)
Mahesh A. Varia, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of North Carolina
Chapel Hill, North Carolina 27599-1350

E-Mail: varia@radonc.unc.edu

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. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)
Purpose: To study if tumor hypoxia is associated with metastatic potential in breast carcinoma.
Scope: Breast cancer patients enrolled in an IRB approved study receive hypoxia marker pimonidazole intravenously. Tumor biopsy specimens are examined for pimonidazole binding (hypoxia) and for the bimolecular markers. Regional node metastases data are recorded.
Major findings: Presence of tumor hypoxia has been demonstrated in 8 of 27 patients studied. Tumor hypoxia ranges from 0-33% by an image analysis system. A semiquantitative grading scale of 0-4 (0=no hypoxia, 4=highest amount of hypoxia) is useful in breast cancer hypoxia assessments. It is less expensive, can be easily learnt, and can be readily adopted by any clinical immunohistochemistry laboratory. Tumor hypoxia as detected by pimonidazole hypoxia marker ranges from 0-4 on this scale. No toxicities related to pimonidazole administration or the hypoxia marker procedures have been observed. Preliminary data of tumor hypoxia and microvessel density have been presented.
Status and Progress Report (in terms of results and significance): The preliminary data show the presence of tumor hypoxia in 30% of the patients studied. Tumor hypoxia in several other cancers is associated with adverse prognosis and tumor aggressiveness.. Pimonidazole hypoxia method can be a useful tool to study the significance of tumor hypoxia in breast cancer prognosis and tumor aggressiveness.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
21
16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT
Unclassified

18. SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

19. SECURITY CLASSIFICATION OF ABSTRACT
Unclassified

20. LIMITATION OF ABSTRACT
Unlimited

Table of Contents

Cover Letter	1
Cover	2
SF 298	3
Table of Contents	4
Foreword	5
Introduction	6
Body	7
Key Research Accomplishments	9
Reportable Outcomes	9
Conclusions	9
References	9
Appendices	10

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

- _____ Where copyrighted material is quoted, permission has been obtained to use such material.
- _____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.
- _____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.
- _____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).
- _____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.
- _____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.
- _____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.
- _____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Mahesh J. Varri
PI - Signature

Novemeber 30, 2001
Date

Annual Report Statement

Grant DAMD17-97-1-7279

INTRODUCTION

Background: Tumor hypoxia predicts for poor prognoses in human tumors independently of whether chemotherapy, radiotherapy or surgery is used as treatments. The hypoxic tumor microenvironment may be a significant factor in cellular processes involved in tumor proliferation, invasion, angiogenesis, and metastases. It has been postulated that this may be due to the induction of oxygen regulated proteins, growth factors, transcription factors, genetic instability, and selection of apoptotic resistant cells.

Purpose: A novel physiological approach using pimonidazole for tumor hypoxia assessment in human breast tumors has been initiated in this clinical study. The purpose is to study its association with metastatic potential of breast cancer cells. Does the presence of hypoxia in the primary breast tumors detected by pimonidazole immunohistochemical binding correlate with the presence of axillary lymph node metastases? Is tumor hypoxia associated with the presence of markers of cell proliferation (PCNA), microvessel density/VEGF, p53, and apoptosis in the primary breast tumor tissue.

Scope: The specific aims are:

- I:** Determine the presence and extent of tumor hypoxia in biopsies of primary breast cancer using pimonidazole binding to hypoxic tumor cells.
- II:** Determine the patterns of pimonidazole binding in the breast cancer biopsies in relation to other landmarks such as blood vessels and necrosis.
- III:** Correlate the presence and extent of tumor hypoxia in primary breast cancer with the presence of axillary node metastases.
- IV:** Correlate the presence and extent of tumor hypoxia with the presence of other biological markers such as micro-vessel density/VEGF, p53, apoptosis, and PCNA.
- V:** Monitor the adverse effects of pimonidazole.

Methods: Breast cancer patients enrolled in an IRB approved study receive pimonidazole intravenous infusion. Breast tumor biopsy specimens are examined with immunohistochemical techniques for pimonidazole binding (hypoxia) and for the above biomolecular markers. Regional node metastases data are recorded.

Research Progress Associated with Tasks outlined in the Statement of Work.

