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 on A031801 who have consented to biobanking. 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) or by the University of Wisconsin, 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 A031801 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A031801 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
UW	University of Wisconsin
EOT	End of Treatment

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 A031801 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 or to the University of Wisconsin. 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.

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
A031801-ST1							
After Registration, Before Treatment on C1D1	N	Primary tumor specimen	1 block OR 15 slides (if block is unavailable)	FFPE tissue block (9.2) OR 5 (five) 5 micron charged unstained slides and 10 (ten), 10 micron non-charged unstained slides (9.3)	Ambient	ABWUSTL	1, 2
After Registration, Before Treatment on C1D1	N	Metastatic tumor specimen	1 block OR 15 slides (if block is unavailable)	FFPE tissue block (9.2) OR 5 (five) 5 micron charged unstained slides and 10 (ten), 10 micron non-charged unstained slides (9.3)	Ambient	ABWUSTL	1, 2

After Registration, Before Treatment on C1D1	Y	Whole blood for serum	3 x 1 ml aliquots	Frozen Serum (10.1)	Dry Ice	ABWUSTL	1, 4
After Registration, Before Treatment on C1D1	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen Plasma (10.2)	Dry Ice	ABWUSTL	1, 4
After Registration, Before Treatment on C1D1	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.3)	Ambient	ABWUSTL	1, 4, 6
After Registration, Before Treatment on C1D1	Y	Whole blood (Streck BCT)	2 x 8.5 ml	Plasma for cfDNA (10.4)	Ambient	ABWUSTL	1, 4
After Registration, Before Treatment on C1D1	Y	Whole blood (CellSave & EDTA tubes)	2 x 10 ml CellSave tubes & 2 x 10 ml EDTA tubes	Whole blood-CTC (10.5)	Ambient	UW	1, 3, 4
Cycle 2 Day 1 (+/- 7 days)	Y	Whole blood (CellSave & EDTA tubes)	2 x 10 ml CellSave tubes & 2 x 10 ml EDTA tubes	Whole blood-CTC (10.5)	Ambient	UW	1, 3, 4

Every 8 weeks (+/- 7 days)	Y	Whole blood for serum	3 x 1 ml aliquots	Frozen Serum (10.1)	Dry Ice	ABWUSTL	1, 4
Every 8 weeks (+/- 7 days)	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen Plasma (10.2)	Dry Ice	ABWUSTL	1, 4
Every 8 weeks (+/- 7 days)	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.3)	Ambient	ABWUSTL	1, 4, 6
Every 8 weeks (+/- 7 days)	Y	Whole blood (Streck BCT)	2 x 8.5 ml	Plasma for cfDNA (10.4)	Ambient	ABWUSTL	1, 4
At progression / EOT	Y	Whole blood for serum	3 x 1 ml aliquots	Frozen Serum (10.1)	Dry Ice	ABWUSTL	1, 4, 5
At progression / EOT	Y	Whole blood for plasma	3 x 1 ml aliquots	Frozen Plasma (10.2)	Dry Ice	ABWUSTL	1, 4, 5
At progression / EOT	Y	Whole blood (EDTA)	2 x 10 ml	Whole blood-EDTA tubes (10.3)	Ambient	ABWUSTL	1, 4, 5, 6
At progression / EOT	Y	Whole blood (Streck BCT)	2 x 8.5 ml	Plasma for cfDNA (10.4)	Ambient	ABWUSTL	1, 4, 5
At progression / EOT	Y	Whole blood (CellSave & EDTA tubes)	2 x 10 ml CellSave tubes & 2 x 10 ml EDTA tubes	Whole blood-CTC (10.5)	Ambient	UW	1, 3, 4, 5

Notes:

1. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.

2. One paraffin tissue block from a bone metastasis and one paraffin tissue block from the primary tumor should be submitted for patients opting for A031801-ST1. If bone metastasis block is not available, a block from another metastatic site can be sent if available. Unstained slides will be accepted as alternative to block submission. A set of 15 unstained slides (5 x 5 micron charged, 10 x 10 micron non-charged) from each block type (i.e. metastasis, primary) should be submitted. If fewer than 15 unstained slides can be submitted, please prioritize the 5 x 5 micron slides and submit as many of the 10 micron slides as possible.
3. Whole blood in CellSave tubes and EDTA tubes for CTC will be shipped directly to the University of Wisconsin on the same day as blood draw for processing. **Please see section 7 for additional details.**
4. **All blood specimens must be collected prior to Radium-223 administration.**
5. Progression and EOT samples may be collected and submitted up to 30 days following progression or treatment discontinuation, but must be collected prior to any intervening treatment. If prior biospecimen collection has occurred within 14 days, there is no need to collect research samples at progression / EOT.
6. Whole blood sample in EDTA tubes will be collected for PBMC isolation and cryopreservation at the biorepository.

7. Biospecimen Collection Kits

7.1 Blood Specimens

7.1.1 To facilitate the proper collection and shipping of whole blood specimens, 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 or to the University of Wisconsin via priority overnight shipping**.**

7.1.2 Kits should be requested at least 10 working days in advance of the anticipated collection date. As many as 3 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.

7.1.3 Kit contents and specific instructions for use of the kit are provided in the kit box. Please return any unused collection materials with the kit.

7.1.4 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.

7.1.5 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.

7.1.6 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.

7.1.7 Note that individual kit components that are expired, damaged, or missing cannot be replaced. 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 some kit components are highly specialized (e.g. Streck BCT) and probably are not available at the institution. If substitutions are not available at the site, please order a complete, new kit. Please note in your request that you are replacing an expired or damaged kit.

7.1.8 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.

7.1.9 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.

