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 A031902. 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) and by Tempus, 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 A031902 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A031902 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
HRR	Homologous-recombination repair

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 questions regarding tissue submission to Tempus for HRR testing, please contact: support@tempus.com.

4.4 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 A031902 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 and to Tempus. 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 **Please confirm that your institutional pathology department is willing to submit a minimum of ten (10) 5 micron unstained slides and maximum of twenty (20) 5 micron unstained slides from the most recent biopsy or resection AND 1 H&E stained slide cut from the same block, OR a minimum of eleven (11) 5 micron unstained slides and maximum of twenty-one (21) 5 micron unstained slides from such a block to Tempus for HRR testing for eligibility. Tissue should contain a minimum of 20% tumor cellularity.**

5.5 **An institution whose pathology department is unwilling to comply with mandatory slide submission should not enroll patients to this study.**

6. Collection Schema

The following biospecimens are to be collected at each of the time points listed in the table below. Please refer to biospecimen collection and processing methods and specific shipping procedures that are detailed in this manual.

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Processing Method	Shipping / Recipient Lab	Notes
Mandatory for <u>all</u> patients registered to A031902						
After pre-registration	N	Archival tissue for HRR testing	20 unstained slides (5 um) AND 1 H&E OR 11-21 unstained slides (5 um)	Fixed tissue slides (9.2)	Ambient / Tempus	1
Cycle 1, Day 1	Y	Whole blood (Streck BCT)	3 x 8.5 ml	Plasma for ctDNA (10.1)	Ambient / ABWUSTL	2, 5
Cycle 2, Day 1	Y	Whole blood (Streck BCT)	3 x 8.5 ml	Plasma for ctDNA (10.1)	Ambient / ABWUSTL	2, 5
At progression / treatment discontinuation	Y	Whole blood (Streck BCT)	3 x 8.5 ml	Plasma for ctDNA (10.1)	Ambient / ABWUSTL	2, 3, 5
A031902 Biobanking						
After pre-registration	N	Fixed tissue block	1	Fixed tissue block (9.3)	Ambient / ABWUSTL	4, 6
After pre-registration	N	H&E stained slide **AND** Tissue sections	1 H&E **AND** 10 (10 um) tissue sections	H&E and tissue sections (9.4)	Ambient / ABWUSTL	4, 6

1. Sites will submit tissue directly to Tempus for HRR testing. Sites are encouraged to submit 20 unstained slides for HRR testing as a higher number of slides offers a better chance of receiving a successful NGS result. If sites are unable to submit 20 unstained slides, Tempus will accept a minimum of ten (10) unstained slides. Please see additional details in **section 9.2**.
2. Whole blood (Streck BCT) for isolation of buffy coat and plasma at ABWUSTL.
3. Progression/treatment discontinuation samples may be collected and submitted up to 2 months after progression or treatment discontinuation for any reason.
4. Collection is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents (question #3).
5. Obtain up to 7 days prior or up to 3 days following this timepoint.
6. Submission of a fixed tissue block is **strongly preferred.** If institutional policy prohibits release of a fixed tissue block, 1 H&E stained slide **AND** 10 (10 um) tissue sections can be submitted as an alternative. Please see additional details in **section 9.4**.

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 blood 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 outgoing 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 ml of whole blood, generally a 10 ml tube is provided in the kit for convenience. If desirable or necessary to collect 8 ml in 3 x 3 ml tubes (for example), that is permissible.

7.11 No kits are provided for submission of mandatory tissue to Tempus for HRR testing or for submission of optional tissue blocks, H&E stained slides, or tissue sections to ABWUSTL for biobanking. Paraffin blocks or slides or sections 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, sections, 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, sections 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 and to Tempus.

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.

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 slides are being submitted instead of a block, each slide or cryovial/tube containing tissue sections should be labeled with the patient study number, institutional surgical pathology number, the block identifier, the section thickness, and serial section number (1, 2, etc.) 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 and to Tempus. 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 tissue can be matched directly to the pathology report.

8.4 In addition to the pathology report, the institution must complete the "Tempus Requisition Form" and submit with the slides to Tempus. Both the de-identified pathology report and the Tempus Requisition Form should be faxed to 1-800-893-0276 or emailed to support@tempus.com at the time the slides are submitted. Physical copies should also be printed and included with the slide shipment. Failure to submit the pathology report or requisition form with the specimens will delay turnaround time for HRR testing. For Alliance members, the form may be found on the A031902 study page on the Alliance website under the "Supplemental Materials" tab. For non-Alliance institutions, the form can be found under the "LPO Documents" tab on the CTSU A031902 study page (www.ctsu.org).

8.5 Label all containers and vials with an indelible, solvent-resistant marker when they are at ambient temperature.

8.6 Do not affix any labels to vials, slides or tubes. Label the collection containers directly with the marking pen.

8.7 All biospecimens that are collected and sent to the Alliance Biorepository or to Tempus 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.

8.8 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.

9.2 Fixed Tissue Slides for HRR Testing

9.2.1 A set of twenty (20) unstained tumor tissue slides AND one (1) H&E stained slide should be submitted for all patients pre-registered to this study. The H&E stained slide should be from the same block from which the unstained slides were cut. If unable to submit an H&E slide, twenty-one (21) unstained slides can be submitted as an alternative. Sites are encouraged to submit 20 unstained slides for HRR testing as a higher number of slides offers a better chance of receiving a successful NGS result. If sites are unable to submit 20 unstained slides, Tempus will accept a minimum of ten (10) unstained slides. Please follow the procedures below for submitting unstained tissue slides. If your pathology department is unwilling or unable to follow these tissue-sectioning instructions, you should not enroll patients to this study.