Task 1: Patient enrolment

To date, 29 breast cancer patients have been enrolled on Institutional Review Board (IRB) approved protocol for this DOD clinical study. Age and ethnic origin data are shown below.

Ages	Number
18-30	0
31-40	2
41-50	9
51-60	8
61-70	5
71-80	3
81-90	2
Total	29
Ethnic Origin	
White	23
African-American	6

For each patient enrolled:

Task 1A

- | | |
|--|-------|
| a. Document eligibility criteria upon enrollment. | Done. |
| b. Have signed informed consent on record prior to study procedures. | Done |
| c. Record clinical data. | Done |

Task 1B

- | | |
|---|----------|
| a. Administer pimonidazole, 0.5 g/m ² in 100 ml N-saline i.v.
3 patients did not wish to return for the pimonidazole infusions after they had given consent for the study. | Done |
| b. Procure breast biopsy specimen at the time of lumpectomy/mastectomy. | Done |
| c. Prepare biopsy material for pimonidazole and biological markers staining. | Done |
| d. Record pathological status of axillary lymph nodes. | On-going |

Task 1C

- | | |
|--|----------------------|
| a. Perform pimonidazole immunostaining. | On-going |
| b. Perform p53, apoptosis, PCNA, Ki-67, and VEGF marker staining | Awaiting batch study |
| c. Record any adverse effects of pimonidazole | Done |

Task 1D

- | | |
|--|----------------------|
| a. Perform Image Analysis/semiquantitative analysis on stained slides. | In progress |
| b. Calculate Hypoxic Fraction (ratio of labeled to unlabeled cells). | In progress |
| c. Record p53, apoptosis, PCNA, Ki-67, and VEGF marker staining. | Awaiting batch study |
| b. Assess hypoxia marker binding relationships to anatomic landmarks. | Awaiting batch study |

**Task 2 Perform Data and Statistical Analysis to obtain
Results of Specific Aims I-V**

See preliminary data
below

Preliminary Data and Results

Pimonidazole labeling of breast cancer cells has been demonstrated in the tumor biopsy specimens of the patients enrolled in this DOD supported research study. Preliminary results have been combined with the results from 16 breast cancer patients enrolled in our own IRB approved study and presented at various meetings (Appendix I).

To date, 29 patients have been enrolled on the study. Tumor hypoxia detected by pimonidazole ranges from 0-33% by an image analysis system. A semiquantitative grading scale of 0-4 (0 = no hypoxia, 4 = highest amount of hypoxia) developed in cervix cancer studies and validated with the image analysis method is also useful in breast cancer hypoxia assessments. Advantages of the semiquantitative hypoxia scoring system are that it is based on the standard light microscopic examination of the tumor section slides rather than an expensive image analysis system requiring video camera, computer and related software. It is less tedious and can be easily learnt. The semiquantitative system is similar to the grading system used for histopathologic grading of tumors in routine pathology. Hence this system can be readily adopted in any clinical pathology laboratory performing immunohistochemistry. Presence of tumor hypoxia has been demonstrated in human breast cancer as detected by pimonidazole hypoxia marker and ranges from 0-4 on the semiquantitative grading scale. No toxicities related to pimonidazole or hypoxia marker related procedures have been observed. Preliminary data of tumor hypoxia and microvessel density have been presented at national scientific meetings (Appendix I).

Administrative problems with regard to the approval of the consent form by the IRBs involved have been reported previously that delayed the enrollment of patients to the study. After the initial award (IRB) of the grant, a number of changes in the Consent Form approved by our Institutional Review Board were requested by the Surgeon General's Human Subjects Research Review Board (HSRRB, HURRAD Log. No. A-7766), USAMRMC Human Subjects Protection Division. Following further discussions on this subject, language acceptable to both Review Boards was developed whereby Department of Defense as the sponsor of the research assumes the financial responsibility.