7.1.10 Note that all tubes must be filled. Incompletely filled tubes provide insufficient sample to conduct the planned analysis and will result in a request to re-draw the sample if possible.

7.2 Tissue Specimens

7.2.1 There is no independent “kit” for submission of paraffin blocks or slides.

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 or to the University of Wisconsin.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (cryovials, vacutainer tubes, 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,” “serum,” etc).

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 or to the University of Wisconsin 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 A031801-ST1, one representative diagnostic block from a bone metastasis and one representative diagnostic block from the primary tumor should be submitted. If bone metastasis block is not available, a block from another metastatic site can be sent if available. If tissue from a metastasis focus is not available, a single block from the primary tumor will be accepted. Similarly, if tissue from the primary tumor is not available, a single block from a metastasis focus will be accepted.

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 unstained slides. Please refer to section 9.3.

9.3 Unstained Slides from Diagnostic Fixed Tissue Blocks

9.3.1 In cases where an institution is unwilling or unable to submit tissue blocks, a set of 15 unstained slides from each block (i.e. metastasis, primary) with adequate tumor may be sent as an alternative. If fewer than 15 unstained slides from a block can be submitted, please submit as many as possible. 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
5	5 micron	Charged	PD-L1 Immunohistochemistry
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.).

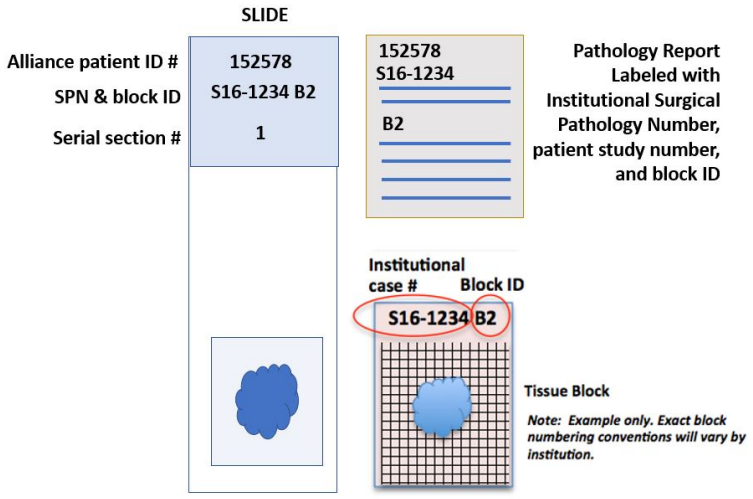
9.3.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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 Serum Processing

10.1.1 **Prior to radium-223 administration**, collect whole blood by standard venous phlebotomy technique into the red top (plain glass with clot activator) tube. Do not collect whole blood into a "tiger top" / "SST" / "gel tube." Invert tube 10 times

10.1.2 Allow blood to clot for 30 minutes.

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

10.1.4 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.

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

10.1.6 Freeze serum 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 Processing

10.2.1 **Prior to radium-223 administration**, collect whole blood by standard venous phlebotomy technique into the purple top (EDTA) tube. Invert tube 10 times.

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

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

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

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

10.2.6 Carefully remove plasma (without touching the pellet) and aliquot 1 ml into each of the labeled cryovials.

10.2.7 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.

10.3 Whole blood (EDTA- no processing)

10.3.1 **Prior to radium-223 administration**, collect whole blood by standard venous phlebotomy technique into each of the EDTA tubes. Invert tubes 10 times.

10.3.2 Store EDTA tubes with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tubes. **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.

10.4 Plasma Nucleic Acid (Streck) Tube Processing

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

10.4.2 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.

10.5 Whole blood for CTC (CellSave & EDTA Tubes - no processing)

10.5.1 **Prior to radium-223 administration**, collect whole blood by standard venous phlebotomy technique into each of the CellSave and/or EDTA tubes. Invert tubes 10 times.

10.5.2 Store CellSave tubes with whole blood at room temperature. Do not freeze or refrigerate the CellSave tubes. The EDTA tubes with whole blood should be stored at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the EDTA tubes. **The tubes must be received at the recipient laboratory within 24 hours of collection.** Ensure that the 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, labeled with the patient study number. Do not send specimens without a completed BioMS Packing Manifest or substitute "BioMS Downtime Form." Biospecimens cannot be accepted without this completed form.

11.1.3 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.**

11.2 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. A blank FedEx airbill is included with the kit for convenience. 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

11.3 Shipping to University of Wisconsin

11.3.1 Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies and using the preferred vendor. A blank FedEx airbill is included with the kit for convenience. Ship to:
Circulating Biomarker Core
K4/517 Clinical Science Center
University of Wisconsin Carbone Cancer Center
600 Highland Ave
Madison, WI 53792
Phone: 608-265-5349

Note: On the day when samples are being shipped (or as far in advance as possible), please send an e-mail notification with the shipment tracking information to the following study team members:

- **UWCTC@medicine.wisc.edu**

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 EDTA specimens will undergo ficoll processing to cryopreserved cells which will be stored under liquid nitrogen vapor.

12.8 Plasma and "buffy coat" will be derived from Streck BCT tubes and stored under liquid nitrogen vapor.

12.9 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
3.0	Updated shipping address for University of Wisconsin	PAA	04/30/2021
2.2	Clarified Cycle 2, Day 1 time point (added +/- 7 day window)	PAA	12/03/2020
2.1	Removed EDTA CTC collection at every 8 week time point to align with protocol Updated hyperlinks	PAA	07/13/2020
2.0	Removed buffy coat Updated CTC collection schema	PAA	10/24/2019
1.1	Updated collection schema to ST1	PAA	09/10/2019
1.0	New	PAA	09/09/2019