CORRELATIVE SCIENCE PROCEDURE MANUAL

1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens from all patients enrolled or registered on A082002. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the Alliance Biorepository (i.e. Siteman Cancer Center Tissue Procurement Core at Washington University), prior to their use for protocol-specified and future, unspecified correlative science research studies. This document should be used by staff involved with any aspect of the A082002 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A082002 only. Please refer to the trial protocol-specific language for additional details regarding eligibility, participant enrollment, data submission, and specific procurement procedures. **Please ensure that you are reading the most updated version of this document. This document may experience minor updates, revisions, and clarifications independent of a formal protocol amendment. The most recent version of this document may be found on the Alliance website and CTSU.**

3. Definitions

Term	Definition
ABWUSTL	Alliance Biorepository at Washington University in St. Louis
FFPE	Formalin fixed, paraffin embedded

4. Contact Information

4.1 For questions and problems related to protocol administration, eligibility, patient registration, and data submission, relevant contact information is listed on protocol pages 1 and 2.

4.2 For information on using the BioMS system, please refer to the 'Help' links on the BioMS webpage to access the online user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org.

4.3 For all other questions regarding biospecimen procurement and shipping procedures, please contact the Alliance Biorepository Program Manager: 1-314-747-4402 or alliance@email.wustl.edu.

5. Site Preparation

5.1 Please refer to A082002 protocol document for any specific requirements related to patient enrollment, registration, and regulatory compliance.

5.2 Please ensure that you have appropriate log on credentials and can successfully access the BioMS application. The BioMS application is used for logging the collection and shipment of biospecimens to the Alliance Biorepository at Washington University. For training and assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or bioms@alliancenctn.org.

6. Collection Schema

The following biospecimens are to be collected at each of the time points. Please refer to biospecimen collection and processing methods and specific shipping procedures below.

Time Point	Biospecimen	Quantity	Collection / Processing Method	Shipping	Notes
For patients registered to A082002-ST1					
After consent, prior to treatment	Fixed tissue block	1 block	Fixed tissue block (9.2)	Ambient	1, 2
After consent, prior to treatment	Whole blood for plasma	12 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1
After consent, prior to treatment	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.2)	Ambient	1, 3
Prior to treatment on Day 1 of Cycle 3	Whole blood for plasma	12 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1
Prior to treatment on Day 1 of Cycle 3	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.2)	Ambient	1, 3
Prior to treatment on Day 1 of Cycle 5	Whole blood for plasma	12 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1
Prior to treatment on Day 1 of Cycle 5	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.2)	Ambient	1, 3
Prior to treatment on Day 1 of Cycle 7	Whole blood for plasma	12 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1
Prior to treatment on Day 1 of Cycle 7	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.2)	Ambient	1, 3

Prior to treatment on Day 1 of Cycle 9	Whole blood for plasma	12 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1
Prior to treatment on Day 1 of Cycle 9	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.2)	Ambient	1, 3
End of treatment	Whole blood for plasma	12 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	1, 4
End of treatment	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.2)	Ambient	1, 3, 4

Notes:

1. Collection is optional for patients but requires all sites offer to patients during consent. Please see protocol-specific consent documents.
2. A paraffin tissue block from a diagnostic core biopsy should be submitted for patients opting in for A082002-ST1.
3. Whole blood (EDTA) for PBMC isolation and cryopreservation at the Biorepository.
4. Once a patient discontinues therapy, obtain an end of treatment sample, if possible.

7. Biospecimen Collection Kits

7.1 Blood Specimens

7.1.1 There are no “kits” provided for submission of blood specimens for this study. Sites are responsible for acquiring materials for collection and shipping of these specimens to the Biorepository.

7.2 Tissue Specimens

7.2.1 There are no “kits” provided for submission of the paraffin block for this study.

7.2.2 Blocks should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam container.

7.2.3 During warm weather months, blocks should be shipped in an insulated container that contains a refrigerant pack, to avoid heat > 25 degrees C (77 degrees F) that may melt paraffin and damage the tissue specimens.

7.2.4 Please see Section 11 – Biospecimen Shipping for specific instructions on shipping to ABWUSTL.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (cryovials and vacutainer tubes) MUST be labeled with the participant study number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type (e.g. “plasma”).

8.2 Surgical pathology tissue blocks should not be labeled in any manner. The institutional surgical pathology number (e.g. “S16-1234”) and the individual block identifier (e.g. “A3”) should be readable on the block. Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the block submitted to **ABWUSTL**. Please ensure the institutional surgical pathology number and block ID are maintained on the surgical pathology report. See **section 9** for additional details.

8.3 Label all containers and vials with an indelible, solvent-resistant marker when they are at ambient temperature.

8.4 Do not affix any labels to vials, slides or tubes. Label the collection containers directly with the marking pen.

8.5 All biospecimens that are collected and sent to the Alliance Biorepository must be **logged and tracked in BioMS**. The BioMS system is a web-based application that tracks the collection and shipping of biospecimens. Once individual biospecimens are logged and 'shipped' in the BioMS system, a packing manifest will be created by the system. This manifest must be printed out and must accompany all biospecimen shipments. To become familiar with the BioMS system and for further information about training, access, and use, please contact the BioMS Help desk at: 1-855-55-BIOMS or bioms@alliancenctn.org.

8.6 In the event that BioMS cannot be accessed, please complete a BioMS Specimen Log and Shipping Manifest form which can be found here- **http://tinyurl.com/alliance-bioms-contingency**.

