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 A091902. 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 A091902 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A091902 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 A091902 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 both requesting biospecimen collection kits and 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 Prior to collection of blood biospecimens, a biospecimen collection kit must be at the collection site. Please see section 7 for requesting biospecimen collection kits. Please allow at least 10 working days to receive the collection kit.

5.4 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 individual collection kit instructions, biospecimen collection and processing methods, and specific shipping procedures below.

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping	Notes
Mandatory for <u>all</u> patients registered to A091902						
Baseline (≤ 14 days after registration)	N	Local diagnostic slides	Case dependent	Stained tissue slides (9.2)	Ambient	1
Baseline (≤ 14 days after registration)	N	Unstained tumor tissue slides	5	Unstained tissue slides (9.3)	Ambient	1
A091902 Biobanking						
Baseline (≤ 14 days after registration)	N	Fresh tissue research biopsy	2 x 4 mm skin punches (1 flash frozen; 1 fixed tissue block)	Research biopsy frozen tissue (9.4); Research biopsy fixed tissue block (9.5)	Dry Ice (Frozen tissue) / Ambient (Fixed tissue block)	2, 3
Baseline (≤ 14 days after registration)	N	Fixed tumor tissue block	1	Fixed tissue block (9.6)	Ambient	2
Baseline (≤ 14 days after registration)	Y	Whole blood for plasma	4 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2, 4
Baseline (≤ 14 days after registration)	Y	Whole blood (Streck BCT)	2 x 8.5 ml	Plasma for cfDNA (10.2)	Ambient	2, 5
Baseline (≤ 14 days after registration)	Y	Whole blood (ACD)	3 x 10 ml	Whole blood-ACD tubes (10.3)	Ambient	2, 6
Cycle 4, Day 1	Y	Whole blood for plasma	4 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2, 4
Cycle 4, Day 1	Y	Whole blood (Streck BCT)	2 x 8.5 ml	Plasma for cfDNA (10.2)	Ambient	2, 5
Cycle 4, Day 1	Y	Whole blood (ACD)	3 x 10 ml	Whole blood-ACD tubes (10.3)	Ambient	2, 6

Cycle 4 or first restaging	N	Fresh tissue research biopsy	2 x 4 mm skin punches (1 flash frozen; 1 fixed tissue block)	Research biopsy frozen tissue (9.4); Research biopsy fixed tissue block (9.5)	Dry Ice (Frozen tissue) / Ambient (Fixed tissue block)	2, 3
Progression	Y	Whole blood for plasma	4 x 1 ml aliquots	Frozen plasma (10.1)	Dry Ice	2, 4
Progression	Y	Whole blood (Streck BCT)	2 x 8.5 ml	Plasma for cfDNA (10.2)	Ambient	2, 5
Progression	Y	Whole blood (ACD)	3 x 10 ml	Whole blood-ACD tubes (10.3)	Ambient	2, 6

Notes:

1. Submission of all local diagnostic slides including, but not limited to H&E stained slide, is required for retrospective central pathology review to confirm local diagnosis. In addition to stained diagnostic slides, five (5) unstained slides from diagnostic tumor tissue must also be submitted.
2. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.
3. Fresh tissue research biopsy should be submitted for cutaneous angiosarcoma patients only. Two (2), 4 mm skin punches are requested. One should be formalin fixed and embedded into paraffin (fixed tissue block) and the other should be flash frozen.
4. Peripheral blood (EDTA) for plasma (4 x 1 ml aliquots), processed and frozen on site and shipped on dry ice.
5. Whole blood (Streck BCT) for isolation of “buffy coat” and cell free DNA from plasma (2 x 8.5 ml).
6. Whole blood (ACD) 3 x 10 ml for PBMC isolation and cryopreservation at the biorepository.

7. Biospecimen Collection Kits

7.1 To facilitate the proper collection and shipping of blood biospecimens, biospecimen collection kits and materials will be provided. The cost of the kit and shipping the kit to the site will be paid for. The institution is expected to pay for shipping the kit with the biospecimens back to the Alliance Biorepository at Washington University in St. Louis via priority overnight shipping.

7.2 Kits should be requested at least 10 working days in advance of the anticipated collection date. As many as 2 kits can be requested at one time. Since the collection materials (vacutainer tubes) have expiration dates, do not request kits more than 90 days prior to their anticipated use. All kits must be requested by using the BioMS system.

7.3 Kit contents and specific instructions for use of the kit are provided in the kit box.

7.4 Once a kit is received, do not discard the outer cardboard overwrap. The kit, containing biospecimens, is to be shipped back in the same box.

7.5 Please return all components of the kit, regardless of whether they have been used or not. Kits and kit components are recycled when possible, minimizing the kit cost.

