CORRELATIVE SCIENCE PROCEDURE MANUAL

1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens from patients enrolled or registered who have consented to participate in A021804. 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 A021804 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A021804 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 on-line 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 A021804 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 individual biospecimen collection and processing methods and specific shipping procedures below.

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping	Notes
Mandatory for all patients registered to A021804						
Prior to initiation of treatment	N	Whole blood for plasma	12 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	1
Prior to initiation of treatment	N	Whole blood for "buffy coat"	3 aliquot	"Buffy Coat" (10.2)	Dry Ice	1
For patients registered to A021804 Biobanking						
Prior to initiation of treatment	N	Fixed tissue block	1	Fixed tissue block (9.2)	Ambient	2, 3
Prior to initiation of treatment	N	Unstained tumor tissue slides	10	Unstained tissue slides + H&E stained slide for tissue QA (9.3)	Ambient	2, 3
At Progression	N	Whole blood for plasma	12 x 1 ml aliquots	Frozen Plasma (10.1)	Dry Ice	1, 2
At Progression	N	Whole blood for "buffy coat"	3 aliquot	"Buffy Coat" (10.2)	Dry Ice	1, 2

Notes:

1. Peripheral blood (EDTA) 3 x 10 ml to be processed for plasma (1 ml aliquots) and "buffy coat," frozen on site and shipped on dry ice.
2. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.
3. A paraffin tissue block with adequate tumor **OR** 10 unstained slides **AND** 1 H&E stained slide from such block should be submitted for patients opting in for A021804 biobanking, if available. If fewer than 10 unstained slides can be submitted, please prioritize the H&E stained slide and submit as many unstained slides as 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 paraffin blocks or slides for this study.

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

7.2.3 During warm weather months, paraffin blocks and slides 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.

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

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (cryovials, tissue bags) MUST be labeled with the participant study number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type.

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 sections are being submitted instead of the block, each tissue section slide should be labeled with the patient study number, institutional surgical pathology number, the block identifier, and the serial section number. Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or slides 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 (needle core biopsy, sampling of surgically resected tumor) 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 Pathology Fixed Tissue Blocks

9.2.1 For patients who consent to A021804 biobanking, one representative diagnostic block from the resection of primary tumor should be submitted prior to initiation of treatment.

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.

9.2.3 In the event that an institution will not release tissue blocks, the institution may instead submit one (1) H&E stained slide and ten (10) unstained slides. Please refer to section 9.3 for more details.

9.3 Unstained Tissue Slides from Diagnostic Fixed Tissue Block

9.3.1 In cases where an institution is unwilling or unable to submit tissue blocks, a set of ten (10) unstained slides from a single block with adequate tumor cellularity may be sent as an alternative. **An H&E stained slide should accompany the unstained slides. The H&E stained slide should be cut from the same block from which the unstained slides were cut.** If fewer than 10 unstained slides from a block can be submitted, please submit as many as possible (up to 10 slides). Please follow the procedures below for submitting unstained slides. If your pathology department is unwilling or unable to follow these tissue-sectioning instructions, please consider submission of tissue blocks, which can be cut at the biorepository and returned to your institution at a later date.

# of slides	Section thickness	Slide type	Purpose
1	5 micron	Charged	H&E stained slide
10	10 micron	Non-Charged	DNA, RNA

9.3.2 Serial tissue sections should be cut fresh from the appropriate formalin fixed, paraffin embedded tissue block.

9.3.3 Cut sections at 5 micron or 10 micron thickness onto glass slides (charged or non-charged) as indicated above.

9.3.4 Ensure that each slide is labeled with the patient study number, the institutional surgical pathology number and block ID, and the slide serial section number (1, 2, 3, etc.).

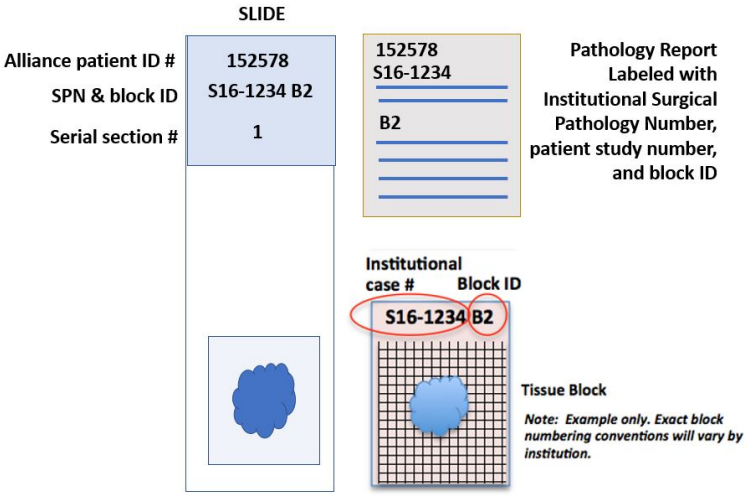
9.3.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

9.3.6 No adhesives or other additives should be used in the water bath.

9.3.7 Mount only one tissue section per slide. Make certain sections are placed on the painted / textured side of the slide.

9.3.8 When placing the sections onto the slides, ensure that the tissue is placed on the bottom third of the slide. Ensure that each serial section from the block is placed in the same orientation on each slide.

9.3.9 See figure below for proper mounting and labeling.



9.3.10 Air dry slides for 12-24 hours prior to shipping. Do not oven dry slides.

9.3.11 Use slide mailers or a slide box to ship slides. Slides should not be touching each other. Ensure that slides from only one patient are placed in one slide mailer.

9.3.12 Include a copy of the **de-identified pathology report with all slide submissions.**

10. Blood Collection Methods

10.1 Plasma Processing

10.1.1 Collect 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. Keep the vacutainer tube containing the white, buffy coat layer for white blood cell isolation **(section 10.2)**.

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 in 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

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

10.2.2 Label cryovials as instructed in section 8 (1 cryovial for each EDTA tube collected).

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

10.2.4 Transfer the buffy coat layer (approximately 0.2 – 0.5 ml) from each EDTA tube into the corresponding, labeled cryovial. 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 Celsius until ready for shipment on dry ice. Buffy coat should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

11. Biospecimen Shipping

11.1 Overview

11.1.1 Frozen plasma and buffy coat 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 **Biospecimens should be shipped Monday—Thursday only. Do not ship on Friday, Saturday, Sunday or the day before a nationally recognized holiday.**

11.2 Shipping to ABWUSTL

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

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
1.0	New	PAA	03/03/2020