

RADIATION THERAPY ONCOLOGY GROUP

RTOG 0612

INVESTIGATING MARKERS OF RADIATION OUTCOME IN PATIENTS WITH INTERMEDIATE-RISK PROSTATE CANCER USING DNA MICROARRAY ANALYSIS: AN RTOG PILOT STUDY

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Required Sample Size: 156

RTOG Institution # _____

RTOG 0612

ELIGIBILITY CHECKLIST (6/20/06)

Case # _____

(page 1 of 2)

- _____(Y) 1. Is the patient eligible for RTOG 0232?
- _____(Y) 2. Is the patient enrolled on RTOG 0232?
- _____(Y) 3. Was the paraffin tissue block from the initial diagnosis submitted or will it be submitted?
- _____(Y) 4. Will the biopsy specimen that contains frozen tissue submitted?
- _____(N) 5. Did the patient receive neoadjuvant hormone therapy?

RTOG Institution # _____

RTOG 0612

ELIGIBILITY CHECKLIST (6/20/06)

Case # _____

(page 2 of 2)

The following questions will be asked at Study Registration:

- _____ 1. Name of institutional person registering this case?
- _____ (Y) 2. Has the Eligibility Checklist (above) been completed?
- _____ (Y) 3. Is the patient eligible for this study?
- _____ 4. Date the study-specific Consent Form was signed? (must be prior to study entry)
- _____ 5. Patient's Initials (First Middle Last)
- _____ 6. Verifying Physician
- _____ 7. Patient's ID Number
- _____ 8. Date of Birth
- _____ 9. Race
- _____ 10. Ethnic Category (Hispanic or Latino; Not Hispanic or Latino; Unknown)
- _____ (Male) 11. Gender
- _____ 12. Patient's Country of Residence
- _____ 13. Zip Code (U.S. Residents)
- _____ 14. Patient's Insurance Status
- _____ 15. Will any component of the patient's care be given at a military or VA facility?
- _____ 16. Calendar base date
- _____ 17. Randomization/registration date: This date will be populated automatically.
- _____ (Y/N) 18. Tissue kept for cancer research
- _____ (Y/N) 19. Tissue kept for other medical research
- _____ (Y/N) 20. Allow contact for future research
- _____ 21. 0232 Case Number

The Eligibility Checklist must be completed in its entirety prior to web registration. The completed, signed, and dated checklist used at study entry must be retained in the patient's study file and will be evaluated during an institutional NCI/RTOG audit.

Completed by _____ Date _____

1.0 INTRODUCTION (6/20/06)

Approximately 70% of men identified with newly diagnosed prostate cancer present with organ-confined disease. Conventional treatment options include radical prostatectomy, external beam radiation (EBRT), interstitial brachytherapy, and watchful waiting.¹ The notion of stratifying patients into risk groups according to stage, Gleason score, and PSA has become helpful for comparing outcomes for various modalities of therapy and selecting appropriate treatment.²⁻¹¹ The definition of low risk most often includes those patients with a Gleason score of 6 or less, PSA of 10 ng/ml or less, and stage T2a or less disease. High-risk patients generally either have a Gleason score of 8 or above, a PSA above 20, or advanced disease on digital exam beyond T2b. Intermediate-risk patients generally have a Gleason score of 7, a PSA up to 20, or palpable tumor to stage T2b, without any other high risk factor. Zelefsky et al.¹² reported a 5-year actuarial PSA relapse-free survival (bNED) rate of 85% for low-risk patients, 65% for intermediate-risk patients, and 35% for high-risk patients, when these patients were treated with 3DCRT. Therefore, given the relatively equivalent percentages of patients with intermediate-risk prostate cancer who will experience biochemical control versus failure, a major clinical dilemma is to prospectively identify which patients will benefit from radiotherapy alone so that the remaining patients may be directed toward alternative therapies (e.g., radiotherapy plus hormonal therapy, surgery).

Molecular markers have been examined as a possible means to this end. The advent of expression microarray technology has further enabled us to examine the prognostic value of thousands of genes simultaneously. Indeed, molecular profiling using cDNA arrays can lead to molecular signatures that reliably “fingerprint” tumors based on their behavior. DNA microarrays are generally constructed in one of two fashions. Spotted cDNA arrays consist of two-dimensional elements on a surface, where each element of the array is an aliquot of some nucleic acid (representing a distinct gene of interest) that has been deposited in its specific location, usually on a glass slide. The nucleic acids may represent short and long oligonucleotides, PCR products, or clones in densities ranging from 100 elements per cm² to 25,000 elements per cm². Oligonucleotide arrays, on the other hand, are constructed using photolithography and light-directed soluble DNA synthesis. Both technologies permit gene expression analysis on a large scale, which can establish genetic fingerprints of the clinical behavior of tumors.

There is presently a lack of data in the literature concerning gene expression patterns associated with clinical outcome in patients with prostate cancer treated by definitive radiotherapy with or without hormone therapy. Further, we do not have a clear understanding of genes and clusters of genes that are radiation inducible in prostate cancer. Acquiring this information may yield valuable information on genetic profiles associated with response to radiotherapy. In the present study, biopsy specimens obtained at the time of brachytherapy from patients on RTOG 0232 will be used to obtain RNA for gene expression analysis. Tissues obtained at the time of brachytherapy must be “snap frozen” in liquid nitrogen to maintain integrity of RNA. Pretreatment paraffin blocks are also required to be submitted. Emerging technology has also enabled RNA isolation from paraffin-embedded tissues as well as frozen tissue. As the vast majority of tissues in cooperative groups are stored as paraffin specimens, for optimal tissue utilization, it is critical to understand the relative accuracy of gene expression measurements including microarray analysis obtained from paraffin tissues versus frozen tissues (which are considered the “gold standard”). The present study allows for such an opportunity by using paraffin blocks obtained at the time of the initial biopsy and comparing to subsequently-acquired frozen specimens at the time of brachytherapy. This analysis can be performed for both tumor and surrounding benign prostatic stromal tissue. As half of patients on RTOG 0232 will have had EBRT before brachytherapy, the variation in microarray data between paraffin and frozen tissue may be best ascertained in patients who will undergo brachytherapy alone. In the case that minimal variation is observed in the brachytherapy-alone group, comparing microarray data obtained before and after EBRT may yield valuable information on which genes are radiation inducible in prostate cancer and which of these are associated with clinical outcome.

