CORRELATIVE SCIENCE PROCEDURE MANUAL

1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens that are being submitted for patients enrolled or registered on A051301. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the Alliance Biorepository at Washington University in St. Louis (ABWUSTL) or by Cleveland Clinic (CC) 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 A051301 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for the A051301 clinical trial study. Please refer to the trial protocol-specific language for additional details regarding eligibility, participant enrollment, and data submission. **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
CC	Cleveland Clinic
IHC	Immunohistochemistry

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:

ABWUSTL

- Program Manager
  Phone: 1-314-747-4402
  Email: alliance@email.wustl.edu

Cleveland Clinic

- Research Coordinator
  Phone: 1-216-445-1753
  Email: fullmea@ccf.org

5. Site Preparation

5.1 Please refer to the A051301 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 and to Cleveland Clinic. 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 Please confirm that your institutional pathology department is willing to submit:
All original diagnostic stained slides OR 6 unstained slides (4-6 micron) from archival paraffin diagnostic biopsy tissue for real time, central histopathology review to confirm eligibility.
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	Notes
Mandatory for <u>all</u> patients pre-registered to A051301							
≤ 7 days after pre-registration	N	Original diagnostic stained slides	Case dependent	Stained Slides for Central Pathology Review (9.2)	Ambient	CC (11.1)	1
≤ 7 days after pre-registration	N	Unstained slides from archival paraffin diagnostic biopsy tissue	6	Unstained Tissue Slides for Central Pathology Review (9.3)	Ambient	CC (11.1)	1
For patients registered to A051301-PP1							
Registration	N	Whole Blood (EDTA/lavender top)	1 x 10 ml	Whole blood- EDTA (10.1)	Ambient	ABWUSTL (11.2)	2, 3, 6
For patients registered to A051301-ST1							
Pre-Registration	N	Fixed tissue block	1	Fixed tissue block (9.4)	Ambient	ABWUSTL (11.2)	3, 4, 7
Pre-Registration	N	Fixed tissue core, sections **<u>AND</u>** unstained slides	Refer to section 9.5	Fixed tissue block alternatives (9.5)	Ambient	ABWUSTL (11.2)	3, 4, 7
At Disease Progression or Relapse	N	Fixed tissue block	1-2	Fixed tissue block (9.4)	Ambient	ABWUSTL (11.2)	3, 4, 5, 7
At Disease Progression or Relapse	N	Fixed tissue core, sections **<u>AND</u>** unstained slides	Refer to section 9.5	Fixed tissue block alternatives (9.5)	Ambient	ABWUSTL (11.2)	3, 4, 5, 7

Notes:

1. Submission of slides for real-time central histopathology review is required for all patients at pre-registration to confirm eligibility. Biopsy can be acquired at the time of initial diagnosis or relapse. Submit original diagnostic stained slides: at least one H&E stained slide and stained slides for CD10, BCL6 and MUM1 used by the enrolling site for COO determination (including positive control stains) **OR** submit 6 unstained FFPE tissue sections (4-6 µm thickness) mounted onto positively charged slides. Slides should be freshly cut and packaged in a manner so that they do not break during shipment. If less than 6 slides are available, submit as many as possible. See additional details in **sections 9.2 and 9.3.**
2. Whole blood to be used for pharmacogenomics analyses. This blood specimen should be collected prior to the initiation of protocol treatment. However, blood specimen collection may take place at a later time point while the patient is on study.
3. Collection for A051301-PP1 and for A051301-ST1 is optional for patients but requires all sites offer to patients to consent. Please see protocol-specific consent documents.
4. **Submission of a block is highly encouraged.** If a block cannot be submitted, one 4 mm punch for TMA construction, three 10-micron sections from excision or five 10-micron sections from core needle biopsy AND 8 4-6-micron unstained slides should be submitted.
5. **Submission of a block is highly encouraged.** Tissue should be submitted within 2 months of progression. For patients randomized to the placebo arm who cross over to treatment with open-label ibrutinib, tissue should be submitted at both progression prior to crossover **AND** progression following crossover.
6. Collect and submit only from patients who answer "yes" to model consent question #1.
7. Collect and submit only from patients who answer "yes" to model consent question #2.

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 these specimens to the Biorepository.

7.2 Tissue Specimens

7.2.1 There are no "kits" provided for submission of paraffin blocks or slides for this study.

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, 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 to Cleveland Clinic or to ABWUSTL.

