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 A032103. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by Natera and 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 A032103 biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A032103 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
ctDNA	Circulating Tumor DNA
FFPE	Formalin fixed, paraffin embedded
H&E	Hematoxylin and Eosin
WES	Whole Exome Sequencing

4. Contact Information

Protocol-related questions may be directed as follows: **Questions**	**Contact (via email)**
Questions regarding patient eligibility, treatment, and dose modification:	Study Chair: Matthew D. Galsky, MD matthew.galsky@mssm.edu Nursing Contact: Archana Ajmera aajmera@health.ucsd.edu Protocol Coordinator: Shiva Baghaie, MPH sbaghaie@bsd.uchicago.edu (where applicable) Data Manager: Kelsey Peterson peterson.kelsey1@mayo.edu
Questions related to data submission, RAVE or patient follow-up:	Data Manager: Kelsey Peterson peterson.kelsey1@mayo.edu
Questions regarding the protocol document and model informed consent:	Protocol Coordinator: Shiva Baghaie, MPH 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 drug supply	PMB: PMBAfterHours@mail.nih.gov
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 questions regarding **mandatory specimens submitted to Natera** for analysis, please contact AllianceMODERN-A032103@natera.com.

4.3 For questions regarding **shipping logistics of mandatory specimens submitted to Natera** for analysis, please contact logistics@natera.com.

4.4 For questions regarding **kits sent by Natera**, please contact CTKits@natera.com.

4.5 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 A032103 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 Natera. 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 mandatory blood specimens sent to Natera, a biospecimen collection kit must be at the collection site. Please see **section 7** for requesting biospecimen collection kits. **Please allow at least 3 calendar weeks to receive the collection kit from Natera.**

5.4 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.

5.5 **Please confirm that your institutional pathology department is willing to submit a minimum of 6 x 10-micron unstained slides and a maximum of 10 x 10-micron unstained slides from an archived diagnostic tumor tissue block AND 1 H&E stained slide cut from the same block to Natera for the Signatera Assay. If your pathology department is unwilling or unable to follow these tissue-sectioning instructions, you should not enroll patients to this study.**

6. Collection Schema

The following biospecimens are to be collected at each of the time points. Please refer to biospecimen collection and processing methods and specific shipping procedures below.

Time Point	Kit (Y/N)	Kit Name	Biospecimen	Quantity	Collection / Processing Method	Shipping / Receiving Lab	Notes
Mandatory for <u>all</u> patients registered to A032103							
At pre-registration	Y	Signatera Tissue Collection Kit- WES	Archival tissue for Signatera assay (WES) - 1 H&E stained slide **AND** unstained slides	1 H&E stained slide **AND** 6-10 x 10 um unstained sllides	H&E stained slide **AND** unstained slides (10.2)	Frozen gel pack / Natera	1, 7
At pre-registration	Y	Signatera Blood Collection Kit- WES	Whole blood in EDTA tubes for Signatera assay (WES)	1 x 6 ml	Whole blood-EDTA tube (11.1)	Refrigerated gel pack / Natera	7
At pre-registration	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	7
Mandatory for patients registered to Cohort A (ctDNA+) (Arms 1 & 2)							
Month 3 (Week 12)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	9
Month 6 (Week 24)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	9
Month 9 (Week 36)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	9
Month 12 (Week 48)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	9
Month 18 (Week 72)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	9
Month 24 (Week 96)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	9

Mandatory for patients registered to Cohort B (ctDNA-) (Arms 3 & 4)							
Cycle 1 (Day 1 for Arm 3 and Day 1 surveillance for Arm 4)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8
Month 3 (Week 12)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 6 (Week 24)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 9 (Week 36)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 12 (Week 48)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 15 (Week 60)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 18 (Week 72)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 21 (Week 84)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 24 (Week 96)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 30 (Week 120)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10
Month 36 (Week 144)	Y	Whole Blood sample-ctDNA	Whole blood in Streck tubes for Signatera assay (ctDNA)	2 x 10 ml	Whole blood-Streck tubes (11.2)	Refrigerated gel pack / Natera	8, 10