To obtain adequate quantities of RNA, linear amplification of the isolated RNA sample will be performed. Hybridized chips will then be scanned to obtain gene expression profiles, which can then be readily associated with various parameters of clinical outcome (bNED, local failure, distant failure, etc.). This information can also be used to classify tumors based on their likely outcome following radiation and to identify whether certain populations (e.g., African Americans) suspected to have more aggressive tumors than other patient populations have underlying differences in tumor biology compared to other populations. Additionally, the information yielded from microarray analysis may reveal candidate genes responsible for local and distant progression following radiation, thereby establishing a list of potential molecular targets. The present study is designed to be a pilot study with a maximum enrollment of 50

patients. Should the present study demonstrate feasibility and reveal important findings, a subsequent study will be performed on a larger patient population.

2.0 OBJECTIVES

2.1 Primary

2.1.1 To demonstrate feasibility of acquiring specimens, both frozen and paraffin embedded, to yield RNA of adequate quality to perform DNA microarray studies within the multi-institutional setting.

2.2 Secondary

2.2.1 To compare the gene expression results from frozen tissue and paraffin-embedded tissue to see the correlation between the two methods in the brachytherapy alone arm of RTOG 0232.

2.2.2 To find genes that are expressed differentially before and after radiation therapy in the brachytherapy plus external beam radiation therapy (EBRT) arm of RTOG 0232.

2.2.3 To find gene expression patterns in the population that have significant associations with biochemical as well as clinically apparent local and distant failure in both arms of RTOG 0232.

3.0 PATIENT SELECTION

3.1 Conditions for Patient Eligibility

3.1.1 Patient must be eligible for RTOG 0232

3.1.2 Patient must be enrolled on RTOG 0232

3.1.3 Submission of the paraffin tissue block from initial diagnosis

3.1.4 Submission of the biopsy specimen that contains frozen tissue

3.1.5 Patient must sign a study-specific informed consent for RTOG 0612 prior to study entry.

3.2 Conditions for Patient Ineligibility

3.2.1 Patient must not have received neoadjuvant hormonal therapy.

4.0 PRE-TREATMENT EVALUATION

Pre-treatment evaluation will be per RTOG protocol 0232.

5.0 REGISTRATION PROCEDURES

Patients can be registered only after eligibility criteria are met.

Institutions must have an RTOG user name and password to register patients on the RTOG web site. To get a user name and password:

- The Investigator must have completed Human Subjects Training and been issued a certificate (Training is available via <http://cme.cancer.gov/clinicaltrials/learning/humanparticipant-protections.asp>).
- The institution must complete the Password Authorization Form at www.rtog.org/members/webreg.html (bottom right corner of the screen), and fax it to 215-923-1737. RTOG Headquarters requires 3-4 days to process requests and issue user names/passwords to institutions.

An institution can register the patient by logging onto the RTOG web site (www.rtog.org), going to "Data Center Login" and selecting the link for new patient registrations. The system triggers a program to verify that all regulatory requirements (OHRP assurance, IRB approval) have been met by the institution. The registration screens begin by asking for the date on which the eligibility checklist was completed, the identification of the person who completed the checklist, whether the patient was found to be eligible on the basis of the checklist, and the date the study-specific informed consent form was signed.

Once the system has verified that the patient is eligible and that the institution has met regulatory requirements, it assigns a patient-specific case number. The system then moves to a screen that confirms that the patient has been successfully enrolled. This screen can be printed so that the registering site will have a copy of the registration for the patient's record. Two e-mails are generated

and sent to the registering site: the Confirmation of Eligibility and the patient-specific calendar. The system creates a case file in the study's database at the DMC (Data Management Center) and generates a data submission calendar listing all data forms, images, and reports and the dates on which they are due.

If the patient is ineligible or the institution has not met regulatory requirements, the system switches to a screen that includes a brief explanation for the failure to register the patient. This screen can be printed.

In the event that the RTOG web registration site is not accessible, participating sites can register a patient by calling RTOG Headquarters, at (215) 574-3191, Monday through Friday, 8:30 a.m. to 5:00 p.m. ET. The registrar will ask for the site's user name and password. This information is required to assure that mechanisms usually triggered by web registration (e.g., drug shipment, confirmation of registration, and patient-specific calendar) will occur.

6.0 RADIATION THERAPY

Not applicable to this study.

7.0 DRUG THERAPY

Not applicable to this study.

8.0 SURGERY

Not applicable to this study.

9.0 OTHER THERAPY

Not applicable to this study.

10.0 TISSUE/SPECIMEN SUBMISSION

10.1 Review of Pre-Treatment Diagnostic Prostatic Biopsy Tissue (7/2/09)

10.1.1 The investigators at the treating institutions are **required** to submit paraffin-embedded tissue blocks from the ***pre-treatment diagnostic prostatic biopsy***, which will be reviewed to confirm Gleason score and to record other histopathologic features, such as the extent of tumor in the biopsies, the number of positive biopsies, and mitotic index. The block must be clearly labeled with the protocol and case number. A P2 form with the identifying numbers must accompany the sample as well as a pathology report from the submitting institution. Patient information should be removed from the pathology report but the pathology accession number must be retained. In many cases, this sample has already been submitted for review as part of RTOG treatment protocol 0232. As part of enrollment into RTOG 0612, please indicate previous study and case number on the 0612 specimen submission form (i.e., RTOG 0232 case 999). Submit paraffin-embedded tissue blocks (or specimen submission form with RTOG 0232 case information) to the RTOG Biospecimen Resource at the University of California San Francisco:

U. S. Postal Service Mailing Address: For Non-frozen Specimens Only

**RTOG Biospecimen Resource
University of California San Francisco
Campus Box 1800
1657 Scott Street, Room 223
San Francisco, CA 94143-1800**

Courier Address (FedEx, UPS, etc.): For Frozen Specimens

**RTOG Biospecimen Resource
University of California San Francisco
1657 Scott Street, Room 223
San Francisco, CA 94115**

Questions: 415-476-RTOG (7864)/FAX 415-476-5271; RTOG@ucsf.edu

10.2 Collection of Tissue For DNA Chip Analysis (7/2/09)

Note: See Appendix II for detailed instructions on specimen collection. These instructions are also included in the tissue collection kit.