7.6 Well in advance of collecting biospecimens, inspect the biospecimen collection kit to ensure that all components are present and not expired, particularly if the kit has been onsite for longer than 30 days.

7.7 Note that individual kit components that are expired, damaged, or missing cannot be replaced. The remedy is to order a complete, new kit (Please note in your request that you are replacing an expired or damaged kit).

7.8 Please return all kits that have expired or missing components. Return the ENTIRE kit using the cheapest possible shipping method at your expense. DO NOT DISCARD kits that have missing or expired components. Recycling kits keeps the cost of kit materials to a minimum. Please note that all out-going and in coming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

7.9 If a biospecimen collection component (e.g. vacutainer collection tube) is missing, damaged, or expired, the institution may substitute a like-kind collection tube from their own supply. However, note that while some kit components are generic (i.e. EDTA tubes), others are highly specialized (e.g. Streck BCT) and probably are not available at the institution.

7.10 Note that protocol requirements are based on blood volumes, not tube sizes. If the protocol requires the collection of 8 cc of whole blood, generally a 10 cc tube is provided in the kit for convenience. If desirable or necessary to collect 8 cc in 3 x 3 cc tubes (for example), that is permissible.

7.11 No kits are provided for submission of mandatory tissue slides, optional tissue blocks, or optional research tissue biopsies to ABWUSTL. Frozen tissue should be packed in an insulated container according to IATA guidelines and standard institutional policies. Paraffin blocks or slides cut from such blocks should be sent independently of other biospecimens using the following guidelines:

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

7.11.2 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.

7.11.3 Blocks and slides may be shipped for standard overnight delivery according to institutional policies and using the preferred vendor.

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

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (vacutainer tubes, cryovials, and 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 (i.e. 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. If tissue sections or cores are being submitted instead of the block, each tissue section slide or tube should be labeled with the patient study number, institutional surgical pathology number, the block identifier, and the serial section number (if applicable). Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the blocks or slides submitted. 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, or tumor 'debulking') 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.

9.1.3 Fresh tissue biopsy biospecimens collected for this protocol are for research purposes only and cannot be used for clinical management. Ensure that all tissue biospecimens needed for routine diagnostic standard of care are collected and deemed adequate PRIOR to submitting tissue specimens for this research study. No clinical pathology diagnosis will be rendered on submitted tissue biospecimens. Research tissue biospecimens (unlike diagnostic surgical pathology blocks- see section 9.6) will not be returned to the institution.

9.2 Local Diagnostic Stained Tissue Slides

9.2.1 Submission of all local diagnostic slides from tumor tissue is required for retrospective central pathology review to confirm local diagnosis. Sites should submit a H&E stained slide and any others produced for histopathologic diagnosis to the Biorepository.

9.2.2 Any stained slide submitted must be labeled as indicated in section 9.3.9.

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

9.3 Unstained Slides from Diagnostic Fixed Tissue Blocks

9.3.1 In addition to the local diagnostic slides, a set of 5 unstained slides from a diagnostic tumor tissue block must also be submitted. Please follow the procedures below for submitting unstained tissue slides.

# of slides	Section thickness	Slide type	Purpose
5	4-6 micron	Positively Charged	Retrospective central pathology review

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 4-6 micron thickness as indicated onto positively charged slides.

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.).

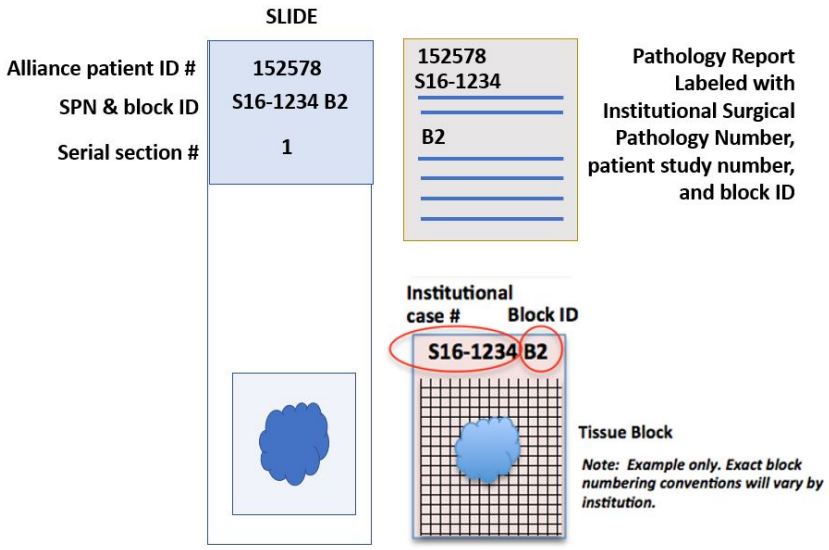
9.3.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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 a de-identified pathology report with all slide submissions.

