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 A012103. 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 A012103 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A012103 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
H&E	Hematoxylin and Eosin

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 the A012103 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**.**

5.3 Identify a reliable source of dry ice for freezing and shipping biospecimens and a -70 to -90 degree Celsius freezer ("ultralow") in which frozen biospecimens may be stored prior to shipment.

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
Mandatory for all patients registered to A012103					
≤7 days after registration	Fixed tissue blocks	1 block from diagnostic core biopsy **AND** 1 block from surgery	Fixed tissue block (9.2)	Ambient	1
≤7 days after registration	H&E stained slides **AND** Tumor tissue scrolls	1 H&E stained slide **AND** 10 x 10 micron scrolls from diagnostic core biopsy **AND** 1 H&E stained slide **AND** 10 x 10 micron scrolls from surgery	H&E stained slide **AND** Fixed tissue scrolls (9.3)	Ambient	1
≤7 days after registration	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2
≤7 days after registration	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	2
27 weeks/End of treatment or observation	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2
27 weeks/End of treatment or observation	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	2
6 months after end of treatment or observation	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2

6 months after end of treatment or observation	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	2
3 years after registration	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2
3 years after registration	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	2
At recurrence (if applicable)	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2
At recurrence (if applicable)	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.2)	Dry Ice	2
At recurrence (if applicable)	Fixed tissue block	1 block	Fixed tissue block (9.2)	Ambient	3
At recurrence (if applicable)	H&E stained slide **AND** Tumor tissue scrolls	1 H&E stained slide **AND** 10 x 10 micron scrolls	H&E stained slide **AND** Fixed tissue scrolls (9.3)	Ambient	3
For patients consented to A012103 optional biobanking					
At recurrence (if applicable)	Whole blood (EDTA tube)	3 x 10 ml	Whole blood- EDTA tube (10.3)	Ambient	4, 5

Notes:

1. A representative, archived tumor tissue block from both diagnostic core biopsy **AND** from surgery should be submitted, if available. If entire tissue block cannot be submitted, one H&E stained slide **AND** ten (10 um) serial tissue scrolls from each block will be accepted as an alternative. If tissue is limited, please submit H&E and as many tissue scrolls as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**
2. A total of 20 mL of peripheral blood in EDTA tubes will be collected. Please submit plasma (6 x 1 ml aliquots) and buffy coat (2 aliquots), processed and frozen on site and shipped on dry ice for ct-DNA analysis and storage at ABWUSTL.
3. Tumor tissue block from a site of tumor recurrence (when applicable), only if tissue was collected per standard of care. No new research biopsy is required. If entire tissue block cannot be submitted, one H&E stained slide **AND** ten (10 um) serial tissue scrolls will be accepted as an alternative. If tissue is limited, please submit H&E and as many tissue scrolls as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**

4. Collection is optional for patients but all sites must ask patients for their consent to participate. Please see protocol-specific consent documents.
5. Whole blood sample in EDTA tubes will be collected for PBMC isolation and cryopreservation at ABWUSTL. **Blood should be collected Monday through Thursday only.** Due to required processing, the tubes MUST be received at the Biorepository within 24 hours of collection.

7. Biospecimen Collection Kits

7.1 There are no "kits" provided for submission of blood or tissue specimens for this study. Sites are responsible for acquiring materials for collection and shipping of these specimens to the Biorepository.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (cryovials, EDTA tubes, and tissue bags) MUST be labeled with the Alliance patient ID number, patient initials (Last, First, Middle), the date and time (if applicable) of collection, and specimen type (e.g.. plasma, "buffy coat").

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. If tissue scrolls are being submitted instead of a block, each tube containing scrolls should be labeled with the Alliance patient ID number, institutional surgical pathology number, the block identifier, section thickness, and the serial section number (if applicable).

8.3 A **de-identified copy of the surgical pathology report**, labeled with the Alliance patient ID number, is required to accompany **all** tissue submissions. Usually, this is generated by obscuring all PHI (names and dates) with white-out or a black magic marker, labeling each page of the report with the Alliance patient ID number, and photocopying the report. However, please make sure to **maintain the pathology accession numbers** so the submitted block can be matched directly to the pathology report.

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

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

8.6 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.

8.7 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.

a. Please refer to protocol-specific instructions for procedures related to actual tissue procurement from individual participants. The method for tissue procurement (needle core biopsy, sampling of surgically resected tumor, or tumor 'debulking') is dependent upon the disease site and the individual patient.

b. 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 Blocks

a. A representative, archived tumor tissue block from both diagnostic core biopsy and from surgery should be submitted, if available. A tissue block from a site of tumor recurrence is also requested, if applicable.

b. 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 endpoints have been met.