- 10.2.1** Submission of frozen tissue cores from biopsy specimens **obtained at the time of brachytherapy is required.** A tissue collection kit is available from the RTOG Biospecimen Resource at the address listed in 10.1.1. Briefly, tissue must be transferred to the provided cryomold and placed flat on the bottom, covered with OCT, and rapidly frozen in isopentane immersed in liquid nitrogen, or on a dry-ice/ethanol bath. Each core should be processed separately. Samples should be stored in liquid nitrogen, or -80C, or on dry ice before shipping. Shipping should be done only Monday-Thursday priority overnight in dry ice using the provided container. Questions regarding shipment should be directed to the RTOG Biospecimen Resource (contact information provided in 10.1.1).

10.3 Sample Acquisition (6/20/06) (7/2/09)

Note: Samples at the time of rebiopsy will include only frozen tissues.

- 10.3.1** Samples including pretreatment paraffin-embedded tissue blocks and subsequently acquired frozen tissues at the time of rebiopsy will be obtained from patients entered into RTOG protocol 0232
- 10.3.2** Re-biopsy requires consent prior to enrollment on this trial (see Section 3.1)
- 10.3.3** Acquisition of biopsies at the time of brachytherapy (Fresh frozen tissue – specimens are processed as described in 10.2.1)
- 10.3.3.1** 4 core biopsies to be obtained at the time of therapy, just prior to intervention and processed as described in 10.2.1
- 10.3.3.2** Biopsies will be taken from locations based upon review of pathology report of primary diagnosis of adenocarcinoma of the prostate (PCa)
- 3 cores will be obtained from region(s) harboring PCa
 - 1 core will be obtained from regions(s) without Pca (remote from cancer)
- 10.3.3.3** The preferred annotation of biopsies is as follows: Three parameters are needed per biopsy — location of biopsy, whether it is from previous cancer site or not (+ or -), and the number from the location (P(pass) 1, P2, P3, P4). Therefore, for example, a slide would be labeled (l. apex(+), P1), meaning left apex biopsy, previously a region with cancer, first biopsy site. Another example would be R. base (-), P2 corresponding to right base biopsy, previously no cancer in this region, second biopsy from this site. If prior knowledge of cancer is not available, then location and number of pass should be specified.
- 10.3.4** Frozen tissue specimens are to be sent to the RTOG Biospecimen Resource. A specimen transmittal form (ST) must accompany the frozen tissue specimens. A specimen collection kit including materials and instructions can be obtained from the Biospecimen Resource. If needed, shipping containers and specimen bags can be obtained by contacting the RTOG Biospecimen Resource.

U. S. Postal Service Mailing Address: For Non-frozen Specimens Only
RTOG Biospecimen Resource
University of California San Francisco
Campus Box 1800
1657 Scott Street, Room 223
San Francisco, CA 94143-1800

Courier Address (FedEx, UPS, etc.): For Frozen Specimens
RTOG Biospecimen Resource
University of California San Francisco
1657 Scott Street, Room 223
San Francisco, CA 94115

Questions: 415-476-RTOG (7864)/FAX 415-476-5271; RTOG@ucsf.edu

- 10.3.5** The frozen sections from each core will be stored at the RTOG Biospecimen Resource. At various intervals, the frozen samples will be forwarded to Dr. Amin (Cedars-Sinai prostate tumor bank) for evaluation.
- 10.3.6** At Cedars-Sinai, one frozen section from each core will be stained with H & E and evaluated for histologic parameters: grade and percentage of biopsy. (Estimate of percent benign epithelium, percent neoplastic epithelium, percent stromal elements in each core). Samples will be immediately placed back into -80°C
- 10.3.7** Dr. Amin will send at least one sample with $>50\%$ PCa and one sample with $>10\%$ PCa to P. Nelson (Fred Hutchinson Cancer Research Center, Seattle). Remaining samples will be returned to the RTOG Biospecimen Resource for storage.

10.4 Expression Analysis (6/20/06)

- 10.4.1** Samples received in Nelson lab and stored at -80°C or liquid nitrogen (long-term).

A four-step quality control (Q/C) procedure will be used to assess sample adequacy for gene expression analysis. Each step will produce a 'yes/no' variable, and each sample must satisfy each step to be considered adequate for analysis for the primary outcome of this study.

- QC STEP 1: Adequate RNA integrity (10.4.2)
- QC STEP 2: Adequate RNA yield after LCM and amplification (10.4.4.)
- QC STEP 3: Maintenance of RNA transcript diversity (high and low abundance genes) (10.4.4.)
- QC STEP 4: Adequate microarray hybridization signal intensity relative to the cell line reference standard (10.4.5).

10.4.2 RNA Quality Analysis

One 7 micron frozen section will be cut from each frozen biopsy, and one 7 micron section will be cut from each paraffin-embedded biopsy.

For frozen tissues: Cell lysis buffer (Stratagene) is placed on the frozen section and allowed to warm to room temperature. RNA extraction is performed using the guanidine isothiocyanate procedure (Picopure RNA isolation kit-Arcturus).

For fixed tissues: Cut sections will be deparaffinized while the RNA is kept intact. RNA extraction will be performed according to the Arcturus Paradise Reagent System, based on the manufacturer's recommendations. Both tumor and benign prostatic epithelium will be analyzed from this procedure.

To assess RNA quality, OD measurements will be taken (260/280 ratio), using an Agilent bioanalyzer for measurements of RNA quantity, ribosomal RNA peaks, and RNA degradation. Samples with at least 28S:18S ratios of 1:5 will be considered adequate (QC Step 1) and will undergo cell acquisition by LCM.