9.2.2 Turnaround time for testing is 14 calendar days (2 weeks) from time of receipt of tissue and all required documents (de-identified pathology report and properly completed requisition form). HRR results will be blinded to the physicians and patients. The results will be sent from Tempus to the Alliance registration/randomization system and an email will be generated to notify sites that HRR results are available. Resubmission of tissue is not allowed in the event of specimen failure (adequate tissue is submitted, but testing unable to be performed) as the patient and site will only be informed of a failed test upon unblinding.

# of unstained slides	Section thickness	Slide type	Purpose
20 (with H&E) OR 21 (without H&E)	5 microns	Non-Charged	HRR testing for eligibility

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

9.2.4 Cut sections at 5 micron thickness as indicated onto non-charged slides.

9.2.5 Ensure that each slide is labeled with the Alliance study number (A031902), Alliance patient ID number, surgical pathology number (SPN) and institutional block ID, slide serial section number (1, 2, 3, etc.), and thickness (5 microns). Please make sure to maintain the pathology accession numbers so the submitted slides can be matched directly to the pathology report.

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

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

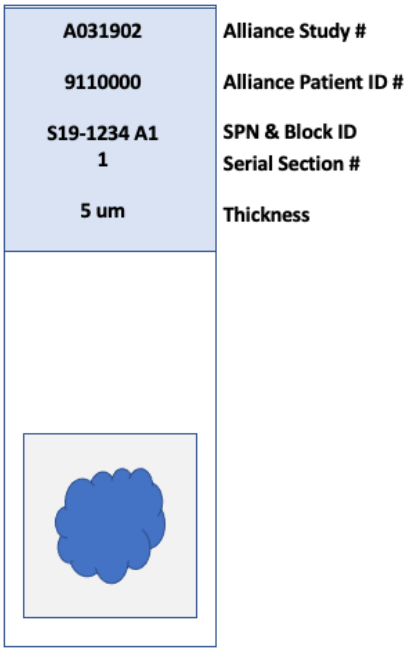
9.2.8 Mount only one tissue section per slide. Make certain sections are placed on the painted / textured side of the slide.

9.2.9 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.

9.2.10 See figure below for proper mounting and labeling.

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

9.2.12 Use slide mailers or a slide box to ship unstained slides. Slides should not be touching each other. Ensure that slides from only one patient are placed in one slide mailer.



9.3 Diagnostic Fixed Tissue Blocks

9.3.1 For patients consenting to biobanking, this protocol requests submission of one representative, diagnostic pathology, formalin fixed paraffin embedded block from the most recent diagnostic biopsy or surgical resection specimen.

9.3.2 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.

9.3.3 In the event that institutional policy prohibits release of tissue blocks, the institution may submit one H&E stained slide **AND** a set of 10 (10 um) tissue sections as an alternative (refer to **section 9.4**). **BLOCK SUBMISSION IS STRONGLY PREFERRED.**

9.4 H&E Stained Slide and Tissue Sections

9.4.1 In cases where institutional policy prohibits release of tissue blocks, one H&E stained slide **AND** 10 (10 um) tissue sections may be submitted as an alternative. The H&E stained slide and tissue sections should be prepared fresh and all cut from the same tissue block.

9.4.2 Tissue sections should be cut serially and placed directly into a cryovial or equivalent container. Tissue sections should not be floated in a water bath. Tubes of tissue should be labeled following the guidelines outlined in **section 8.1**.

10. Blood Collection Methods

10.1 Plasma Nucleic Acid (Streck) Tube Processing

10.1.1 Collect ~8.5 ml of whole blood by standard venous phlebotomy technique into each of the Streck tubes. A total of ~25.5 ml of whole blood should be collected into the Streck tubes (3 x 8.5 ml). Following collection, 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. Ensure that the Streck 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 Please see the Directions for Use (DFU) document that is included in each kit for specific directions on how to package and ship blood biospecimens.

11.1.2 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. Tissue sent to Tempus for HRR testing should additionally be accompanied by the Tempus Requisition Form. 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 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.

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

11.2 Shipping to Tempus

11.2.1 Ship container for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies. **Shipping can be charged to the Tempus FedEx account 787724361. Please include "A031902" in the billing reference section of the waybill.**

Ship to:
Tempus, Inc.
Attention: Accessioning Lab
600 West Chicago Avenue
Suite 510
Chicago, IL 60654
Phone: 800-739-4137

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. 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

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
4.1	Updated quantity of unstained slides requested by Tempus for HRR Testing	AAW	09/06/2022
4.0	Updated time point names Added block alternative for biobanking Updated instructions for Streck BCT Minor grammatical corrections	PAA	01/20/2022
3.0	Updated assay lab for mandatory tissue to Tempus Provided detailed instructions for tissue submission to Tempus Removed PK collection	PAA	04/15/2021
2.0	Revised biospecimen collection schedule, moved mandatory tissue submission to phase III patients only Fixed minor typos and grammatical errors	PAA	10/23/2020
1.0	New	PAA	08/21/2020