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 A082101. 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 A082101 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A082101 only. Please refer to the trial protocol-specific language for additional details regarding eligibility, participant enrollment, data submission, and specific procurement procedures. **Please ensure that you are reading the most updated version of this document. This document may experience minor updates, revisions, and clarifications independent of a formal protocol amendment. The most recent version of this document may be found on the Alliance website and CTSU.**

3. Definitions

Term	Definition
ABWUSTL	Alliance Biorepository at Washington University in St. Louis
FFPE	Formalin fixed, paraffin embedded

4. Contact Information

4.1 For questions and problems related to protocol administration, eligibility, patient registration, and data submission, relevant contact information is listed on protocol pages 1 and 2.

4.2 For information on using the BioMS system, please refer to the 'Help' links on the BioMS webpage to access the 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 A082101 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.

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	Biospecimen	Quantity	Collection / Processing Method	Shipping	Receiving Lab	Notes
For Patients Registered to A082101-ST1						
At Registration	Fixed tissue block	1	Fixed tissue block (9.2)	Ambient	Mayo	1, 2
At Registration	H&E stained slide **AND** unstained slides	1 H&E stained slide **AND** 10 x 5 micron unstained slides	Fixed tissue slides (9.3)	Ambient	Mayo	1, 2
At Registration	Whole blood (EDTA tube)	2 x 10 ml	Whole blood-EDTA tube (10.1)	Ambient	ABWUSTL	1, 3
Cycle 2 Day 1 Prior to Treatment	Whole blood (EDTA tube)	2 x 10 ml	Whole blood-EDTA tube (10.1)	Ambient	ABWUSTL	1, 3
At Completion of Immunotherapy	Whole blood (EDTA tube)	2 x 10 ml	Whole blood-EDTA tube (10.1)	Ambient	ABWUSTL	1, 3

Notes:

1. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.
2. A representative, archived diagnostic tumor tissue block from biopsy or surgery should be submitted, if available. If entire tissue block cannot be submitted, one H&E stained slide **AND** ten (5 um) serial tissue sections will be accepted as an alternative. If tissue is limited, please submit H&E and as many tissue sections as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED.**
3. 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 There are no "kits" provided for submission of blood specimens for this study. Sites are responsible for acquiring materials for collection and shipping of biospecimens to the Biorepository.

7.2 Tissue Specimens

7.2.1 There is no independent "kit" for submission of paraffin blocks, H&E stained slides, or unstained slides.

7.2.2 Paraffin blocks, H&E stained slides, and unstained 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, H&E stained slides, and unstained 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.

7.2.4 Please see **Section 11 – Biospecimen Shipping** for specific instructions on shipping.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens (vacutainer tubes 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.

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 slides are being submitted instead of the block, each H&E stained slide and unstained slide should be labeled with the patient study number, institutional surgical pathology number, and the block identifier. Provide **a de-identified copy of the surgical pathology report**, labeled with the patient study number, corresponding to the block, H&E stained slide, or unstained 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 Mayo Clinic or to the Alliance Biorepository must be **logged and tracked in BioMS**. The BioMS system is a web-based application that tracks the collection and shipping of biospecimens. Once individual biospecimens are logged and 'shipped' in the BioMS system, a packing manifest will be created by the system. This manifest must be printed out and must accompany all biospecimen shipments. To become familiar with the BioMS system and for further information about training, access, and use, please contact the BioMS Help desk at: 1-855-55-BIOMS or bioms@alliancenctn.org.

8.6 In the event that BioMS cannot be accessed, please complete a BioMS Specimen Log and Shipping Manifest form which can be found here- **http://tinyurl.com/alliance-bioms-contingency**.

9. Tissue Collection

9.1 Overview.

9.1.1 Please refer to protocol-specific instructions for procedures related to actual tissue procurement from individual participants. The method for research tissue procurement (needle core biopsy, sampling of surgically resected tumor) is dependent upon the disease site and the individual patient.

9.1.2 When procuring tissue biospecimens by any method, when possible, avoid tissue that is grossly necrotic, hemorrhagic, fatty, or fibrous. If in doubt, briefly (1 min or less) place the tissue segment in a sterile specimen cup containing physiologic (normal) saline to rinse the tissue. Necrotic, hemorrhagic, and fatty tissue will generally dissolve or float on the surface while tumor and parenchymal tissue will remain intact and sink to the bottom of the cup.

9.2 Fixed Tissue Block

9.2.1 For patients who consented to sub-study A082101-ST1, a representative diagnostic block with adequate tumor should be submitted, if applicable.