10.4.3 Microdissection by Laser Capture Microscopy

Frozen sections are dehydrated in ethanol gradients followed by xylene rinse and stored in a desiccator until use. Regions of the biopsy containing neoplastic cells are identified by microscopy and 2,000 cells, each of benign epithelium and cancerous epithelium, are separately captured by laser activation of the thermoplastic cap (Arcturus) to induce cell adherence. Following capture, cells are placed into RNA buffer and processed for total RNA using the Picopure RNA method (Arcturus). The procedure is repeated for the acquisition of benign epithelium. Quality control will include image capture before and after cell acquisition. The Paradise System (Arcturus) will be used to purify and amplify RNA from paraffin sections.

10.4.4 RNA Amplification

Total RNA purified from microdissected cells will be subjected to two rounds of linear RNA/cDNA amplification according to the Eberwine protocol using T7 RNA polymerase. Quality of cDNA will be assessed by agarose gel electrophoresis after each round. cDNA yield and quality will be measured by spectrophotometry following the second round of amplification. Adequate yield is determined to be 200 ng RNA (QC Step 2).

Quantitative RT-PCR for four genes of high, medium, and low abundance (PSA, beta-actin, CHDH, and HoxB13) will be performed on each amplified sample. The expression of each test gene should not vary more than 3-fold in the benign epithelium captured from each specimen (QC Step 3).

10.4.5 cDNA Probe Preparation and Microarray Hybridization

Fluorescence-labeled probes will be made from ≥ 100 ng of amplified RNA (aRNA) in a reaction volume of 20 μ l containing 1 μ l anchored oligo-dT primer (Amersham), 0.05 mM Cy3-dCTP or Cy5-dCTP (Amersham), 0.05 mM dCTP, 0.1 mM each of dGTP, dATP, dTTP, and 200 U Superscript II reverse transcriptase (Life Technologies). Reactants will be incubated at 42°C for 120 minutes followed by the hydrolysis of RNA and cDNA probe purification by chromatography (Qiagen, Valencia, CA) as previously described. Labeled probes will be placed onto a microarray slide with a coverslip, hybridized in a humid chamber at 52°C for 16 hours, and washed with SSC gradients. Tumor probes and benign probes will be hybridized against a reference standard probe with a case-assignment of Cy3 and Cy5 labeling to either the tumor or benign probe and the reference probe (see Section 13.2). cDNA microarrays are constructed with ~22,000 distinct oligonucleotides corresponding to cDNAs derived from prostate cDNA libraries (or biochemical pathways of interest—e.g., apoptosis) and thus represent a cohort of genes relevant for the analysis of prostate biology. Individual oligonucleotides (Operon) are spotted onto glass microscope slides using a GeneMachine robotic spotting tool. Fluorescence intensities of the immobilized array targets will be measured using an Axon slide scanner. Quantitative data are obtained with GenePix software. Local background hybridization signals are subtracted before spot intensities are compared and expression ratios are determined. For each experiment, each oligonucleotide or cDNA target, corresponding to a unique gene, is represented once on each slide. Intensity ratios for each gene hybridized with experimental and control probes are calculated. For each experimental sample, microarray analysis demonstrating at least 50% of genes exhibiting hybridization signal above background compared with the cell line reference standard is considered adequate (QC Step 4).

10.5 Reimbursement and Case Credit (7/2/09)

RTOG will reimburse submitting institutions \$300 per case for flash frozen tissue. After confirmation from the RTOG Biospecimen Resource that appropriate materials have been received, RTOG Administration will prepare the proper paperwork and send a check to the institution. Pathology payment cycles are run twice a year in January and July and will appear on the institution's summary report with the institution's regular case reimbursement.

Institutions will receive .5 credit per case.

11.0 PATIENT ASSESSMENTS

Patient assessments will be per RTOG protocol 0232.

12.0 DATA COLLECTION (7/2/09)

Data (specimens) should be submitted to the RTOG Biospecimen Resource as described in Sections 10.1 and 10.3.

Patients will be identified by initials only (first middle last); if there is no middle initial, a hyphen will be used (first-last). Last names with apostrophes will be identified by the first letter of the last name.

12.1 Summary of Data (Specimen) Submission

<u>Item</u>	<u>Due</u>
Slides/Blocks (P2)*	Within 2 weeks of study entry.
Specimen Transmittal Form (ST)	Within 1 week of brachytherapy.

*If slides/blocks have been previously submitted for RTOG 0232, they will be credited to RTOG 0612.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Endpoints

13.1.1 Primary

The number of frozen and paraffin-embedded specimens that yield adequate RNA (see Section 10.4) for tumor tissue microarray analysis.

13.1.2 Secondary

- 13.1.2.1** Comparison of the gene expression patterns between frozen and paraffin-embedded tissue in the brachytherapy alone arm (Arm 2) of RTOG 0232. This will be done only if the primary endpoint analysis shows that it is feasible to acquire an adequate number of tumor tissue specimens for successful microarray analysis.
- 13.1.2.2** Comparison of genes to determine which are expressed differentially pre- and post-external beam radiation therapy (EBRT) in the EBRT plus brachytherapy arm (Arm 1) of RTOG 0232. This will be done only if the 13.1.2.1 analysis shows fewer than 50% of the genes have a statistically significant difference between frozen and paraffin-embedded tissue expression.
- 13.1.2.3** Exploration of any natural subgroups of genes to see if those subgroups associated with biochemical and clinical (local and distant metastasis) failure and non-failure patient populations. This analysis will be done only if at least 25 DNA microarrays assays are successfully completed.

13.2 Experimental Design (6/20/06)

Experimental tissue samples will be allocated to two-color microarrays according to a common reference design¹³ to allow for the greatest flexibility in making different comparisons (e.g., differences due to tissue preparation, treatment effect, benign versus cancer). The reference sample to be used for each array is composed of equal quantities of RNA purified from three prostate cancer cell lines (DU145, PC3, LNCap) that provide reproducible hybridization signals covering a large component of the prostate transcriptome. For the microarrays that will be used in this study, 80% of the clones exhibit signal above background using this 3 cell line reference standard.¹⁴ Each array hybridization will use a combination of a test sample: either benign or cancerous epithelium, and the reference sample. Table 1 presents the basic experimental design in terms of test sample groups that will be analyzed. All test samples from the eight groups represented in Table 1 will be labeled with Cy5 (red) and the reference sample will be labeled with Cy3 (green). Each test sample will be cohybridized with the reference sample to an array.