9.4 Fresh Tissue Research Biopsy-Frozen Tissue

9.4.1 For patients with cutaneous angiosarcoma who have consented to a fresh tissue research biopsy, 1 x 4 mm skin punch should be submitted as flash frozen tissue. The frozen tissue punch should be submitted in addition to the fixed tissue research biopsy block **(see section 9.5).**

9.4.2 Prior to procurement, prepare tissue for freezing by placing approximately six pounds of crushed dry ice into the bottom compartment of a Styrofoam cooler. Place a metal freezing plate on top of the dry ice and allow the surface of the plate to reach the approximate temperature of the dry ice.

9.4.2.1 An alternative method is to use the freezing plate found on a pathology cryostat.

9.4.2.2 An alternative method is to use a flat surface of a dry ice block.

9.4.2.3 An alternative method is to use a commercially available Cryocooler (OPS Diagnostics) which uses a metal platform and a liquid nitrogen saturated “pillow” to achieve freezing temperatures of -130 degrees C.

9.4.2.4 Do not freeze tissue by placing warm tissue in a -70 to -90 degree Celsius ultralow freezer.

9.4.2.5 Do not freeze tissue using a dry ice ethanol bath.

9.4.2.6 Do not freeze tissue by submersion in an isopentane cryobath.

9.4.3 Label one tissue cryomold for the tissue punch that is to be frozen. Ensure that the cryomold and tissue bag are labeled with the participant study number as instructed in section 8.

9.4.4 Working quickly, gently place the tissue length-wise in the mold. Place the cryomold on the level cold plate or flat, level surface of dry ice. Allow the tissue to freeze for 3-5 minutes.

9.4.5 Record the time when the tissue sample is first procured from the patient and the time at which the individual tissue segment is frozen. This time difference is warm ischemia time (WIT). The WIT should be less than 30 minutes, but even if it is not, accurate record keeping of this time is essential.

9.4.6 Once frozen, quickly wrap the mold with the tissue block in cooled foil and place the block in the corresponding labeled tissue bag. Maintain the tissue block buried in dry ice, in a -70 to -90 degree C freezer, or in liquid nitrogen vapor (not liquid phase) until ready for shipment.

9.5 Fresh Tissue Research Biopsy- Fixed Tissue Block

9.5.1 For patients with cutaneous angiosarcoma who have consented to a fresh tissue research biopsy, 1 x 4 mm skin punch should be formalin fixed and embedded into paraffin according to standard institutional policies.

9.5.2 This fixed tissue block should be submitted in addition to the flash frozen tissue **(see section 9.4).**

9.6 Diagnostic Pathology Fixed Tissue Block

9.6.1 This protocol requests submission of one representative, diagnostic pathology, formalin fixed paraffin embedded tumor tissue block for patients consenting to biobanking.

9.6.2 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 whole blood by standard venous phlebotomy technique into the purple top (EDTA) tube. Invert tube 10 times.

10.1.2 Within 30 minutes of collection, spin the vacutainer tube at room temperature in a clinical centrifuge at 2500 xG for 15 minutes.

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

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

10.1.5 Label 4 cryovials as instructed in section 8. Make certain each vial is labeled completely and identically.

10.1.6 Carefully remove 4 ml of plasma (without touching the pellet) and divide into 4, 1 ml 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 C until ready for shipment on dry ice.

10.2 Plasma Nucleic Acid (Streck) Tube Processing

10.2.1 Collect 8.5 ml of blood into each of the Streck BCT tubes using standard venous phlebotomy. Invert tubes 10 times.

10.2.2 Store Streck tubes with whole blood at room temperature. Do not freeze or refrigerate the tubes. The tubes must be received at the Biorepository within 24 hours of collection. Ensure that the Streck tubes are shipped at ambient temperature to avoid freezing.

10.3 Whole blood (ACD- no processing)

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

10.3.2 Store ACD tubes with whole blood at room temperature. Do not freeze or refrigerate the tubes. The tubes must be received at the Biorepository within 24 hours of collection (**i.e. Friday, Saturday, or holiday collections are not allowed).** Ensure that the ACD tubes are shipped at ambient temperature to avoid freezing.

11. Biospecimen Shipping

11.1 Overview

11.1.1 Please see the Directions for Use (DFU) document that is included in each kit for specific directions on how to package and ship biospecimens.

11.1.2 Place the original, completed copy of the BioMS packing manifest in the shipment. If sending tissue, include a copy of the de-identified surgical pathology report. 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 All biospecimens should be shipped on the same day that they are collected (Monday – Thursday). Biospecimens must be received by the recipient lab within 24 hours of collection. If collected biospecimens cannot be shipped on the same day that they are collected (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.

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

11.1.5 Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor. A blank FedEx Air Bill is provided with the kit for convenience.

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. 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 tissues and 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	08/14/2020