9. Tissue Collection

9.1 Overview.

9.1.1 Please refer to protocol-specific instructions for procedures related to actual tissue procurement from individual participants. The method for research tissue procurement is dependent upon the disease site and the individual patient.

9.1.2 When procuring tissue biospecimens by any method, when possible, avoid tissue that is grossly necrotic, hemorrhagic, fatty, or fibrous. If in doubt, briefly (1 min or less) place the tissue segment in a sterile specimen cup containing physiologic (normal) saline to rinse the tissue. Necrotic, hemorrhagic, and fatty tissue will generally dissolve or float on the surface while tumor and parenchymal tissue will remain intact and sink to the bottom of the cup.

9.2 Diagnostic Fixed Tissue Block

9.2.1 For patients who consent to A082002-ST1, a representative diagnostic core biopsy block should be submitted, if applicable.

9.2.2 Any clinical surgical pathology block that is submitted for research studies will not be exhausted or rendered unsuitable for future diagnostic use. Any clinical surgical pathology block that is submitted will be returned within ten working days of written request, when needed for clinical management or clinical trial enrollment for a specific patient. Otherwise, all blocks will be returned to the submitting institution when the trial and correlative science study end points have been met.

10. Blood Collection Methods

10.1 Plasma Processing

10.1.1 Collect 30 ml of whole blood by standard venous phlebotomy technique into the purple top (EDTA) tubes. Invert tubes 10 times.

10.1.2 Within 2 hours of collection, spin the vacutainer tubes at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.

10.1.3 Carefully remove the plasma layer from each tube (~4 ml each), without touching the white, buffy coat layer, and transfer to new 15 ml conical centrifuge tubes.

10.1.4 Spin the centrifuge tubes containing plasma at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.

10.1.5 Label 4 cryovials per tube of blood collected (i.e. if 3 x 10 mL collected, label 12 cryovials) as instructed in **section 8.** Make certain each vial is labeled completely and identically.

10.1.6 Carefully remove plasma (without touching the pellet) and aliquot 1 ml into each of the labeled cryovials.

10.1.7 Freeze plasma containing cryovials on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees Celsius until ready for shipment on dry ice. Frozen plasma should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

10.2 Whole blood (EDTA- no processing)

10.2.1 Collect whole blood by standard venous phlebotomy technique into each of the EDTA tubes. Invert tubes 10 times.

10.2.2 Store EDTA tubes with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tubes. **Blood should be collected Monday—Thursday only. Due to required processing, the tubes MUST be received at the Biorepository within 24 hours of collection.** Ensure that the EDTA tubes are shipped at ambient temperature to avoid freezing. **During warm weather months (i.e. June—August), a refrigerated pack (not frozen) should be included in the shipment to maintain ambient temperature. When shipping during other months of the year, a room temperature pack should be included in the shipment.**

11. Biospecimen Shipping

11.1 Overview

11.1.1 Frozen plasma aliquots should be placed in a biohazard bag inside of a Styrofoam cooler and covered with 3 to 4 lbs (2 kg) of commercially-prepared dry ice. Pellets or chunks are preferred. Make sure the box is filled with dry ice and the weight of the dry ice is noted on the dry ice label on the outside of the shipping container. It is the local sites' responsibility to obtain dry ice when shipping frozen specimens. Specimens should be shipped according to IATA guidelines. **Frozen aliquots should be shipped to the Biorepository within 30 days of collection. Batch shipment of frozen aliquots is allowed.**

11.1.2 A completed copy of the BioMS packing manifest must accompany all shipments. Do not send specimens without a completed BioMS Packing Manifest or substitute "BioMS Downtime Form." Biospecimens cannot be accepted without this completed form.

11.1.3 If sending tissue, include a copy of the de-identified surgical pathology report.

11.1.4 **<u>Biospecimens should be shipped Monday—Thursday only. Do not ship on Friday, Saturday, Sunday or the day before a nationally recognized holiday.</u>**

11.2 Shipping to ABWUSTL

11.2.1 Ship container according to IATA guidelines and standard institutional policies via **<u>FedEx priority overnight shipping.</u>**

Ship to:

Alliance Biorepository
c/o Siteman Cancer Center Tissue Procurement Core
Washington Univ. School of Medicine
425 S. Euclid Ave.
Room 5120
St. Louis, MO
63110-1005
Phone: 314-454-7615

12. ABWUSTL Biospecimen Receipt and Quality Assurance Measures

12.1 Upon receipt, all biospecimens will be accessioned into the TPC informatics system, OpenSpecimen.

12.2 All biospecimens will be logged, associated, and tracked by the unique patient biopsy control number.

12.3 Each individual biospecimen will receive and be physically labeled with a unique biospecimen identifier, associated with the biopsy control number in the TPC informatics system.

12.4 Upon receipt, all physical biospecimens received will be reconciled with what is recorded on the BioMS packing manifest. Any discrepancies noted will be communicated to the Program Manager who will contact the submitting site for reconciliation.

12.5 Upon receipt, any biospecimen received that is not in appropriate physical condition (broken vials, frozen samples that are thawed, ambient samples that are frozen) will be reported to the Program Manager, who will contact the submitting site for reconciliation.

12.6 Frozen aliquoted biofluids will be stored under liquid nitrogen vapor.

12.7 All biospecimens will remain in storage until additional processing or review is requested in writing by the appropriate protocol PI.

13. Document History

Version	Description and Justification of Change	Author	Effective Date
2.0	Removed alternatives to tissue block submission to align with protocol	PAA	12/08/2021
1.0	New	PAA	10/19/2021