Table 1. Test Sample Groups

Tissue type	Brachytherapy alone (Arm 2)		EBRT+ Brachytherapy (Arm 1)	
	<i>Paraffin-embedded (baseline)</i>	<i>Frozen (baseline /at implant)</i>	<i>paraffin-embedded (baseline /pre-EBRT)</i>	<i>Frozen (post-EBRT/at implant)</i>
<i>Benign</i>	<u>IIA</u>	<u>IIC</u>	<u>IA</u>	<u>IC</u>
<i>Cancer</i>	<u>IIB</u>	<u>IID</u>	<u>IB</u>	<u>ID</u>

For purposes of between-group comparisons, it is not necessary to perform reverse dye experiments when a common reference design is used because gene-specific biases cancel out in class comparison analyses.^{15, 16}

13.3 Gene Expression Data Pre-Processing (6/20/06)

13.3.1 Pre-Processing Procedure

A careful examination of array images of each gene's spots on the array images will be carried out to find the spots affected by experiment artifacts. We will not include genes whose intensity is less than 100^{15,17} in both green and red intensities. If one of the two intensities in a spot is less than 100, that intensity will be set to 100.

13.3.2 Expression Ratio Calculation

Red and green fluorescence intensities will be measured for each gene on each microarray. Local background hybridization signals will be subtracted from the intensities. Let R_j be the background-adjusted fluorescence intensity for the cancer or benign sample and G_j be the background-adjusted fluorescence intensity for the reference sample for gene j on a particular

array. The gene expression ratio is computed as R_j / G_j and undergoes normalization and transformation to the log-2 scale. Normalization will be applied to remove systematic differences due to extraneous factors such as array effects, global dye effects, print tip effects, etc. Simple normalization methods such as global median centering¹⁵ will be considered as well as more complex methods such as print tip-specific corrections and intensity-based normalization methods such as lowess smoothing¹⁸ if diagnostic plots (e.g., M vs A plots¹⁹) suggest they are needed. These log-transformed, normalized gene expression ratios are used as the basic data in subsequent analyses.

13.3.3 *Missing Data*

Genes with greater than 20% of spots missing intensities will be removed from the analysis. For remaining genes, individual missing log ratio values will be imputed using the k-nearest neighbors approach, with $k = 10$.²⁰

13.4 **Power Calculation and Sample Size Calculation**

13.4.1 *Primary Endpoint*

The primary endpoint for the study is the proportion of tumor tissue samples for which there is adequate quality and quantity of tumor tissue (see criteria in Section 10.4) to successfully perform a microarray analysis (Stage 1). Fifty patients, 25 patients from each arm, will be studied for the frozen and paraffin-embedded tissue acquisition for the primary endpoint analysis. Paired frozen and paraffin embedded tissue will be obtained from each of the fifty patients, resulting in a total of 100 specimens for analysis. This feasibility endpoint will be assessed separately for each of the frozen and paraffin-embedded tissue groups. The null hypothesis that the proportion of adequate tissue samples is less than or equal to 0.5 will be tested using an exact test for a binomial proportion. If the true adequate tissue samples proportion is 0.7, then the statistical power of a one-sided 0.0325 level exact binomial test of proportion would be 85.9% if the sample size is 50. This corresponds to a rule of 32 or more tissue samples out of 50 yielding acceptable RNA for successful microarray analysis. If the success proportion is less than 32 out of 50, there will be no further accrual to the study.

13.4.2 *Secondary Endpoints*

The first secondary aim (13.1.2.1) is to compare gene expression patterns in matched frozen and paraffin-embedded tumor tissues (Stage 2). Paired samples of frozen and paraffin-embedded tumor tissue from patients in Arm 2 will be analyzed. For each patient, the difference in normalized log gene expression ratio of frozen and paraffin-embedded tissue will be computed for each gene. For each gene, the mean difference will be tested to determine if it is statistically significantly different from zero. A multiple comparisons procedure will be used to control the false discoveries proportion.²¹ For purposes of calculating the minimum number of frozen and paraffin-embedded tumor tissue pairs, we will base the sample size target on a paired t-test with a Type I error of α and statistical power of $1-\beta$ using

$$n = [(t_a + t_b) / (|\mu_d| / \sigma_d)]^2$$

where μ_d = mean difference for a single gene, σ_d = standard deviation of paired gene expression differences, $a = 1 - \alpha/2$, $b = 1 - \beta$ and $t_q = 100(q)$ percentile of the t distribution with $n-1$ degrees of freedom. We assume the variance will be similar in the frozen and paraffin-embedded tissue groups. The maximum standard deviation of the difference in the two expressions is $\sigma_d \leq 2 * \sigma$. Using the median sample standard deviation of genes $s = \sqrt{0.45}$ from a previous experiment,²² we assume the maximum standard deviation of the difference in the matched expression groups is 1.34. We expect 5700 out of 6000 genes will not be differentially expressed between frozen and paraffin-embedded tissues, and we wish to control the expected number of false positives (defined as being differentially expressed when actually it is not) to be no more than 5. With this expectation, the probability of a gene falsely declared as differentially expressed is set to $\alpha = 5/5700 \approx 0.001$. To detect a true mean difference greater than or equal to 1 (corresponding to a fold-change of 2 or more) in population mean differences with statistical power of 90% and a two-sided Type I error of 0.001 requires at least 43 pairs.

We expect that there will be some genes that exhibit expression differences between frozen and paraffin-embedded tissues, but ideally the number will not be too large. If 50% or more of the genes tested show significant differences between frozen and paraffin-embedded tissues, we will not proceed to aim 13.1.2.2 out of concern that the substantial differences in gene expression

due to tissue preservation method would invalidate comparisons between pre- and post-EBRT samples.

