# 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 A032101. 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 A032101 biospecimen collection, processing, and submission; including staff at satellite institutions.

## 2. Scope

This document applies to all biospecimens collected specifically for A032101 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 |
| FNA | Fine needle aspiration |
| H&E | Hematoxylin and Eosin |

## 4. Contact Information

| **Protocol-related questions may be directed as follows:**<br>**Questions** | **Contact (via email)** |
|---|---|
| Questions regarding patient eligibility, treatment, and dose modification: | Study Chair: Atish D. Choudhury, MD PhD achoudhury@partners.org<br>Nursing Contact: Archana Ajmera aajmera@health.ucsd.edu<br>Protocol Coordinator: Shiva Baghaie sbaghaie@bsd.uchicago.edu<br>(where applicable) Data Manager: Kayla Kroll kroll.kayla@mayo.edu |
| Questions related to data submission, RAVE or patient follow-up: | Data Manager: Kayla Kroll kroll.kayla@mayo.edu |
| Questions regarding the protocol document and model informed consent: | Protocol Coordinator: Shiva Baghaie sbaghaie@bsd.uchicago.edu |
| Questions related to IRB review | Alliance Regulatory Inbox regulatory@allianceNCTN.org |
| Questions regarding CTEP-AERS reporting: | Alliance Pharmacovigilance Inbox pharmacovigilance@alliancenctn.org |
| Questions regarding specimens/specimen submissions: | Alliance Biorepository alliance@email.wustl.edu |
| Questions regarding drug administration | Pharmacy Contact: Jerline Hsin jhsin@coh.org |

**4.1** 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.

**4.2** 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 A032101 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** 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 / Recipient Lab | Notes |
|---|---|---|---|---|---|---|
| **For patients registered to A032101-ST1** | | | | | | |
| ≤ 60 days after registration | N | Fixed tissue block (primary) | 1 | Fixed tissue block (9.2) | Ambient / ABWUSTL | 3, 6 |
| ≤ 60 days after registration | N | Fixed tissue slides (primary) | 1 (5 um) unstained slide **AND** 15 (10 um) unstained slides | Fixed tissue slides (9.3) | Ambient / ABWUSTL | 3, 6 |
| ≤ 60 days after registration | N | Fixed tissue block (metastasis) | 1 | Fixed tissue block (9.2) | Ambient / ABWUSTL | 3, 6 |
| ≤ 60 days after registration | N | Fixed tissue slides (metastasis) | 1 (5 um) unstained slide **AND** 15 (10 um) unstained slides | Fixed tissue slides (9.3) | Ambient / ABWUSTL | 3, 6 |
| | | | | | | |
| End of treatment | N | Fresh tissue research biopsy | 1-4 cores embedded in fixed tissue block | Research biopsy fixed tissue block (9.4) | Ambient / ABWUSTL | 2, 6, 7 |
| **For patients consented to A032101 Biobanking** | | | | | | |
| Prior to treatment interruption | N | Whole blood for plasma | 6 x 1 ml aliquots | Frozen plasma (10.1) | Dry Ice / ABWUSTL | 4, 6 |
| Prior to treatment interruption | N | Whole blood for "buffy coat" | 4 aliquots | "Buffy Coat" (10.2) | Dry Ice / ABWUSTL | 4, 6 |
| Prior to treatment interruption | N | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.3) | Dry Ice / ABWUSTL | 5, 6 |
| | | | | | | |
| 6 months after treatment interruption | N | Whole blood for plasma | 6 x 1 ml aliquots | Frozen plasma (10.1) | Dry Ice / ABWUSTL | 1, 4, 6 |
| 6 months after treatment interruption | N | Whole blood for "buffy coat" | 4 aliquots | "Buffy Coat" (10.2) | Dry Ice / ABWUSTL | 1, 4, 6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 months after treatment interruption | N | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.3) | Dry Ice / ABWUSTL | 1, 5, 6 |
| | | | | | | |
| 18 months after treatment interruption | N | Whole blood for plasma | 6 x 1 ml aliquots | Frozen plasma (10.1) | Dry Ice / ABWUSTL | 1, 4, 6 |
| 18 months after treatment interruption | N | Whole blood for "buffy coat" | 4 aliquots | "Buffy Coat" (10.2) | Dry Ice / ABWUSTL | 1, 4, 6 |
| 18 months after treatment interruption | N | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.3) | Dry Ice / ABWUSTL | 1, 5, 6 |
| | | | | | | |
| Prior to treatment resumption | N | Whole blood for plasma | 6 x 1 ml aliquots | Frozen plasma (10.1) | Dry Ice / ABWUSTL | 4, 6 |
| Prior to treatment resumption | N | Whole blood for "buffy coat" | 4 aliquots | "Buffy Coat" (10.2) | Dry Ice / ABWUSTL | 4, 6 |
| Prior to treatment resumption | N | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.3) | Dry Ice / ABWUSTL | 5, 6 |
| | | | | | | |
| End of treatment | N | Whole blood for plasma | 6 x 1 ml aliquots | Frozen plasma (10.1) | Dry Ice / ABWUSTL | 2, 4, 6 |
| End of treatment | N | Whole blood for "buffy coat" | 4 aliquots | "Buffy Coat" (10.2) | Dry Ice / ABWUSTL | 2, 4, 6 |
| End of treatment | N | Whole blood for serum | 3 x 1 ml aliquots | Frozen serum (10.3) | Dry Ice / ABWUSTL | 2, 5, 6 |