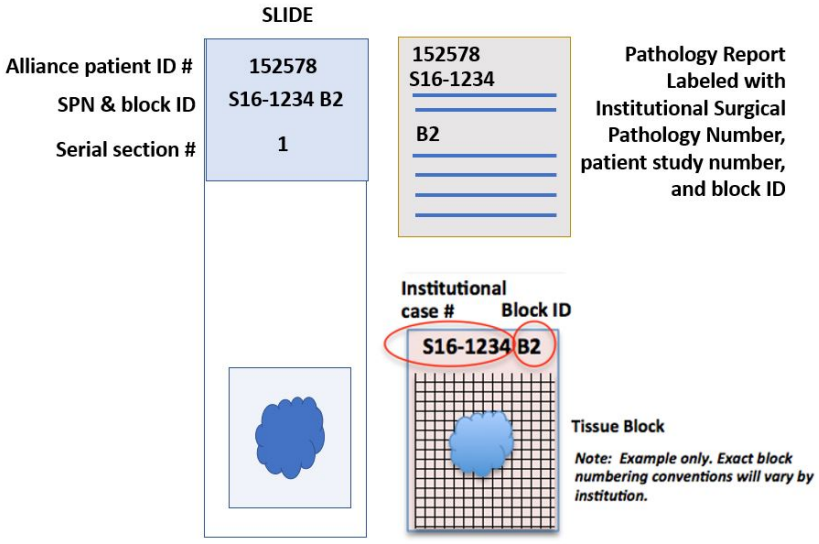
9.2.2 Any clinical surgical pathology block that is submitted for research studies will not be exhausted or rendered unsuitable for future diagnostic use. Any clinical surgical pathology block that is submitted will be returned within ten working days of written request, when needed for clinical management 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 Include a copy of a de-identified pathology report with block submission.

9.3 Fixed Tissue Slides

9.3.1 In cases where an institution is unwilling or unable to submit the requested tissue block, one H&E stained slide and ten (5 micron) unstained slides cut from a single diagnostic tumor tissue block may be submitted as an alternative. Block submission is highly preferred.

9.3.2 Cut and perform routine H&E stain on a single section from the tumor tissue block. See figure below for proper mounting and labeling.



9.3.3 Serial tissue sections should be cut fresh from the appropriate formalin fixed, paraffin embedded tissue block. The serial tissue sections must be cut from the same block from which the H&E stained slide was cut.

9.3.4 Cut sections at 5 micron thickness onto positively charged glass slides. See figure above for proper mounting and labeling.

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

9.3.7 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.

9.3.8 Include a copy of a **de-identified** pathology report with all tissue submissions.

10. Blood Collection Methods

10.1 Whole blood (EDTA- no processing)

10.1.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the EDTA tubes. Invert tubes 10 times.

10.1.2 Store EDTA tubes with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tubes. **Please collect blood on Monday through Thursday only. 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. **During warm weather months (i.e. June – August), a refrigerated pack (not frozen) should be included in the shipment to maintain ambient temperature. When shipping during other months of the year, a room temperature pack should be included in the shipment.**

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 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.

11.1.2 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 Mayo

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

Ship to:

ATTN: Ezequiel Tolosa
Mayo Clinic
200 First St. SW
Stabile 12-14
Rochester, MN 55905
Phone: 507-538-0902

11.3 Shipping to ABWUSTL

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

Ship to:
Alliance Biorepository
c/o Siteman Cancer Center Tissue Procurement Core
Washington Univ. School of Medicine
425 S. Euclid Ave.
Room 5120
St. Louis, MO
63110-1005
Phone: 314-454-7615

12. ABWUSTL Biospecimen Receipt and Quality Assurance Measures

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

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

12.3 Each individual biospecimen will receive and be physically labeled with a unique biospecimen identifier, associated with the biopsy control number in the TPC informatics system.

12.4 Upon receipt, all physical biospecimens received will be reconciled with what is recorded on the BioMS packing manifest. Any discrepancies noted will be communicated to the Program Manager who will contact the submitting site for reconciliation.

12.5 Upon receipt, any biospecimen received that is not in appropriate physical condition (broken vials, frozen samples that are thawed, ambient samples that are frozen) will be reported to the Program Manager, who will contact the submitting site for reconciliation.

12.6 Frozen aliquoted biofluids will be stored under liquid nitrogen vapor.

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

13. Document History

Version	Description and Justification of Change	Author	Effective Date
1.1	Updated Trial Title	AAW	03/20/2023
1.0	New	AAW	11/16/2022