If all conditions are met for proceeding to aim 13.1.2.2 (Stage 3), we will accrue more patients to have 78 matched frozen and paraffin-embedded specimen pairs from Arm 2 and 78 matched frozen and paraffin-embedded specimen pairs from Arm 1. These sample sizes are justified as follows. Group 1 is the differences of each gene expression from pre- and post-EBRT for each specimen in Arm 1. Group 2 is the differences of each gene expression from frozen and paraffin embedded tissue for each specimen in Arm 2. We assume the variance will be similar in the two groups. The minimum number of specimens required to achieve statistical power of $1 - \beta$ at a Type I error probability of α is calculated with a two sample t-test assuming equal sample sizes in the two groups.

$$n = 2 * [(t_a + t_b) / (|\mu|/\sigma)]^2$$

where μ = mean difference (of the differences) for a single gene, σ = the standard deviation of the gene expression difference for a single gene among samples within the group, $a = 1 - \alpha/2$, $b = 1 - \beta$ and $t_q = 100(q)$ percentiles of the t distribution with $n-2$ degrees of freedom. A sample size of 78 in each group will have 90% statistical power to detect at least a difference $\mu = 1$ (a fold-change of 2) at a 0.001 two-sided Type I error probability assuming the standard deviation of the gene expression for a single gene among samples within the group $\sigma = 1.34$.

13.5 Patient Accrual (6/20/06) (10/9/09)

Patients for this study are restricted to RTOG 0232 patients who have not been treated with neoadjuvant hormonal therapy. The rate of patients on RTOG 0232 who have not been treated with neoadjuvant hormonal therapy is 88%. We assume the accrual to RTOG 0612 will be the same from each arm of RTOG 0232. Patient accrual is projected to be 3 cases per month based on 6 months of accrual in RTOG 0232 (7.3 cases/month from July to December 2005); we expect about half of the RTOG 0232 patients will participate in this study. Based on patient accrual in previous RTOG randomized prostate cancer studies, there will be relatively few entries during the initial 6 months after activation while institutions are obtaining IRB approval. We expect to complete accrual necessary for the primary endpoint in 2 years. If we proceed to aim 13.1.2.1, we plan to accrue 18 more patients to have a total of 43 specimen pairs from Arm 2 of RTOG 0232, which is estimated to take an additional 6 months. If we proceed to aim 13.1.2.2, we plan to accrue to a total of 78 specimen pairs for each arm of RTOG 0232, which is estimated to take an additional 30 months for both arms. Obtaining frozen tissue in radiation-treated prostate cancer patients is a difficult prospect, even in single institution studies. The study design of RTOG 0232 affords us this unique opportunity. Although accrual is projected to be slower than we would like for a pilot study, there are no other RTOG studies from which we can prospectively obtain frozen tissue. Further, to mandate frozen tissue acquisition from a non-therapeutic trial would require an additional series of biopsies purely for research purposes, at which most men with prostate cancer and their clinicians would balk.

If the average monthly accrual rate during the first year beyond the quiet period is less than 1 case per month, the study will be re-evaluated for its feasibility.

13.6 Analysis Plan (6/20/06)

Table 2 presents possible comparisons that could be made in this study.

Table 2. Possible Comparisons

Comparison	Test Sample Groups
<u>Paraffin-embedded vs. Frozen</u>	<u>IIA vs. IIC</u> <u>IIB vs. IID</u>
<u>pre-EBRT vs. post-EBRT</u>	<u>IA vs IC</u> <u>IB vs. ID</u>

*Test sample groups are from Table 1

The comparison between frozen versus paraffin-embedded tissues assumes no biological changes occur in the 4 weeks between baseline and time of implant in Arm 2. The comparison between pre-EBRT versus post-EBRT will assume no interaction between EBRT and tissue preservation method; the analysis will adjust for any effect due to the tissue preservation method by considering the differences between frozen and paraffin-embedded tissue gene expressions into the differences between pre-EBRT versus post-EBRT gene expressions.

The analysis will be conducted in three stages, some of which are conditional on the results of others.

STAGE 1: This stage investigates the feasibility of acquiring specimens, both frozen and paraffin-embedded, to yield RNA of adequate quality and quantity to perform DNA microarray studies on tumor tissue. The primary study result is the feasibility of performing DNA microarrays with tumor tissue (13.1.1).

Exploratory analyses will be done to look for the natural subgroups of genes related to treatment failure (13.1.2.3) if at least 25 DNA microarrays are generated in any one of the following microarray types: paraffin-embedded benign tissue, paraffin-embedded tumor tissue, frozen benign tissue, frozen tumor tissue microarray. If it is determined that it is not feasible to perform DNA microarray hybridization, the analyses for secondary endpoints 13.1.2.1 and 13.1.2.2 will not be done.

STAGE 2: This analysis will only be done if it is determined that it is feasible to perform DNA microarrays from the acquired biopsy tissues. We will accrue more patients to have 43 specimens of each tissue preservation method from the same patients on Arm 2. This stage will examine gene expression differences between matched frozen and paraffin-embedded specimens in Arm 2 only. This analysis will be performed on tumor and benign tissues (as applicable, 13.1.2.1).

STAGE 3: If fewer than 50% of the genes show statistically significant differences between frozen and paraffin-embedded tissue expression, gene expression patterns will be compared between pre-EBRT and post-EBRT in Arm 1. This analysis will be performed on tumor and benign tissues (as applicable, 13.1.2.2).

13.6.1 *Primary Endpoint*

The null hypothesis is that no more than 50% of patients yield the proper frozen (paraffin-embedded) tumor tissue to generate the microarray data. The alternative hypothesis is that more than 50% of patients yield the proper frozen (paraffin-embedded) tumor tissue to generate the microarray data. Each of the 4 QC criteria (Section 10.4) must be met for a sample to be considered adequate to generate the microarray data.

We will test the null hypothesis separately for frozen and paraffin-embedded tissues using the exact binomial test of proportions at a significance level $0.0325 \times 2 = 0.065$. The overall rate of incorrectly rejecting at least one the hypotheses is no more than 0.065 because this test will be carried out for both the frozen and paraffin-embedded tissues. We will conclude that it is feasible to acquire specimens, in the form of frozen tissue or paraffin-embedded tissue, to yield RNA of adequate quality to perform DNA microarray studies on tumor tissues within the multi-institutional setting if 32 or more out of 50 patients yield the proper frozen tissue or paraffin-embedded tissue to generate the microarray data.