Patient enrolment is progressing gradually. As a result of screening and early diagnosis by mammography, increasing number of women are presenting with clinically occult and non-palpable but mammographic abnormalities. Some of these are found to be invasive cancer on biopsy evaluation. These women are not eligible for the hypoxia marker study as the biopsy has already been performed leaving no or limited residual cancer tissue. The limited tissue is required for diagnostic evaluation and not available for hypoxia marker studies. Also a number of patients are receiving neoadjuvant therapy prior to lumpectomy or mastectomy. They are also not candidates for this study as primary tissue removal and node dissection are performed after the neoadjuvant therapy thus pretherapy hypoxia assessment cannot be made. These changes in clinical presentations and treatment management have slowed the patient accrual rate to this study. To address the patient recruitment requirements, a Research Nurse is involved in patient recruitment, attends the Breast Cancer Conferences where new patients are discussed, and provides information about the study to the Breast Cancer Clinic staff. She assists with patient education, recruitment, clinical procedures involved in pimonidazole infusions, patient follow-ups, and clinical data management

KEY RESEARCH ACCOMPLISHMENTS AND REPORTABLE OUTCOMES:

The study is ongoing and results are updated as more breast cancer patients are enrolled on the study. The following significant observations have been made from the preliminary results:

1. Pimonidazole detects hypoxic regions in human breast carcinoma (Appendix I).
2. Preliminary data show 30% of breast cancers have tumor hypoxia at the time of initial presentation.
3. Innovative correlative study of tumor hypoxia detection with microvessel density analysis has been performed using a double staining technique on the tumor section on the same slide.
4. Abstracts 1-4 listed in Appendix I were submitted with 2000 Annual Report. Copy of abstract number 5 is submitted in Appendix I.
5. Dr. Ballenger was awarded a Travel Fellowship by the Radiation Research Society to present the results from this research at their April 2000 meeting.
6. Dr. Ballenger's research work on this project has been accepted as the research requirement for her Radiation Oncology training in the University of North Carolina Hospitals Residency Program.
7. Dr. Ballenger has been recruited to a faculty position in the Department of Radiation Oncology at Duke University Medical Center, Durham, North Carolina.
8. Current updated Consent Form is attached in Appendix II.

CONCLUSIONS:

Presence of tumor hypoxia is observed in 30 % of human breast cancer. Dr. Ballenger was provided with research training and her contributions led to 5 abstracts and a Travel Award Fellowship of the Radiation Research Society. Results from this breast cancer research will be updated as more patients are entered on the study and further analysis of the tumor biopsies is completed. There have been no adverse effects related to the pimonidazole infusions in these patients. Pimonidazole based tumor hypoxia detection method can be a useful tool to demonstrate hypoxia in breast cancer to study hypoxia related adverse prognosis and the role of hypoxia in tumor physiology.

REFERENCES: Not Applicable.

APPENDICES

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples of appendices include journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Appendix I.

1. Ballenger CA, Varia MA, Chou S-C, Novotny DB, Haroon Z and Raleigh JA Hypoxia and microvessel Density in human breast adenocarcinomas. 22nd Annual San Antonio Breast Cancer Symposium, San Antonio, Texas, Dec 8-11, 1999.
2. Ballenger CA, Chou S-C, Calkins-Adams DP, Novotny, DB, Raleigh JA, Varia MA. Hypoxia And Microvessel Density In Human Breast Adenocarcinomas. American Radium Society Meeting, April 1-5, 2000, London, United Kingdom.
3. Ballenger CA, Varia MA, Chou S-C, Novotny DB, Haroon Z and Raleigh JA.. Hypoxia and microvessel density in human breast adenocarcinomas Radiation Research Society Meeting, Albuquerque, New Mexico, April 29-May 3, 2000.
4. Varia MA, Ballenger CA, , Chou S-C, Novotny DB, Haroon Z and Raleigh JA. Demonstration of Hypoxic Regions with Pimonidazole in Human Breast Cancers and Relationship to Microvascular Density. Era of Hope meeting, Atlanta, June 8-11, 2000.
5. Varia, MA, Chou, SC, Ballenger CA, Novotny DB, Lerea LS, Haroon Z, Murphy BJ, Raleigh, JA. An Approach To Study Mechanisms Of Hypoxia-Associated Poor Prognosis Using Hypoxia Marker Pimonidazole In Cancers Of The Head And Neck, Cervix, And Breast. American Society of Therapeutic Radiology and Oncology Meeting, Boston, October 23-25, 2000.

Appendix I (continued)

Abstract

An Approach To Study Mechanisms Of Hypoxia-Associated Poor Prognosis Using Hypoxia Marker Pimonidazole In Cancers Of The Head And Neck, Cervix, And Breast.

