

RTOG 0837/ACRIN 6689

BIOMARKER PROCESS MANUAL

(FOR ACRIN 6689 BLOOD COLLECTION)

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1. INTRODUCTION

1.1 Plasma Biomarker Analysis

Plasma samples will be analyzed for potential biomarkers using multiplexed enzyme-linked immunosorbent assay (ELISA) kits (96-well plates, 4-10 analytes) or single cytokine ELISA (for analytes unavailable in multiplex, e.g., collagen IV, SDF1 α). Using these technology, we will be able to assess VEGF family members and their soluble receptors, and collagen IV, SDF1 α , bFGF, sICAM-1, sVCAM-1, PDGF-C, PDGF-B, thrombospondin-1, Ang1, Ang2, IL-1, IL-6, IL-8, and TNF- α . This broad array of proteins to be tested will permit complete evaluation of the most promising known angiogenic biomarkers.

2. BLOOD COLLECTION CRITERIA

2.1 Blood Collection Time Points

Blood samples will be obtained from each participant prior to imaging at each time point described below for protein analysis of potential biomarkers for anti-angiogenic therapy. If the site is capable of drawing blood and processing prior to the MRI, then proceed as normally. If the site is not set up for blood draw or processing blood, it is acceptable to draw the blood within 24 hours of MR imaging at a facility of your choice.

- T0: Baseline (within 7 days prior to initiation of chemoradiation)
- T1: Between doses (within 2 to 24 hours after the first dose of placebo or cediranib, but prior to the second dose of placebo or cediranib/radiation/TMZ);
- T2: Week 4 of chemoradiation;
- T3: Week 10 (Week 4 after completion of chemoradiation);
- T4: Week 16 (Week 10 after completion of chemoradiation);
- T5: Week 24 (Week 18 after completion of chemoradiation);
- T6: Progression (whenever disease progression occurs; progression is defined as > 25% increase in tumor area [two diameters]).

2.2 Confidentiality

The confidentiality of the participant's identity will be maintained. All collected information will be protected per ACRIN policies and procedures and federal regulatory guidelines. Access to study data will be limited to the RTOG 0837/ACRIN 6689 staff working on the study. All computer data will be maintained in a manner consistent with Title 21 Code of Federal Regulations Part 11 (21 CFR Part 11). In addition, access to the data management system will be limited to designated staff through use of individualized, confidential login ID and password. Designated staff will not share login IDs or passwords.

The data from this study will be maintained until 10 years following completion of the study or until the data are no longer required for research. Data will be destroyed as required by the ACRIN Record Retention Policy and federal regulatory guidelines. Human research subjects are protected in accordance with Title 45 CFR Part 46 and Title 21 CFR Part 50. ACRIN is not a covered entity according to the Privacy Rules of the 1996 Health Insurance Portability and Accountability Act (HIPAA); therefore, HIPAA regulations should be followed according to institutional standards.

2.3 Informed Consent

Human research subjects are protected by informed consent procedures in accordance with Title 45 CFR Part 46 and Title 21 CFR Part 50. The Biomarker consent component of the institution-specific, IRB-approved informed consent form grants permission for study investigators to request and obtain blood and blood fractionates, and

to use those samples for research involving molecular studies on the development of cancers and/or for other diseases.

All participants will already have provided a written consent for the collection of blood for this biomarker assessment using the institution-specific, IRB-approved informed consent form at the time of enrollment (a sample informed consent form template is provided as Appendix I in protocol RTOG 0837/ACRIN 6689). If blood collection was not included in the original institution-specific, IRB-approved informed consent form, please inform the ACRIN Protocol Development and Regulatory Compliance department and refer to your local IRB and institutional policy for further guidance.

3. BLOOD COLLECTION PROCESS

Each participant will have **2** vials of blood (~8 mL each) collected at the time points listed in Section 2.1. ACRIN will provide the labels that will be used to label the blood tubes and the plasma cryovials. The blood must be processed and stored within 30-45 minutes after collection.

3.1 Materials and Labeling of Blood Tubes and Cryovials

ACRIN will provide the labels for the vacutainer blood tubes and the cryovials. All labels will have space for sites to provide the following information:

- Participant study number;
- Participant initials;
- Time point when sample was collected;
- For the blood tubes, a “BL” and for the plasma cryovials, a “PL” to distinguish which label should be used for each tube.