8. Biospecimen Labeling and Tracking

8.1 All research biospecimens, with exception of surgical pathology blocks and tissue slides, MUST be labeled with the Alliance study number (A051301), Alliance patient ID number, patient initials (Last, First, Middle), the date and time (if applicable) of collection and specimen type. Specimen labels must match BioMS records.

8.2 Surgical pathology tissue blocks should be labeled according to institutional standards, including the surgical pathology number (e.g. "S16-1234") and the individual block identifier (e.g. "A3"). **Adhesive labels should not be used to cover the institutional label.** If tissue section slides are being submitted instead of the block, each tissue section slide should be labeled with the procurement date, Alliance study number (A051301), Alliance patient ID number, patient initials (Last, First, Middle), surgical pathology number (SPN) and institutional block ID, slide serial section number (1, 2, 3, etc.), and thickness. Stained slides should additionally be labeled to indicate stain type (i.e. H&E, BCL6, CD10, MUM1).

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. 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 slides can be matched directly to the pathology report. **For mandatory tissue submitted to Cleveland Clinic, ancillary study reports such as flow cytometry or molecular studies should also be submitted, if available.**

8.4 In addition to the pathology report, the institution must complete the "Central Pathology Results Form" and submit with the diagnostic slides to Cleveland Clinic. Failure to submit this form with the specimens will delay turnaround time for central review. The top portion of the form must be completed by typing and cannot be handwritten. For Alliance members, the form may be found on the A051301 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 A051301 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 Cleveland Clinic or to ABWUSTL must be **logged and tracked in BioMS** under A051301. Do not send samples to the Biorepository or to Cleveland Clinic in which the patient has not been registered onto the study. 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) 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 Stained Slides for Central Pathology Review

9.2.1 Diagnosis confirmation and quality assessment are mandatory for all patients pre-registered to this study.

9.2.2 Submission of stained tissue slides from the diagnostic or relapse biopsy is required for real-time central review to confirm eligibibiity. Incisional or excisional biopsy is strongly preferred over a core needle biopsy. Diagnosis by fine needle aspirate (FNA) is **NOT** acceptable for enrollment. Bone marrow samples should not be submitted for registration.

9.2.3 Original diagnostic stained slides should be submitted to include at least one H&E stained slide and stained slides for CD10, BCL6, and MUM1 (to include positive control slides/tissue). Slides should be labeled as indicated in **section 8.**

9.2.4 Include a copy of the Central Pathology Results form and a de-identified pathology report with slide submission. The pathology report should be labeled with Alliance patient ID number, surgical pathology number and block ID. Ancillary study reports such as flow cytometry or molecular studies, if performed, should also be included.

9.3 Unstained Slides for Central Pathology Review

9.3.1 If unable to submit original diagnostic stained slides, sites must submit a set of six (6) unstained slides directly to Cleveland Clinic. If less than 6 unstained slides are available, please submit as many as possible.

9.3.2 Unstained slides should be prepared following instructions below.

# of slides	Section thickness	Slide type	Sample Types	Purpose
6	4-6 microns	Positively Charged	Biopsy from initial diagnosis or relapse	Central Pathology Review

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

9.3.2.2 Cut sections at 4-6 micron thickness as indicated onto positively-charged slides.

9.3.2.3 Ensure that each slide is labeled with the procurement date, Alliance study number (A051301), Alliance patient ID number, patient initials (Last, First, Middle), surgical pathology number (SPN) and institutional block ID, slide serial section number (1, 2, 3, etc.), and thickness (4-6 microns). Please make sure to maintain the pathology accession numbers so the submitted slides can be matched directly to the pathology report.

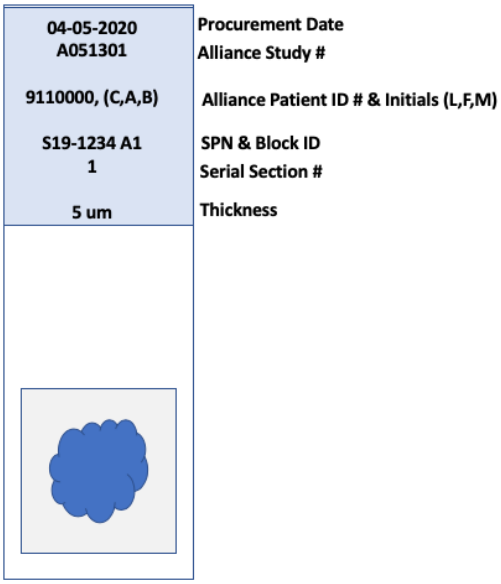
9.3.2.4 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

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

9.3.2.7 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.