Varia, MA, Chou, SC, Ballenger CA, Novotny DB, Lerea LS, Haroon Z, Murphy BJ, Raleigh, JA. An Approach To Study Mechanisms Of Hypoxia-Associated Poor Prognosis Using Hypoxia Marker Pimonidazole In Cancers Of The Head And Neck, Cervix, And Breast. American Society of Therapeutic Radiology and Oncology Meeting, Boston, October 23-25, 2000.

Purpose: The molecular mechanisms underlying poor prognosis and treatment outcome associated with tumor hypoxia are not well understood. The purpose of this study is to use the hypoxia marker pimonidazole to investigate hypoxia's role in factors associated with tumor aggressiveness and treatment resistance including cell proliferation, angiogenesis, differentiation, and oxygen regulated protein (ORP) expression such as metallothionein (MT), a chemo- and radio-protectant.

Materials & Methods: Thirty-six patients (20 cervix and head and neck squamous cell carcinoma-SCC, 16 breast adenocarcinoma-BR,) enrolled in an IRB-approved study were given pimonidazole hydrochloride 0.5 g/m² intravenously. Twenty-four hours later, 3 or more random biopsy samples were obtained from each tumor. Biopsies were formalin fixed, paraffin embedded and sectioned at 4 microns. Contiguous sections were immunostained for hypoxia marker pimonidazole, proliferating cell nuclear antigen (PCNA), and for MT I, MT II and the cell differentiation marker involucrin. A double staining technique was also developed on the same sections for pimonidazole and Factor VIII to study microvasculature density (MVD). Riboprobes for stress inducible MT-I and MT IIa mRNA and non-inducible MT- IV mRNA labeled with 35S were prepared and applied to formalin-fixed, paraffin-embedded selected sections of SCC patients. The extent of immunohistochemical staining with each of these markers and microregional relationships to hypoxic cells were investigated.

Results: In the cervix and head and neck SCC patients, the average values for %S-phase PCNA for hypoxia above and below the median hypoxia values were not significantly different ($p = 0.26$, t-test). An 8-fold change in hypoxia existed in the absence of a change in proliferation. In breast cancer patients there was very little overlap between PCNA and hypoxia marker but high MVD correlated with low amount of hypoxia ($p = 0.001$). Of the 84 sets of tissue sections studied for hypoxia and MT expression, 64 demonstrated the presence of hypoxia. Forty-three of these sixty-four (67%) possessed little or no overlap between hypoxia and MT. Immunostaining for involucrin overlapped with that for hypoxia in 82 % of these 64 sections and was primarily in non-PCNA positive regions. Autoradiography revealed strong zonal expression for inducible MT-I and IIa mRNAs but weak uniform expression for non-inducible MT-IV mRNA. In general, MT-IV mRNA is expressed in hypoxic regions of SCC.

Conclusions: These observations suggest that the association between hypoxia and poor prognosis is not mediated by increased tumor cell proliferation and that hypoxia does not lead to high MVD. The majority of hypoxic cells do not express MT. However, molecular markers for hypoxia and terminal differentiation co-localize, suggesting that this may exert some control on ORP expression in hypoxic cells. This biopsy-based immunohistochemical method using pimonidazole to detect cellular hypoxia and the detection of biomarkers of interest in the same microenvironment is a valuable tool to investigate the mechanisms of hypoxia-associated poor prognosis in human tumors. Additionally, this method readily allows the study of microregional relationships between hypoxia and biomarkers of tumor physiology.

Supported by DHHS R42 CA68826, R21 CA74069, RR00046 and DOD BC962506. and the State of North Carolina

APPENDIX II

- 1. Informed Consent Form.**
- 2. Tissue Consent Form**

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects

Medical IRB Study # GCRC 1425
Consent Form Version Date: 5/11/01

Title of Study: **Correlative Study of Tumor Hypoxia and Metastatic Potential
in Breast Cancer**

Principal Investigator: **Mahesh Varia, M.D.**
UNC-CH Department: **Department of Radiation Oncology**
Phone number: **919-966-1101**

Co-Investigators: **James Raleigh, Ph.D., Susan Maygarden, M.D., William
Cance, M.D., Mark Graham, M.D., Carolyn Sartor, MD**

Sponsor: **US Department of Defense**

You are being asked to take part in a research study. The investigators listed above are in charge of the study; other professional persons may help them or act for them.