For all patients consenting to biobanking							
Within 60 days after randomization	N	None	Fixed tissue block	1 block	Fixed tissue block (10.3)	Ambient / ABWUSTL	2, 3
Within 60 days after randomization	N	None	H&E stained slide **AND** Tumor tissue scrolls	1 H&E stained slide **AND** 10 x 10 micron scrolls	H&E stained slide **AND** Fixed tissue scrolls (10.4)	Ambient / ABWUSTL	2, 3
Day 1 (Cycle 1 for Arms 1-3 and surveillance for Arm 4)	N	None	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (11.3)	Dry Ice / ABWUSTL	2, 4
Day 1 (Cycle 1 for Arms 1-3 and surveillance for Arm 4)	N	None	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (11.4)	Dry Ice / ABWUSTL	2, 5
Month 3 (Week 12)	N	None	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (11.3)	Dry Ice / ABWUSTL	2, 4
Month 6 (Week 24)	N	None	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (11.3)	Dry Ice / ABWUSTL	2, 4
Month 12 (Week 48)	N	None	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (11.3)	Dry Ice / ABWUSTL	2, 4
Confirmed recurrence	N	None	Fixed tissue block	1 block	Fixed tissue block (10.3)	Ambient / ABWUSTL	2, 6
Confirmed recurrence	N	None	H&E stained slide **AND** Tumor tissue scrolls	1 H&E stained slide **AND** 10 x 10 micron scrolls	H&E stained slide **AND** Fixed tissue scrolls (10.4)	Ambient / ABWUSTL	2, 6
Confirmed recurrence	N	None	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (11.3)	Dry Ice / ABWUSTL	2, 4

Notes:

1. Sites will submit one (1) H&E stained slide **AND** between six (6) and ten (10) unstained slides directly to Natera for the Signatera Assay. Sites are encouraged to submit ten unstained slides. However, if sites are unable to submit 10 unstained slides, Natera will accept a minimum of six (6) unstained slides. Please see additional details in **section 10.2.**
2. Collection is optional for patients but all sites must ask patients for their consent to participate. Please see protocol-specific consent documents.
3. A representative, archived diagnostic tumor tissue block should be submitted, if available. If entire tissue block cannot be submitted, one H&E stained slide **AND** ten (10 um) serial tissue scrolls will be accepted as an alternative. If tissue is limited, please submit H&E and as many tissue scrolls as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED. Unstained slides will not be accepted.**
4. A total of 20 mL of peripheral blood in an EDTA tube will be collected. Please submit plasma (6 x 1 ml aliquots), processed and frozen on site and shipped on dry ice for storage at ABWUSTL.
5. Please submit buffy coat (2 aliquots) processed and frozen on site and shipped on dry ice for storage at ABWUSTL. Buffy coat only needs to be collected from a single time point. Collection of the buffy coat from a C1D1 / Day 1 of surveillance timepoint is preferred, but other timepoints are also acceptable. Please contact the BioMS help desk for assistance with logging non-C1D1 / Day 1 of surveillance buffy coat collections.
6. Tumor tissue block from a site of confirmed tumor recurrence (when applicable), only if tissue was collected per standard of care. No new research biopsy is required. If entire tissue block cannot be submitted, one H&E stained slide **AND** ten (10 um) serial tissue scrolls will be accepted as an alternative. If tissue is limited, please submit H&E and as many tissue scrolls as possible. **BLOCK SUBMISSION IS STRONGLY PREFERRED. Unstained slides will not be accepted.**
7. Pre-registration specimens can be sent up to 12 weeks after cystectomy. Sites are encouraged to submit Streck tubes for ctDNA Analysis as early as possible to allow the possibility for resubmission. Patients must be registered / randomized within 18 weeks of cystectomy.
8. Patients in cohort B who convert from ctDNA (-) to ctDNA (+) and initiate nivolumab therapy will continue assessment based on the timing since initial enrollment to the study (i.e. every 3 months through year 2, then every 6 months through year 3).
9. For Cohort A (ctDNA+) (Arms 1 & 2), blood collection every 3 months (+/- 1 week) through year 1, then every 6 months (+/- 2 weeks) through year 2.
10. For Cohort B (ctDNA-) (Arms 3 & 4), blood collection every 3 months (+/- 1 week through year 1, +/- 2 weeks through year 2) through year 2, then every 6 months through year 3 (+/- 2 weeks).