**Notes:**

1. 6-month and 18-month specimens are only collected if a patient is in the off-treatment interval at the time. If a patient has resumed treatment prior to 6 months or 18 months after treatment interruption, then biospecimens do not need to be collected at these time points. If treatment is resumed within 30 days of the 6 or 18 month collection, then new samples do not need to be collected at the treatment resumption time-point.
2. End of treatment samples should be collected between time of permanent discontinuation from ARPI (including day of last dose) and initiation of next systemic therapy. If a new systemic therapy is planned during an off-treatment interval, the end of treatment sample should be collected prior to initiation of next systemic therapy.

3. Representative, archived tumor tissue blocks from previous surgery or biopsy should be submitted, if available. Tissue should be submitted from both primary and metastatic sites if possible. If entire tissue blocks cannot be submitted, 1 unstained slide cut at 5 um **AND** 15 unstained slides cut at 10 um from each representative tissue block should be submitted. If tissue is limited, please submit (from each block) 1 unstained slide cut at 5 um and as many unstained slides cut at 10 um as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**
4. Peripheral blood (EDTA) 2 x 10 ml to be processed for plasma (6 x 1 ml aliquots) and "buffy coat," frozen on site and shipped on dry ice.
5. Peripheral blood (no additive) 1 x 10 ml for serum (3 x 1 ml aliquots), processed and frozen on site and shipped on dry ice.
6. Collection is optional for patients but all sites must ask patients for their consent to participate. Please see protocol-specific consent documents.
7. For patients who undergo a standard of care biopsy at end of treatment, tissue is requested from metastatic site.

## 7. Biospecimen Collection Kits

### 7.1 No kits are provided for submission of serum, plasma, buffy coat or tissue to ABWUSTL.

**7.2** 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.

## 8. Biospecimen Labeling and Tracking

**8.1** All research biospecimens (vacutainer tubes, cryovials, 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, serum, 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 sections are being submitted instead of a block, each tissue section slide 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 to ABWUSTL. 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.

9.1.1 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.

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 Blocks

9.2.1 Within 60 days of registration, this protocol requests submission of up to two representative, diagnostic pathology, formalin fixed paraffin embedded (FFPE) tissue blocks. Blocks should be submitted from either a previous surgery or biopsy from a primary and/or metastatic site.

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 endpoints have been met.

**9.2.3** In the event that an institution will not release tumor tissue blocks, the institution may instead submit one (1) unstained slide cut at 5 um and fifteen (15) unstained slides cut at 10 um from representative tissue blocks containing primary and/or metastatic tissue. **Although slides will be accepted, tissue block submission is strongly preferred.**

**9.2.4 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 (77degrees F) that may melt paraffin and damage the tissue specimens.**

### 9.3 Fixed Tissue Slides

9.3.1 In cases where an institution is unwilling or unable to submit the requested tissue blocks, sets of sixteen (16) unstained tissue slides can be submitted as an alternative. A set of unstained tissue slides is requested from both primary and metastatic sites, if possible.

9.3.2 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 tissue blocks, which can be cut at the biorepository and returned to your institution at a later date.