What are some general things you should know about research studies?

Research studies are designed to gain scientific knowledge that may help other people in the future. You may not receive any direct benefit from participating. There may also be risks associated with participating in research studies.

Your participation is voluntary. You may refuse to participate, or may withdraw your consent to participate in any study at any time, and for any reason, without jeopardizing your future care at this institution or your relationship with your doctor. If you are a patient with an illness, you do not have to participate in research in order to receive treatment.

Details about this particular study are discussed below. It is important that you understand this information so that you can decide in a free and informed manner whether you want to participate. You will be given a copy of this consent form. You are urged to ask the investigators named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this research study is to detect and learn more about the behavior of cancer cells with low amounts of oxygen. Such cells are referred to as hypoxic cells. Hypoxic cells can survive the treatment effects of radiation and some types of chemotherapy. This could be one reason why some cancers recur after these treatments.

Knowledge gained from this research can advance our understanding of cancer and lead to improvements in cancer treatment.

You have the option not to participate in this research study if you so desire. You have been selected as a possible subject in this study because you have been diagnosed with cancer or there is a possibility that the tests your doctor has recommended may show cancer. This research does not involve treatment.

This study involves the testing of a drug called pimonidazole. The Food and Drug Administration (FDA) has approved its use as an Investigational New Drug (IND#036,783) for the following purpose. Investigational New Drug approval does not mean that the drug is approved for routine use.

- a. To identify cancers with low oxygen (hypoxic cells)
- b. To study the relationships between such hypoxic cells and the growth and the spread of cancer cells
- c. To monitor side effects of pimonidazole.

How many subjects will participate in this study?

If you decide to participate, you will be one of approximately 50 subjects in this research study.

How long will your participation last?

Your participation in this study will last for approximately 4 months. Your medical care will be continued with your doctors.

What will happen if you take part in the study?

During the course of this study, the following will occur:

You will have physical examinations, blood, urine and laboratory tests to determine if you are eligible for the study. The blood and urine tests are to check your blood counts, liver and kidney functions and is similar to the blood taken when admitted to the hospital.

You will receive pimonidazole solution through your vein (IV). This takes about 30 minutes. This will be done in the Radiation Oncology clinic, or it can be done in the hospital where you are admitted for you medical care or you may be admitted for one night at no financial cost to you to the General Clinical Research Center at UNC Hospitals.

You will have a procedure (biopsy) done to obtain samples of the tumor for this study. This procedure may be done the same day or the next day. If you are having surgery or other procedures for evaluation and treatment of you cancer, the biopsies may be obtained from the cancer and suspected areas of cancer spread at the time of the operation. The size of the sample will usually be small about the size of rice grains depending upon location and the size of the tumor.

After the diagnostic and treatment purpose has been met as certified by the pathologist-in-charge or clinical laboratory director, excess tissue from these procedures may be collected for this Study. These tissues will be tested in the laboratory with special tests to detect the hypoxic cells and some of the biological features of the tumor.

You will be asked to return one time for a medical checkup in 4 months after your pimonidazole infusion. Whenever possible this will be coordinated with the checkup for you medical condition with your doctor. At this time you will be checked for side effects. You will have blood and urine tests to check your blood counts, liver and kidney functions.

Approximately 10cc of blood (about tenth of a 4-ounce cup) will be drawn at your 4-month check-up visit. You will be asked to report any side effects of pimonidazole, biopsy procedures or any other side effects during the study period.

You will remain under the care of your doctors for medical care of your condition.

Are there any reasons you should not participate?

You should not participate in this study if you believe you are pregnant, or have received another investigational drug in the past 4 weeks, have severe infection, have nerve damage, or are completely disabled and cannot perform self care

What are the possible risks or discomforts?

This study might involve the following risks and/or discomforts to you: For the dose of pimonidazole used, this study might involve the following risks and/or discomforts to you:

Common side effects of pimonidazole: None
Uncommon side effects of pimonidazole: None
Rare side effects of pimonidazole:

None have been noted.