7. Biospecimen Collection Kits

7.1 Specimens Shipping to Natera

7.1.1 To facilitate the proper collection and shipping of mandatory blood and tissue biospecimens, biospecimen collection kits and materials will be provided by Natera. Kits from Natera contain a pre-paid waybill for return shipping. Kits will be sent at no additional cost to the participating institutions.

7.1.2 Collection kit orders take up to 3 calendar weeks to manufacture and ship to the sites so the collection kit requests should be sent to Natera a minimum of 3 calendar weeks before the required delivery date. As many as 5 kits can be requested at one time. Natera is **unable** to expedite kit shipment requests.

7.1.3 Sites should request collection kit orders using the fillable kit order form **(Appendix 4)** and email the order request to CTKits@natera.com. BioMS will **NOT** be used for kits for this study and the BioMS help team is unable to assist with kits. All questions regarding Natera kits should be sent to CTKits@natera.com.

7.1.4 Kits are shipped to the sites based on the address and contact information provided in the form that is emailed to Natera.

7.1.5 Sites should maintain a minimum of 2 collection kits of each kit type in inventory and can order up to 5 kits per kit type per order.

7.1.6 Natera kits that have expired or are missing components should be discarded and documented according to institutional policy. Discarded kits should be recorded on the Natera Kit Destruction Log **(Appendix 5)** to be maintained as part of the site's records. Discard the entire kit; do not keep any individual components. This log should be completed and returned within 30 calendar days of the kit expiration date to Natera at AllianceMODERN-A032103@natera.com.

7.1.7 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.

7.1.8 Note that individual kit components that are expired, damaged, or missing cannot be replaced. The remedy is to order a complete, new kit. Kit tubes should not be swapped from the site's own stock or from another Natera kit as each tube is barcoded uniquely to the patient and must be maintained.

7.1.9 Each kit and all components are uniquely barcoded. Do not combine kit components from different kits. Contents between kits must not be mixed. Do not combine shipments from different kits. Please keep all barcoded items together.

7.1.10 Kits with biospecimens should be received at Natera within 2 days of the kit expiration date.

7.2 Specimens Shipping to ABWUSTL

7.2.1 No kits are provided for submission of optional blood, tissue blocks, H&E stained slides, or tissue scrolls to ABWUSTL.

7.2.2 Paraffin blocks, H&E stained slides or scrolls cut from such blocks should be sent independently of other biospecimens using the following guidelines:

7.2.3 Blocks, H&E stained slides, and scrolls should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam container.

7.2.4 During warm weather months, paraffin blocks, scrolls, and H&E stained 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.

7.2.5 Blocks, scrolls and H&E stained slides may be shipped for standard overnight delivery according to institutional policies and using the preferred vendor.

8. Biospecimen Labeling and Tracking for Natera Specimens

8.1 Blood tubes (EDTA and Streck BCT) come pre-labeled with a unique barcode. These tubes MUST be additionally labeled with the Alliance patient ID number (in the "Subject ID" blank), the date of collection (DDMMMYYYY, i.e. 01APR2023), and patient year of birth (YYYY).

8.2 The A032103 Whole Blood Sample WES Requisition Form must be submitted along with the EDTA blood tube to Natera. Failure to submit this form with the blood may delay turnaround time for central testing. The A032103 Whole Blood Sample WES Requisition Form can be located in **Appendix 2** of this manual or on the A032103 protocol-specific page on the CTSU and Alliance websites. Please see additional details in **section 11.1**. An extra label is provided in the kit. Affix this extra barcode label to the upper right corner of the requisition form.

8.3 The A032103 Whole Blood Sample ctDNA Requisition Form must be submitted along with the Streck blood tubes to Natera. Failure to submit this form with the blood may delay turnaround time for central testing. The A032103 Whole Blood Sample ctDNA Requisition Form can be located in **Appendix 3** of this manual or on the A032103 protocol-specific page on the CTSU and Alliance websites. Please see additional details in **section 11.2**. An extra label is provided in the kit. Affix this extra barcode label to the upper right corner of the requisition form.

