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 A051901. 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 A051901 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A051901 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
CSF	Cerebrospinal Fluid
FFPE	Formalin Fixed, Paraffin Embedded
LP	Lumbar Puncture

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 A051901 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
For patients who consent to A051901 Biobanking						
Registration	N	Fixed tumor tissue block	1	Fixed tissue block (9.2)	Ambient	1, 2
Registration	N	Fixed tissue cores	2	Fixed tissue cores (9.3)	Ambient	1, 2
Registration	N	Unstained tissue slides	5	Fixed tissue slides (9.4)	Ambient	1, 2
Registration	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient	1, 3
Registration	Y	Whole blood (EDTA)	1 x 10 ml	Whole blood- EDTA tubes (10.2)	Ambient	1
Registration	Y	CSF – frozen supernatant	Up to 10 x 1 ml aliquots	CSF- frozen supernatant (11.1)	Dry Ice	1, 7
Registration	Y	CSF – frozen cell pellet	1	CSF- frozen cell pellet (11.2)	Dry Ice	1, 7
End of Induction Cycle 3	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient	1, 3, 4
End of Induction Cycle 3	Y	Whole blood (EDTA)	1 x 10 ml	Whole blood- EDTA tubes (10.2)	Ambient	1, 4
End of Last Induction Cycle	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient	1, 3, 4
End of Last Induction Cycle	Y	Whole blood (EDTA)	1 x 10 ml	Whole blood- EDTA tubes (10.2)	Ambient	1, 4
End of Last Induction Cycle	Y	CSF – frozen supernatant	Up to 10 x 1 ml aliquots	CSF- frozen supernatant (11.1)	Dry Ice	1, 4, 7
End of Last Induction Cycle	Y	CSF – frozen cell pellet	1	CSF- frozen cell pellet (11.2)	Dry Ice	1, 4, 7
Maintenance Cycle 6	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient	1, 3, 4, 5

Maintenance Cycle 6	Y	Whole blood (EDTA)	1 x 10 ml	Whole blood- EDTA tubes (10.2)	Ambient	1, 4, 5
Maintenance Cycle 6	Y	CSF – frozen supernatant	Up to 10 x 1 ml aliquots	CSF- frozen supernatant (11.1)	Dry Ice	1, 4, 5,7
Maintenance Cycle 6	Y	CSF – frozen cell pellet	1	CSF- frozen cell pellet (11.2)	Dry Ice	1, 4,5,7
Maintenance Cycle 12	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient	1, 3, 4, 5
Maintenance Cycle 12	Y	Whole blood (EDTA)	1 x 10 ml	Whole blood- EDTA tubes (10.2)	Ambient	1, 4, 5
At Progression	Y	Whole blood (Streck BCT)	2 x 10 ml	Plasma for cfDNA (10.1)	Ambient	1, 3, 6
At Progression	Y	Whole blood (EDTA)	1 x 10 ml	Whole blood- EDTA tubes (10.2)	Ambient	1, 6

Notes:

1. Collect and submit only from patients who answer "yes" to model consent question #1. Collection is optional for patients but requires all sites offer patients to consent. Please see protocol-specific consent documents.
2. Submission of an archival tumor tissue block **OR** block alternative (2 x 4 mm fixed tissue cores **AND/OR** 5 x 10 um unstained slides) is requested. **Block submission highly preferred.**
3. Whole blood (Streck BCT) for isolation of cell free DNA from plasma at the Biorepository
4. Collect within 7 days from induction cycle 3 day 14, last day of induction cycle (cycle 6 day 14 or earlier if proceeding with maintenance after at least 4 cycles of induction as per protocol section 7.2), maintenance cycle 6 day 1, maintenance cycle 12 day 1.
5. Patients who discontinue all study treatment due to any of the reasons specified in protocol section 12.2 except for disease or clinical progression will proceed to clinical follow-up. Specimen should be submitted at 6 months and 12 months of clinical follow-up according to months 6 and 12 of maintenance.
6. Progression samples may be collected and submitted up to 1 month after progression.
7. CSF will be spun at the collection site and both pellet and supernatant will be collected and submitted to the Biorepository.

7. Biospecimen Collection Kits

7.1 To facilitate the proper collection and shipping of all 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 incoming 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 Because paraffin blocks, slides cut from such blocks, or fixed tissue cores may be requisitioned and received from the surgical pathology department at a different time than the day of procurement for other biospecimens, paraffin blocks, cut slides, or fixed tissue cores may be sent independently of other biospecimens using the following guidelines:

7.11.1 There is no independent “kit” for submission of paraffin blocks, cores, or slides.

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

7.11.3 During warm weather months, paraffin block, cores, 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.

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

7.12 Please see **Section 12 – Biospecimen Shipping** for specific instructions on shipping to ABWUSTL.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (vacutainer tubes, 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 (i.e. CSF pellet, CSF supernatant).

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 slide 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.

9.1.3 Include a copy of the **de-identified pathology report with all tissue submission.**

9.2 Diagnostic Pathology Fixed Tissue Block

9.2.1 This protocol requests submission of one representative, diagnostic pathology, formalin fixed paraffin embedded tumor tissue block.

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 a tissue block, the institution may instead submit fixed tissue cores **AND/OR** tissue sections, mounted and unstained to glass slides.