With the higher and more frequent doses of pimonidazole uncommon side effects include nausea, vomiting, sensation of heat and sweating. If the side effects do occur they are usually reversible. Other side effects of pimonidazole such as skin rashes and mental disorientation are uncommon and treatment of these symptoms will be available. This study does not use this higher dose and does not involve giving the lower dose more than once.

Pimonidazole is a new investigational drug in the USA and has been studied for its side effects in the United Kingdom. We have given pimonidazole to over 120 subjects at UNC with no side effects. Twice as much as the dose given in this study was found to be safe and well tolerated in subjects in the United Kingdom. Very few, if any, side effects would be expected from the amount of the pimonidazole that will be used for you in this study.

Risks such as bleeding, infection and surgical injury are uncommon with blood tests and biopsy procedures performed for this study. Qualified personnel will perform biopsies and steps will be taken to minimize any discomfort to you. Special studies to be performed in the laboratory on the tissue samples from the biopsy procedures are not expected to cause any risks.

Subjects who are pregnant or breastfeeding should not participate in this study. All women of childbearing age will have a pregnancy test done prior to administration of pimonidazole.

In addition, there may be uncommon or previously unrecognized risks that might occur.

What are the possible benefits?

The benefits to you of participating in this study are the knowledge that you are helping with Cancer research. Information gained from this participation will be helpful to better understand hypoxic cells and other growth behaviors of tumors. This knowledge may assist future patients and the society by improvements in cancer treatments.

If you choose not to participate, what other options do you have?

Your choice of not participating will not have any effect on the care you will receive.

What if we learn about new risks during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will your privacy be protected?

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-CH will take all steps allowable by law to protect the privacy of personal information.

Your records will be reviewed and analyzed by physicians, other personnel and their designees associated with this study. These records are kept confidential and are stored in a locked file. No names are used for identification; numbers are assigned for each participant. Information collected is kept confidential and in your medical records which may be audited by the representatives of the UNC School of Medicine, the UNC Hospitals and applicable research grant funding agencies (National Institutes of Health (NIH) and National Cancer Institute (NCI) or the U.S. Army Medical Research and Material Command (USAMRMC)]. As pimonidazole is an investigational drug, the FDA may inspect these records.

Because this study involves the injection of a drug, a copy of this consent form will be placed in your medical record. This will allow the doctors caring for you to obtain information about what drugs or procedures you are receiving in the study and treat you appropriately, if you have other health problems or needs during the study.

Will you be paid for participating?

You will receive up to \$200 after the completion of the pimonidazole infusion and the biopsy procedure.

Will it cost you anything to participate?

There will be no costs to you for the research related procedures. You will not be billed for the research costs of your stay in the General Clinical Research Center, the blood tests, the pimonidazole infusion, the biopsy procedure, the research laboratory test done for this study or for the follow-up visits and blood tests. You will not be billed for the cost of the pimonidazole. You will be responsible for the costs of your medical care and incidental expenses.

Who is sponsoring this study?

This research is funded by the US Department of Defense. This means that the sponsor is compensating the research team for conducting the study. The researchers do not, however, hold a direct financial interest in the sponsor or in the product being studied.

What will happen if you are injured by this research?

In the event of personal injury resulting directly from the research procedures, financial compensation cannot be provided by The University of North Carolina at Chapel Hill. All forms of medical diagnosis and treatment, whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study. If such complications arise, the researchers will assist you in obtaining appropriate medical treatment but The University of North Carolina at Chapel Hill does not provide financial assistance for medical or other costs. You do not waive any liability rights for personal injury by signing this form.

The United States Department of Defense is funding this research project. Should you be injured as a direct result of participating in this research project, you will be provided medical care, at no cost to you, for that injury. The cost of such medical care will be provided by the United States Department of Defense. You will not receive any injury compensation, only medical care. You should also understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with the Principal Investigator or his designee before you enroll in this study

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have further questions, or if a research-related injury occurs, you should call Mahesh Varia, M.D. at 919-966-1101.

What if you have questions about your rights as a subject?

This research has been reviewed and approved by the Committee on the Protection of the Rights of Human Subjects (Medical IRB) at the University of North Carolina at Chapel Hill. If you have any questions or concerns regarding your rights as a research subject, you may contact the Chairman of the Committee at (919) 966-1344.