9.3.3 Include a copy of the corresponding, de-identified pathology report with all slide submissions.

9.3.4 Please follow the procedures below for submitting fixed tissue slides.

Primary tumor:

| # of slides | Section thickness | Slide type | Purpose |
|---|---|---|---|
| 1 | 5 um | Positively Charged | H&E staining |
| 15 | 10 um | Positively Charged | Tumor RNA profiling |

Metastatic site:

| # of slides | Section thickness | Slide type | Purpose |
|---|---|---|---|
| 1 | 5 um | Positively Charged | H&E staining |
| 15 | 10 um | Positively Charged | Tumor DNA profiling |

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

9.3.6 Cut sections at 5 or 10 um thickness as indicated onto positively charged slides.

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

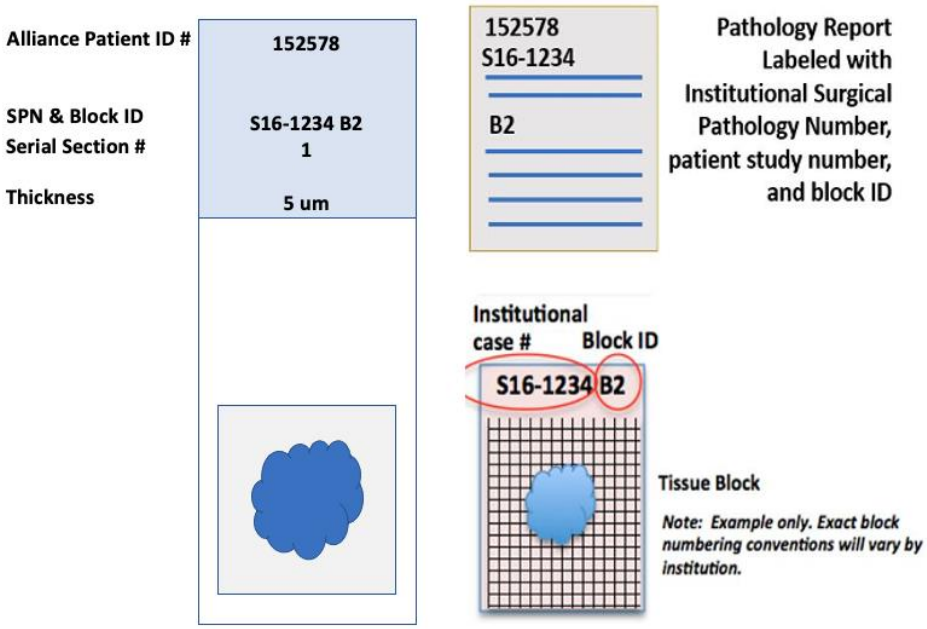
9.3.8 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

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

9.3.11 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.

9.3.12 See figure below for proper mounting and labeling.




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

9.3.14 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.

9.3.15 **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 (77degrees F) that may melt paraffin and damage the tissue specimens.**

### 9.4 Fresh Tissue Research Biopsy- Fixed Tissue Block

9.4.1 For patients who undergo a standard of care biopsy and have consented to A032101-ST1, 1-4 tissue cores from the biopsied site should be formalin fixed and embedded into paraffin according to standard institutional policies. **FNAs are not acceptable.**

## 10. Blood Collection Methods

### 10.1 Plasma Processing

10.1.1 Collect 20 ml of whole blood by standard venous phlebotomy technique into lavender 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 (~3 ml from each EDTA 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 10.3)**.

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 6 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.

10.1.6 Carefully remove 6 ml of plasma (without touching the pellet) and divide into 6, 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 "Buffy Coat" (White Blood Cell) Processing

10.2.1 Follow procedures in **section 10.2** for collecting and processing plasma from EDTA tubes.

10.2.2 Label 4 cryovials as instructed in **section 8**.

10.2.3 After removing the plasma, carefully remove the white, "buffy coat" white blood cell layer from each EDTA tube, 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 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 Celsius until ready for shipment on dry ice.

**10.3 Serum Processing**

10.3.1 Collect whole blood by standard venous phlebotomy technique into red top (plain glass with clot activator) tube. Do not collect whole blood into a "tiger top" / "SST" / "gel tube." Invert tube 10 times

10.3.2 Allow blood to clot for 30 minutes.

10.3.3 Label 3 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.

10.3.4 Spin blood in vacutainer tube at 4 degrees in a clinical centrifuge using standard programming for serum separation. Usually this is 1200 xG (actual speed will depend upon the centrifuge) for 10 minutes.

10.3.5 Carefully remove 3 ml of serum (without touching the clot layer) and divide into 3, 1 ml labeled cryovials.

10.3.6 Freeze serum containing cryovials 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. Biospecimen Shipping

### 11.1 Overview

11.1.1 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.

11.1.2 All biospecimens should be shipped within the timeframes indicated above in **sections 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.

**11.1.3 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 |
|---|---|---|---|
| 2.0 | Removed mandatory specimens to epic and all other references to epic sciences, added contact table, | KL | 08/15/2023 |
| 1.1 | Updated collection schema footnotes | PAA | 06/29/2022 |
| 1.0 | New | PAA , AW, AC, MR | 05/11/2022 |