The site will be responsible for obtaining the appropriate tubes for the blood and plasma specimens. The list of supply options are as follows:

For Blood Draw Tubes (3 Options)

1. SARSTEDT Monovette® EDTA KE (9 mL), Part # 02.1333.001; or
2. Becton-Dickinson Vacutainer™ K2E (10 mL), Part # 367525; or
3. Greiner Bio-One Vacuette® K3E EDTA K3 (9 mL), Part # 455036; and

For Plasma Cryovials (1 Option)

4. Nunc, Internally Threaded Cryotube, (3.6 mL vials), Fischer Scientific, Part # 12-565-162N.

In the event that a clinical site cannot obtain the appropriate blood and storage tubes, please contact, [Rosa Medina](#), at ACRIN headquarters via e-mail at rmedina@acr.org. The e-mail should list the name of the person requesting the material, a list of materials needed, and the shipping address for shipment to the site. Make sure to put “Materials Requested, Study ACRIN 6689 and Site Number” in the e-mail ‘Subject’ line.

3.2 Blood Draw and Centrifugation

- Prior to the blood draw, the blood vacutainer should be pre-cooled by placing tube in a bucket with wet ice.
- Directly from the participant’s IV line, draw the 2 vials of blood using 2 pre-labeled blood vacutainer tubes.
- Record time of blood draw on Blood Processing Form (BL).
- Immediately invert the blood tube several times to ensure proper mixing with the preservative.
- Place the blood tube in a bucket filled with wet ice.

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- Centrifuge the blood tube at 700g (2000rpm) for 20 minutes at 4°C with no break at the end of centrifugation for plasma extraction.
 - Using a sterile pipette, pipette the top clear layer (careful not to disturb the bottom red layer) and aliquot equally into 3 pre-labeled 1.8 mL Nunc cryovials.
 - Visually check the plasma for hemolysis.
 - If the plasma appears to be yellow and clear, please proceed with processing the plasma and record the observation on the BL form.
 - If the plasma appears to be dark red, please discard the plasma and sign and date the BL form.
 - After the plasma has been extracted, check the red blood cells by sticking two wooden applicator sticks in the tube and observe the sticks for clots. Record if there was clotting observed, check yes or no on the Blood Processing Form (BL).
 - Record numbers of vials on Blood Processing Form (BL).
 - Immediately store the cryovials into a -80°C freezer*.
 - Record time of freeze, location of the vials (i.e., freezer number, shelf, box number, and room #, as applicable) on the Blood Processing Form (BL).

* If a -80°C freezer is not available on site, the plasma specimens should be shipped to the core facility on dry ice the same day as processing. See Section 4.0 for shipping procedures.

3.3 Labeling and Storing Specimens

For tracking purposes, each specimen and its associated forms should be labeled with the 6689 site number and case number. All cryovials should be stored in a monitored -80° C freezer. A separate freezer box should be set aside for the storage of all the plasma vials. They will be shipped in bulk to the testing core facility at completion of study.

4. SHIPPING SPECIMENS TO CORE FACILITY

When blood collection has been completed for all participants and after the participants go off trial the plasma samples should be shipped to the Steele Laboratory at Massachusetts General Hospital on dry ice in a Styrofoam box. The Styrofoam container should be packed with at least 7 pounds of dry ice, and make sure the top is completely covered with dry ice. Seal the Styrofoam container within a tight-fitting cardboard shipping box. A copy of the Plasma Shipping (PS) Form for each set of samples should be placed within a separate zip-lock plastic bag and placed on top of the Styrofoam container lid before the external shipping box is sealed.

If the site does not have -80°C storage capability, the plasma samples should be sent to the Steele Laboratory at Massachusetts General Hospital after the blood has been processed. The Styrofoam container should be packed with at least 2 inches of dry ice on the bottom, and completely covered on the top with an additional 2 inches or more of dry ice. Seal the Styrofoam container within a tight-fitting cardboard shipping box. A copy of the Plasma Shipping (PS) Form for each set of samples should be placed within a separate zip-lock plastic bag and placed on top of the Styrofoam container lid before the external shipping box is sealed.

If the site does not have facilities and trained personnel for plasma separation, the blood samples should be shipped to the Steele Laboratory at Massachusetts General Hospital with cold packs in a Styrofoam box within 24 hours (DO NOT FREEZE). Seal the Styrofoam container within a tight-fitting cardboard shipping box. A copy of the Plasma Shipping (PS) Form for each set of samples should be placed within a separate zip-lock plastic bag and placed on top of the Styrofoam container lid before the external shipping box is sealed.

Specimens should be shipped Monday to Wednesday only by overnight FedEx to the Testing Core Facility with the original Sample Submission Form (ACRIN web site | Protocol 6689 Forms). On the day of shipment, the study coordinator will notify the Testing Core Facility via e-mail at sylvie@steele.mgh.harvard.edu or

christina@steele.mgh.harvard.edu or FAX (617-724-5841, ATTN: S. Roberge or C. Koppel) so they know to expect the upcoming shipment. Include the estimated date of arrival and the FedEx tracking number.