Subject's Agreement:

I have read the information provided above. I voluntarily agree to participate in this study.

Signature of Research Subject

Date

Printed Name of Research Subject

Signature of Person Obtaining Consent

Date

Printed Name of Person Obtaining Consent

Rev./11/01

**UNC-CH SCHOOL OF MEDICINE / UNC HOSPITALS
INFORMATION ABOUT STORAGE AND USE OF SPECIMENS
WITH IDENTIFYING INFORMATION**

This brochure provides information that may help you decide whether to allow some of your blood, tissue and/or body fluid (specimens) which will be collected as part of this research study to be stored and used for future medical research.

WHAT WILL HAPPEN TO THE SPECIMEN?

The specimens will be processed for storage, catalogued and placed in a secured facility at the UNC-CH School of Medicine, UNC Hospitals, or another site. All identifying information, including your name and medical record number, will be removed from the specimens. The specimens will be given a unique identifier (code).

The researcher in this study and his/her associates will have access to the specimens and the code which links the specimens to you.

WILL RESEARCH RECORDS AND PERSONAL INFORMATION BE KEPT PRIVATE?

Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of those records, including personal information about you. When disclosure is required, the UNC School of Medicine and/or UNC Hospitals will take all steps allowable by law to protect the privacy of your personal information.

IS THERE ANY COST FOR STORAGE OF THE SPECIMENS?

There is no cost to you or your insurance company for the storage and use of the specimens.

WHO OWNS THE SPECIMENS?

By signing the consent form, you will donate the specimens for medical research purposes. Your donation does not entitle you to compensation from any commercial use of the products that may be derived from the specimens.

HOW WILL THE SPECIMENS BE USED IN THE FUTURE?

The research studies in which the specimens may be used have not yet been determined. The studies may involve genetic research. Genetic research is about finding the specific location of genes, learning how genes work, and developing treatments and cures for diseases which are genetically based.

Before any research involving the specimens is conducted, a committee at the UNC School of Medicine called The Committee on the Protection of the Rights of Human Subjects will review and approve the research proposal. The Committee includes scientists and non-scientists, including community representatives. The purpose of the Committee is to assure that the interests of individuals participating in research studies are well protected.

WILL RESEARCHERS SEEK CONSENT TO DO FUTURE STUDIES INVOLVING THE SPECIMENS?

In some cases, the Committee may require that you be contacted and asked for your consent to participate in the specific research study in which the specimens will be used. You have the right not to participate in any research study for which your consent is sought. Refusal to participate will not jeopardize your medical care or result in loss of benefits to which you are entitled.

WILL YOU RECEIVE STUDY RESULTS OF RESEARCH INVOLVING YOUR SPECIMENS?

There may be times when the Committee will require that you be notified about the results of a research study in which your specimens were used. You have the right to be told of the results and their meaning, or to decide not to be told of those results, or to have the information sent directly to your personal physician.

HOW WILL RESEARCHERS FIND YOU IN THE FUTURE?

If you decide to allow the specimens to be stored and used in future medical research studies, you will be asked to provide your social security number. Your social security number will be used by the researchers and their associates in this study when it is necessary to contact you to seek your consent to participate in a specific research study or to notify you about the results of that study.

If you allow your specimens to be stored with identifying information, you will be asked to choose, at the time you sign the consent form, a course of action that will be taken in the event that the researchers are unable to locate you in the future, even with your social security number. The options include allowing continued storage and use of your specimens with the identifying code remaining, continued storage and use of the specimens after removing the identifying code, and disposing of the specimens according to standard medical procedures.

WILL THE SPECIMENS BE SHARED WITH OTHER INSTITUTIONS?

The specimens may be shared with researchers from other institutions. Research studies may be conducted at several locations at the same time.

No identifying personal information about you will be provided to researchers from other institutions who will use the specimens.

HOW LONG WILL THE SPECIMENS BE STORED?

The specimens will be stored indefinitely. Specimens may also be disposed of at any time at the discretion of the investigators, using standard medical procedures. If in the future you should decide that you no longer wish for the specimens to be stored, you may contact the researcher and/or his/her associates on the study in which you are participating. You may also contact The Committee on the Protection of the Rights of Human Subjects at (919) 966-1344 and request that the specimens be disposed of.