13.6.2 *Secondary Endpoints*

13.6.2.1 *Stage 2*

The array data from patients who yield both frozen and paraffin-embedded tumor and benign (as applicable) tissue specimens will be used. A test will be conducted for each gene to test the hypothesis that there is no mean difference in gene expression versus the alternative hypothesis that there is a difference in mean gene expression between frozen and paraffin-embedded tissue. We will control the proportion of false positive gene expression differences out of those genes that are identified as being differentially expressed. A paired t-test will be used to calculate the unadjusted univariate p-value for each gene. We will identify all genes with adjusted p-values²¹ \leq

0.05 as being differentially expressed to be 95% confident that the false discovery proportion is no more than 10%.

13.6.2.2 Stage 3

The array data from patients in both arms who yield both frozen and paraffin-embedded tumor and benign (as applicable) tissue specimens will be used for this analysis. The variability from the tissue preservation type difference will be minimized in the comparison of pre- and post-EBRT gene expression differences by the following procedure. Let m be the number of genes that will be tested. Let d_{1ij} be the gene expression difference between pre- and post-EBRT for patient i and gene j ($i = 1, 2, \dots, 78$ and $j=1, 2, \dots, m$) on Arm 1. Let d_{2ij} be the gene expression difference between frozen tissue and paraffin-embedded tissue for patient i and gene j ($i = 1, 2, \dots, 78$ and $j=1, 2, \dots, m$) on Arm 2. Denote the mean difference between pre- and post-EBRT gene expression in Arm 1 as D_{1j} for gene j and the mean difference between frozen and paraffin-embedded gene expression in Arm 2 as D_{2j} . A test will be conducted to test the following null (H_0) and alternative (H_A) hypotheses for each gene:

$$H_0: D_{1j} - D_{2j} = 0 \text{ vs. } H_A: D_{1j} - D_{2j} \neq 0$$

We will control the false discovery proportion when a test for a gene is called significant. A two-sample t-test will be used to calculate the unadjusted univariate p-value for each gene. We will identify all genes with adjusted p-values²¹ ≤ 0.05 as being differentially expressed between pre- and post-EBRT to be 95% confident that the false discovery proportion is no more than 10%.

13.6.2.3 Exploratory Analysis

There are four types of DNA microarrays; paraffin-embedded benign tissue, paraffin-embedded tumor tissue, frozen benign tissue, and frozen tumor tissue microarrays. If at least 25 DNA microarrays are constructed in any one of the four types, exploratory analyses will be conducted to identify groups of genes corresponding to failure (biochemical, local failure and distant metastasis) and non-failure populations. Several clustering methods (e.g., hierarchical clustering,¹⁶ k-means clustering,^{23,24} and the Self-Organizing Map,²⁵ etc.) will be used to explore gene expression patterns. The results from each of the clustering methods will be compared to assess the stability of the clustering results. The scientific value of any clusters identified will be assessed by the study chairs.

13.6.3 CDUS Monitoring

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly by electronic means. Reports are due January 31, April 30, July 31, and October 31.

13.7 Gender and Minorities

Planned Gender and Minority Inclusion

	Gender		
Ethnic Category	Females	Males	Total
Hispanic or Latino		6	6
Not Hispanic or Latino		150	150
Ethnic Category: Total of all subjects*		156	156
	Gender		
Racial Category	Females	Males	Total
American Indian or Alaskan Native		0	0
Asian		0	0
Black or African American		28	28
Native Hawaiian or other Pacific Islander		0	0
White		128	128
Racial Category: Total of all subjects*		156	156

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APPENDIX I (6/20/06)

RTOG 0612

Informed Consent Template for Cancer Treatment Trials **(English Language)**

INVESTIGATING MARKERS OF RADIATION OUTCOME IN PATIENTS WITH INTERMEDIATE-RISK PROSTATE CANCER USING DNA MICROARRAY ANALYSIS: AN RTOG PILOT STUDY

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

You are being asked to take part in this study because you have prostate cancer and are being treated in RTOG study 0232. The investigators of RTOG study 0612 would like to analyze your tissue to see whether they can help improve treatment for prostate cancer by learning more about the genetic make-up of prostate cancer tissue.

Why is this study being done?

The purpose of this study is to try find better ways to treat prostate cancer by collecting and analyzing prostate cancer tissue taken from tumor biopsies at diagnosis and during treatment with brachytherapy (a type of radiation therapy that involves surgically placing radioactive material close to the tumor). The tissues from both sets of biopsies may provide useful information on genes that radiation therapy impacts. The study doctors want to determine: (1) whether there are differences in the genetic make-up of certain prostate cancer tumors, and (2) if these differences can help predict how patients with prostate cancer will respond to treatment.

Tissue for this study is being collected from patients who are currently enrolled on RTOG study 0232. The study doctors want to know whether it is possible to successfully collect tissue in this manner; if this study is successful, the study doctors would like to try to conduct a larger study using tissue from other patients as well.

How many people will take part in the study?

About 156 people will take part in this study.

What will happen if I take part in this research study? (6/20/06)

You have had a biopsy to see if you have prostate cancer. Your doctor has removed some of your tissue to do some tests. The results of these tests will be given to you by your doctor and will be used as part of your enrollment requirements for RTOG study 0232. If you agree to participate in this study, a small sample of the tissue that has been removed in the biopsy will

be collected and shipped to study doctors who work at a pathology laboratory at Cedars-Sinai Medical Center, so that the doctors can analyze the genetic make-up of that tissue.

In addition, as part of your treatment on RTOG 0232, you are receiving brachytherapy. If you agree to enroll on this study, the study doctors will also biopsy some of the tumor tissue at the time that you receive the brachytherapy. This tissue will also be shipped to the doctors at Cedars-Sinai Medical Center for analysis.

Your tissue will not be used for research about other diseases unless you give your permission.

How long will I be in the study?

Your tissue will be kept indefinitely unless you request that your tissue either be returned to you or your designee or that the study doctors dispose of the tissue.

Can I stop being in the study?

Yes. You can decide to stop participating at any time. If you decide now that your tissue can be kept for research, you can change your mind at any time. If you change your mind, contact your study doctor and let him/her know.

What side effects or risks can I expect from being in the study?