NOTE: The subject line of the e-mail/FAX should include the following so that the Testing Core Facility staff can distinguish plasma specimens sent by ACRIN sites.

ACRIN 6689 Plasma Specimen Shipment--Site Name

Upon receipt of specimens, the Testing Core Facility will reconcile the materials and notify the ACRIN 6689 site study coordinator of missing specimens, damaged specimens, or any concerns to be addressed.

4.1 Shipping Materials and Process

The appropriate shipping materials for ACRIN 6689 specimens are the following:

- Storage boxes for plasma tubes (Fisherbrand, 5 ^{3/4}" x 5 ^{3/4}" x 4 ^{7/8}", Part # 03-395-01, and dividers, Part # 03-395-11);
- Large size zip-locked bags;
- Multi-purpose insulated bio-shippers (Thermosafe Bio-Shippers; dimensions 14" x 10" x 14");
- Biohazard bags;
- M3 carton sealing tape;
- Shipping labels to indicate: "Notice: Keep Frozen" use only for **Dry Ice** shipments, Upright arrows, "Diagnostic Specimens – Not restricted, Packed in Compliance with IATA Packing Instruction 650", and "Class 9 – Dry Ice" label; and "Keep Refrigerated" use only for **Cold Pack** shipments.
- Dry ice or Cold Packs

The packing process for dry ice shipments includes the following:

- Place all plasma tubes in storage freezer boxes, tape the sides of the boxes;
- Place one freezer box, each separately, in a large zip-locked bag;
- Pack the Styrofoam container with at least 7 pounds of dry ice;
- Place the bagged freezer boxes in the middle of the bio-shipper (you can fit as many as the box allows);
- Pack the Styrofoam container with an additional dry-ice on the top of the boxes to cover the top;
- Place a copy of the Plasma Shipping Form (PS) for a single participant inside one biohazard bag, in the form slot (use as many forms/bags as necessary to cover the contents of the box to be shipped);
- Close the lid, place all bagged shipping forms on top of the lid, and seal the shipping container with tape.
- Maintain a copy of the transmittal log at the site.

The packing process for cold pack shipments includes the following:

- Place all blood tubes in storage freezer boxes, tape the sides of the boxes
- Place one freezer box, each separately, in a large zip-locked bag.
- Place the bagged freezer boxes in the middle of the bio-shipper (you can fit as many as the box allows)
- Pack the Styrofoam container with 4-6 cold packs surrounding the boxes with the blood tubes.
- Place a copy of the Plasma Shipping Form (PS) for a single participant inside one biohazard bag, in the form slot (use as many forms/bags as necessary to cover the contents of the box to be shipped).
- Close the lid, place all bagged shipping forms on top of the lid, and seal the shipping container with tape.
- Maintain a copy of the transmittal log at the site.

4.2 Labeling Shipping Containers

Label each shipping container with the FedEx shipping label to include the following:

1. The study coordinator's return address.
2. The Testing Core Facility address:

ATTN: Sylvie Roberge or Christina Koppel

MGH, Cox-734

100 Blossom St.

Boston, MA 02114

Phone: (617) 726-8143 or (617) 724-1353

Fax: (617) 724-5841

Pager: 14082

Email: sylvie@steele.mgh.harvard.edu or christina@steele.mgh.harvard.edu

3. "Notice: Keep Frozen", "Class 9 – Dry Ice" stickers or "Keep Refrigerated", Upright arrows, and "Diagnostic Specimens – Not restricted, Packed in Compliance with IATA Packing Instruction 650".

4.3 Summary Shipping Task List

The following summarizes the tasks to be completed by the site for a scheduled shipment:

- Prepare transmittal paperwork and retain copies at the ACRIN 6689 site.
- **PS- Plasma Specimen Shipment Form:** This form is used to track specimens submitted to the Testing Core Facility. A copy of the tracking form should be faxed to the ACRIN Data Management center as notification of the specimen shipment.
- Send a notification e-mail or FAX to the Testing Core Facility listing the items being shipped, including: the FedEx tracking number, total number of plasma tubes in the shipment, and the expected date of arrival. Please note "**ACRIN 6689 Specimen Shipment—Site Name.**" in the e-mail or FAX 'Subject' line.
- Pack the plasma specimens according to instructions above.
- Label each shipping container with the FedEx shipping label and affix all appropriate shipping labels.
- Maintain a copy of the transmittal log at the site.

CHECKLIST CRITERIA FOR BLOOD DRAW:

- 1. The site has appropriate personnel for drawing blood samples**
- 2. The site has appropriate facilities for processing blood samples into fractionates**
 - a. LIM lab or equivalent**
- 3. The site has appropriate personnel for separating plasma specimens**
- 4. The site has -80°C storage capability**