9.3 Unstained Slides from Diagnostic Fixed Tissue Block

9.3.1 In cases where institutions are unable or unwilling to submit the requested tissue block, a set of 5 unstained slides **AND/OR** two additional tissue cores may be submitted. See **section 9.4** for core submission. If tissue is limited, please submit as many slides and/or cores as possible. Please follow the procedures below for submitting unstained tissue slides. If your pathology department is unwilling or unable to follow these tissue-sectioning instructions, please consider submission of a tissue block, which can be cut at the Biorepository and returned to your institution at a later date.

# of slides	Section thickness	Slide type	Purpose
5	10 micron	Positively Charged	DNA

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 10 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.).

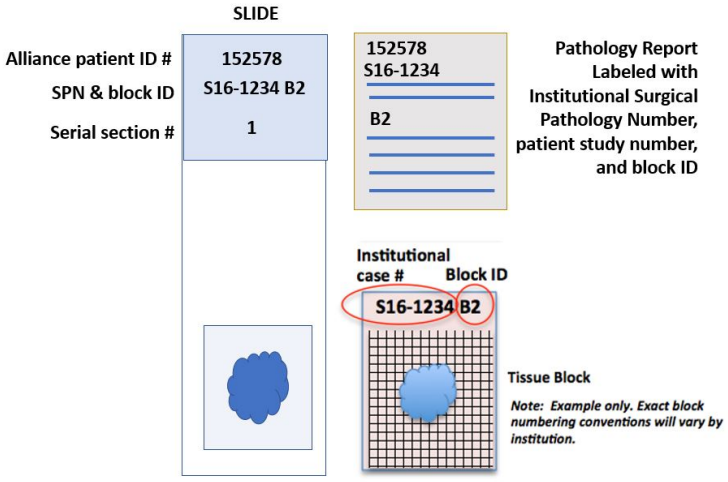
9.3.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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 Tissue Cores from Diagnostic Fixed Tissue Blocks

9.4.1 In cases where an institution is unwilling or unable to submit a tissue block, two (2), 4 mm cores may be submitted **in place of OR in addition to** the unstained tissue slides.

9.4.2 Place the tissue cores directly into a microcentrifuge tube or any other suitable container. Label the tube of tissue following the guidelines outlined in **section 9.3.**

10. Blood Collection Methods

10.1 Plasma Nucleic Acid (Streck) Tube Processing

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

10.1.2 Store Streck tubes with whole blood at room temperature. Do not freeze or refrigerate the tubes. The tubes may be stored for up to 72 hours at ambient temperature before shipment (i.e. if blood must be collected on Friday, it should be stored at ambient temperature over the weekend until Monday shipment). Ensure that the Streck tubes are shipped at ambient temperature to avoid freezing.

10.2 Whole blood (EDTA- no processing)

10.2.1 Collect 10 ml of whole blood into the EDTA tube using standard venous phlebotomy. Invert tube 10 times.

10.2.2 Store EDTA tube with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tube. The tube may be stored for up to 72 hours at refrigerated temperature before shipping (i.e. if blood must be collected on Friday, it should be stored at 4 degrees Celsius over the weekend until Monday shipment). Ensure that the EDTA tube is shipped at ambient temperature to avoid freezing.

11. CSF Collection

11.1 Frozen Supernatant

11.1.1 Collect between 3—10 ml of CSF using standard institutional procedure. CSF can be collected into polypropylene tube provided within the biospecimen collection kit or any standard collection tube included in LP kit.

11.1.2 Spin CSF at ambient temperature in a clinical centrifuge at 1600 xG for 10 minutes.

11.1.3 Label 1 cryovial for each ml of CSF collected. Cryovials should be labeled as indicated in **section 8**. Make certain each vial is labeled completely and identically.

11.1.4 Remove the supernatant from the tube and aliquot 1 ml into each of the labeled cryovials, leaving a small amount of fluid in the tube with the cell pellet. Process cell pellet following instructions in **section 11.2.**

11.1.5 Freeze cryovials with supernatant on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees until ready for shipment on dry ice.

11.2 Frozen Cell Pellet

11.2.1 Follow procedures in **section 11.1** for collecting and processing supernatant.

11.2.2 Label 1 cryovial as instructed in **section 8.**

11.2.3 After removing the supernatant, resuspend any cell pellet in the remaining fluid.

11.2.4 Transfer cell suspension to labeled cryovial. Immediately freeze the cryovial on dry ice or in liquid nitrogen vapor. Do NOT freeze by placing a warm tube in a -70 to -90 degree Celsus ultralow freezer. Once completely frozen, the cryovial containing the cell pellet may be stored at -70 to -90 degrees Celsius until ready for shipment on dry ice.

12. Biospecimen Shipping

12.1 Overview

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

12.1.2 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.

12.1.3 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.

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

12.1.5 Ship container for **PRIORITY OVERNIGHT DELIVERY** according to IATA guidelines and standard institutional policies and using the preferred vendor. A blank FedEx waybill 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

13. Biospecimen Receipt and Quality Assurance Measures

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

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

13.3 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.

13.4 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.

13.5 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.

13.6 Aliquoted biofluids will be stored under liquid nitrogen vapor.

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

14. Document History

Version	Description and Justification of Change	Author	Effective Date
3.0	Updated to remove disclaimer regarding discrepancy between protocol and CSM. Protocol has been amended to match	PAA	04/14/2021
2.0	Updated biospecimen collection schedule and processing instructions	PAA	12/09/2020
1.0	New	PAA	10/23/2020