Participation in this study will involve additional biopsies at the time of brachytherapy that would not normally be part of your care. Each biopsy will be performed by the insertion of a separate needle that would not be needed if additional biopsies were not being obtained. Possible side effects from these additional biopsies and needle insertions include:

- Infection that can be treated with antibiotics
- Soreness in the biopsied area
- Bleeding
- Swelling

Are there benefits to taking part in the study?

The benefits of research using your tissue include learning more about what causes prostate cancer and other diseases, how to prevent them, and how to treat them. You may or may not benefit directly from the research.

What other choices do I have if I do not take part in this study?

Your other choices may include:

- Getting treatment or care for your cancer without being in a study
- Taking part in another study

Talk to your study doctor about your choices before you decide if you will take part in this study.

Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The Radiation Therapy Oncology Group
- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), involved in keeping research safe for people

What are the costs of taking part in this study?

Your permission to use the tissue from the biopsy taken for your diagnosis will not lead to any additional expenses. You or your insurance company may have to pay for the expenses of the biopsies taken at the time of brachytherapy. Storage of your tissue and the research involving it will not result in cost to you.

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

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at <http://www.cancer.gov/clinicaltrials/understanding/insurance-coverage>. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

What happens if I am injured because I took part in this study?

It is important that you tell your study doctor, _____ [*investigator's name(s)*], if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ [*telephone number*].

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

What are my rights if I take part in this study?

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

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

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

Who can answer my questions about the study? (7/2/09)

You can talk to your study doctor about any questions or concerns you have about this study. Contact your study doctor _____ [name(s)] at _____ [telephone number].

For questions about your rights while taking part in this study, call the _____ [name of center] Institutional Review Board (a group of people who review the research to protect your rights) at _____ (telephone number).

Please read the information sheet called "How is Tissue Used for Research" to learn more about tissue research. This information sheet is available to all at the following web site: http://www.rtog.org/tissue%20for%20research_patient.pdf

Making Your Choice

Please read each sentence below and think about your choice. After reading each sentence, circle "Yes" or "No". If you have any questions, please talk to your doctor or nurse, or call our research review board at IRB's phone number.

No matter what you decide to do, it will not affect your care.

1. My tissue may be kept for use in this research study to learn about, prevent, or treat prostate cancer.

Yes No

2. My tissue may be kept for use in research to learn about, prevent, or treat other cancers.

Yes No

3. My tissue may be kept for use in research to learn about, prevent or treat other health problems (for example: diabetes, Alzheimer's disease, or heart disease).

Yes No

4. Someone may contact me in the future to ask me to take part in more research.

Yes No

Where can I get more information?

You may call the National Cancer Institute's Cancer Information Service at:

1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615

You may also visit the NCI Web site at <http://www.cancer.gov/>

- For NCI's clinical trials information, go to <http://www.cancer.gov/clinicaltrials/>
- For NCI's general information about cancer, go to <http://cancer.gov/cancerinfo/>

You will get a copy of this form. If you want more information about this study, ask your study doctor.

Signature

I have been given a copy of all _____ *[insert total of number of pages]* pages of this form. I have read it or it has been read to me. I understand the information and have had my questions answered. I agree to take part in this study.

Participant _____

Date _____

APPENDIX II (7/2/09)
RTOG 0612: SPECIMEN COLLECTION INSTRUCTIONS

Instructions for frozen tissue collection:

This kit includes:

- Cryomold(s)
- Biohazard bag(s)
- Absorbent shipping material
- Styrofoam container (inner)
- Cardboard shipping (outer) box
- Fed Ex shipping label

Materials Needed:

- OCT compound
- Cryomold
- Isopentane (2-Methylbutane)/liquid nitrogen, or ethanol/dry ice combinations
- Small container to hold isopentane/ethanol
- Long handled forceps
- Ring stand and clamp
- Ice bucket for dry ice

Preparation:

- Keep biohazard wipes nearby to keep area clean throughout process.
- Label cryomold(s) with RTOG study and case numbers

Procedure (Each core must be processed separately):

1. Fill dewar or other suitable container with liquid nitrogen or dry ice
2. Fill other small container with approximately 1-2 inches of isopentane or ethanol
3. Suspend isopentane container from clamp on ring stand and lower the container into the liquid nitrogen (or alternatively lower ethanol onto dry ice). The base of the container should just touch the top of the liquid nitrogen/dry ice and cause solution to bubble as it cools the container. If you insert the container too far into the liquid nitrogen/dry ice the isopentane/ethanol will freeze. If this happens, just remove the container and allow the solution to thaw and then place it back in the liquid nitrogen/on dry ice
4. Label a cryomold with the identifying data of the tissue (Study and case numbers). Make sure to use a marker resistant to organic solvents!
5. Place the tissue oriented with the side you are interested in at the bottom of the cryomold
6. Fill the cryomold with OCT compound. Use caution not to move the tissue when adding the OCT and be sure to remove any bubbles that form near the tissue with a forceps as these will cause problems with sectioning (cracking and fragmentation).
7. Place the cryomold into the bubbling isopentane or ethanol solution. Use caution not to tip the cryomold or dislodge the tissue
8. The OCT will freeze into a solid white color. The tissue should be covered and not visible as you look down on the mold. Allow approximately 1 minute for freezing of the entire block
9. Place frozen samples into biohazard bags and store
10. Use RTOG labels* to label biohazard bags
11. Store samples at -80°C in liquid nitrogen, or on dry ice until shipped
12. Ship on dry ice

*RTOG labels are obtained at the time of registration. **PLEASE ASSURE THAT EVERY SPECIMEN IS LABELED.**

Shipping/Mailing:

- Include all RTOG paperwork in pocket of biohazard bag.
- Place specimens and the absorbent shipping material in Styrofoam cooler filled with dry ice (if appropriate; double-check temperature sample shipping temperature). Place Styrofoam cooler into outer cardboard box, and attach shipping label to outer cardboard box.
- Multiple cases may be shipped in the same cooler, but make sure each case is in a separate bag.*
- For questions regarding collection/shipping please contact the RTOG Biospecimen Resource by phone (415) 476-RTOG (7864); Fax (415) 476-5271; E-mail RTOG@ucsf.edu