9.3.2.8 See figure below for proper mounting and labeling.



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

9.4 Diagnostic Pathology Fixed Tissue Blocks

9.4.1 For patients who consent to A051301-ST1, paraffin blocks of primary and, when available, relapse tissue obtained from archival tumor specimens should be sent. Please specify the source of the tumor block (primary or relapse).

9.4.2 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.

9.5 Fixed Tissue Block Alternatives

9.5.1 In the event that an institution will not release tissue blocks, the institution may instead submit one (1) 4mm punch for TMA construction, three (3) 10 micron sections from excision or five (5) 10 micron sections from core needle biopsy, **AND** eight (8) unstained slides (4-6 micron, cut onto charged slides).

9.5.2 Punch for TMA Construction

9.5.2.1 One (1) 4mm punch should be submitted from the primary and, when available, relapse tissue block.

9.5.2.2 Place the tissue punches directly into microcentrifuge tubes or any other suitable container. It is important that the source of the tissue be indicated on the tube (i.e. primary, relapse) and that only one punch be added to each tube. Label the tube of tissue following the guidelines outlined in **section 8.**

9.5.3 Tissue Sections

9.5.3.1 Tissue sections should be submitted from the primary and, when available, relapse tissue block. Three (3) 10 micron sections should be submitted from excision and five (5) 10 micron sections should be submitted from core needle biopsy.

9.5.3.2 Place the tissue sections directly into microcentrifuge tubes or any other suitable container. Do not float the tissue sections in a water bath.

9.5.3.3 It is important that the source of the tissue be indicated on the tube (i.e. primary, relapse). Label the tube of tissue following the guidelines outlined in **section 8.**

9.5.4 Unstained Tissue Slides

9.5.4.1 Unstained slides from the primary and, when available, relapse tissue block should be submitted as indicated below.

# of slides	Section thickness	Slide type	Sample Types	Purpose
8	4-6 microns	Positively Charged	Primary and relapse tissue block	IHC

9.5.4.2 Refer to **section 9.3** for sectioning and labeling instructions.

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 a lavender top, EDTA tube. Invert tube 10 times. Tube should be labeled according to instructions in **section 8.**

10.1.2 Store EDTA tube with whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze the tube. The tube must be received at the Biorepository within 24 hours of collection.

11. Biospecimen Shipping

11.1 Overview

11.1.1 Place the original, completed copy of the BioMS packing manifest in the shipment. 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 If sending tissue, include a de-identified copy of the surgical pathology report labeled with the Alliance patient ID number. Ancillary study reports such as flow cytometry or molecular studies should also be included, if available. **Tissue sent to Cleveland Clinic must also be accompanied by the Central Pathology Results Form available for download on the Alliance website under A051301 or CTSU.**

11.1.3 All biospecimens collected on the study should be shipped on the same day that they are collected. Biospecimens must be received by the recipient lab within 24 hours of collection. If biospecimens cannot be shipped on the same day they are collected, please contact appropriate personnel listed in **section 4.3** for further instructions, at least 24 hours prior to anticipated collection.

11.2 Shipping to Cleveland Clinic

11.2.1 Include the following items together in the shipment container (Review will not be initiated until all specimens and completed paperwork are received):

- The BioMS shipping manifest.
- Central Pathology Results Form
- A copy of the de-identified pathology report and any relevant ancillary reports corresponding to the tissue submitted for testing. **Please maintain the pathology accession numbers so the submitted slides can be matched directly to the pathology report.**

11.2.2 Ship for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies. **Ship specimens to Cleveland Clinic on Monday through Thursday only. Do not ship on Fridays, Saturdays, or on the day before a national holiday.**

Using the preferred vendor (i.e. FedEx), ship to:

Cleveland Clinic
9410 Carnegie Ave
Desk L25
Cleveland, OH 44106
Attn: A051301
Phone: 216-445-1753

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 specimens to ABWUSTL on Monday through Thursday only. Do not ship on Fridays, Saturdays or on the day before a national holiday.**

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 Fixed tissue blocks will be vacuum packed to prevent oxidation and stored at 4 degrees Celsius (refrigerated) to minimize degradation of cellular antigens.

12.8 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	Corrected minor typos and grammatical errors Added contact information for Cleveland Clinic	PAA	03/04/2021
1.0	New	PAA	07/24/2020