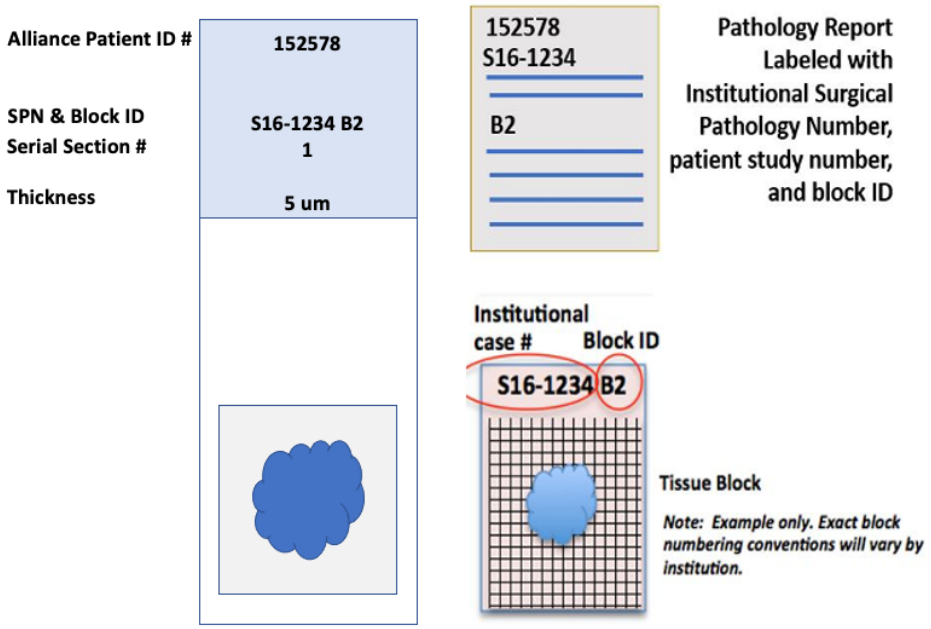
c. In the event that an institution will not release fixed tissue blocks, the institution may instead submit 1 H&E stained slide **AND** tissue scrolls from each of the requested blocks as an alternative (see **section 9.3**). **BLOCK SUBMISSION IS STRONGLY PREFERRED.**

d. Include a de-identified copy of the surgical pathology report with all tissue submissions.

9.3 H&E Stained Slide and Fixed Tissue Sections

a. In cases where an institution is unwilling or unable to submit a tissue block, a single H&E stained slide for reference and serial tissue sections (scrolls, ribbons, curls) from the same block may be submitted.

b. Cut and perform routine H&E stain on a single section from the tumor tissue block. See figure below for proper mounting and labeling.



c. Cut a ribbon (scroll) of 10 paraffin tissue sections at 10 microns. Place the ribbon of tissue directly into a single microcentrifuge tube or any other suitable container. Do not float the tissue ribbon or sections in a water bath. Label the tube of tissue following the guidelines outlined above.

d. Include a de-identified copy of the surgical pathology report with all tissue submissions.

10. Blood Collection Methods

10.1 Plasma Processing

a. Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the purple top (EDTA) tubes. A total of 20 ml of whole blood should be collected into the EDTA tubes (2 x 10 ml). Following collection, invert tubes 10 times.

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

c. Carefully remove the plasma layer from each vacutainer tube (~3—5 ml in volume per tube), without touching the white, buffy coat layer, and transfer to new 15 ml conical centrifuge tubes. Keep the vacutainer tubes containing the white, buffy coat layers for white blood cell isolation (**section 10.2**).

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

e. Label 6 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically. Please use 2 ml cryovials with threaded caps. Do not use flip-top tubes or cryovials larger than 2 ml.

f. Carefully remove 6 ml of plasma (without touching the pellet) and divide into six (6) 2 ml labeled cryovials. Each aliquot should be between 1—1.5 ml in volume.

g. Freeze plasma containing cryovials on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees C 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 "Buffy Coat" (White Blood Cell) Processing

a. Follow procedures in **section 10.1** for collecting and processing plasma from EDTA tubes.

b. Label 2 cryovials as instructed in **section 8**. Please use 2 ml cryovials with threaded caps. Do not use flip-top tubes or cryovials larger than 2 ml.

c. After removing the plasma, carefully remove the white, "buffy coat" white blood cell layer, avoiding the red blood cell mass as much as possible.

d. Transfer the buffy coat layer (approximately 0.2 – 0.5 ml) from EDTA tubes into the labeled cryovials. Immediately freeze the cryovials of buffy coat on dry ice or in liquid nitrogen vapor. Do NOT freeze buffy coat cells by placing a warm tube in a -70 to -90 degree Celsius ultralow freezer. Once completely frozen, the cryovials containing the buffy coat cells may be stored at -70 to -90 degrees C until ready for shipment on dry ice. Frozen buffy coat should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

10.3 Whole blood (EDTA- no processing)

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

b. 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

a. Place the original, completed copy of the BioMS packing manifest in the shipment. If sending tissue, include a de-identified copy of the surgical pathology report labeled with the Alliance patient ID number. Do not send specimens without a completed BioMS Packing Manifest or substitute "BioMS Downtime Form." Biospecimens cannot be accepted without this completed form.

b. All biospecimens should be shipped within the timeframes indicated above in **section 9** and **10.** If collected biospecimens cannot be shipped within the specified timeframe (e.g. Friday – Saturday or Holiday collections), please contact the Alliance Biorepository Program Manager: 1-314-747-4402 or alliance@email.wustl.edu for further instructions, at least 24 hours prior to anticipated collection.

c. **Do not ship on Friday, Saturday, Sunday or the day before a nationally recognized holiday.**

11.2 Shipping to ABWUSTL

Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor.
Ship to:

Alliance Biorepository at Washington University in St. Louis
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 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
1.1	Changed wording of tissue sections to scrolls	KAL	3/24/2023
1.0	New	AAW	02/27/2023