8.4 For tissue slides, use the slide mailers provided in the biospecimen collection kit to submit slides. Slide mailers come pre-labeled with a unique barcode. The Alliance patient ID number, patient year of birth (YYYY), and date of tissue slide preparation (DDMMMYYYY) should be handwritten on the label adhered to the slide mailers. Slides should not be touching each other.

8.5 Each slide should be labeled with the Alliance patient ID number, institutional surgical pathology number (e.g. S16-1234), the block identifier (e.g. "A3"), section thickness, and the serial section number. Refer to figure in **section 10.2** for example of properly labeled slide.

8.6 **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. Please ensure the institutional surgical pathology number (SPN) and block ID that match the SPN on the physical label on the tissue are maintained on the surgical pathology report. See **section 10** for additional details on tissue submission.

8.7 The A032103 Tumor Tissue Sample WES Requisition Form must be submitted along with the tumor tissue slides to Natera. Failure to submit this form with the slides may delay turnaround time for central testing. The A032103 Tumor Tissue Sample WES Requisition Form can be located in **Appendix 1** of this manual or on the A032103 protocol-specific page on the CTSU and Alliance websites. Please see additional details in **section 10.2**. An extra label is provided in the kit. Affix this extra barcode label to the upper right corner of the requisition form.

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

8.9 Do not affix any labels to vials, slides or tubes. Label the pre-labeled blood collection containers with the marking pen.

8.10 All biospecimens that are collected and sent to Natera 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.

8.11 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-biomscontingency.**

9. Biospecimen Labeling and Tracking for ABWUSTL Specimens

9.1 All research biospecimens (vacutainer tubes, cryovials, 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 (i.e. plasma, buffy coat).

9.2 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 scrolls are being submitted instead of the block, each tube containing tissue scrolls should be labeled with the Alliance patient ID number, patient initials (Last, First, Middle), and specimen type (FFPE scrolls). Provide a de-identified copy of the surgical pathology report, labeled with the Alliance patient ID number, corresponding to the blocks or scrolls submitted to ABWUSTL. **Please ensure the institutional surgical pathology number and block ID are maintained on the surgical pathology report.** See **section 10** for additional details.

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

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

9.5 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.

9.6 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-biomscontingency.**

10. Tissue Collection

10.1 Overview

10.1.1 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.

10.1.2 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.

10.2 Diagnostic Tumor Tissue Slides for Signatera Assay at Natera

10.2.1 One (1) H&E stained slide and between six (6) and ten (10) unstained slides must 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.

10.2.2 Tissue should meet the following guidelines:

-Fine Needle Aspirates (FNA) are not accepted
-Resection tumor tissue is preferred for optimal results
-Tumor content requirement is $\geq$ 20%

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

10.2.4 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.**

# of slides	Section thickness	Slide Type	Purpose
6-10	10 microns	Charged	Signatera Assay for eligibility

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

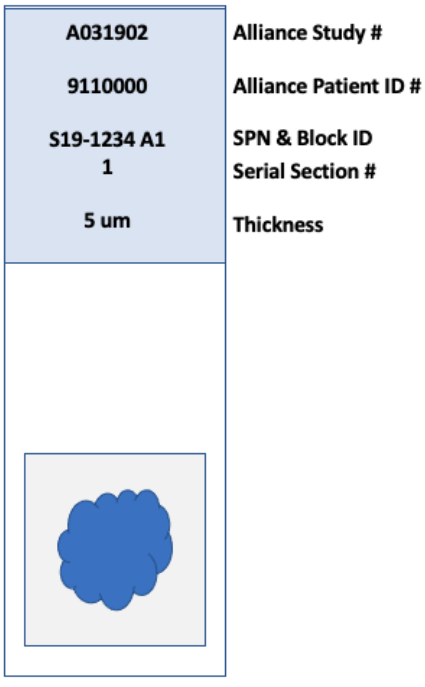
10.2.6 Cut sections at 10 micron thickness as indicated onto charged slides.

10.2.7 Ensure that each slide is labeled with the Alliance patient ID number, surgical pathology number (SPN) and institutional block ID, slide serial section number (1, 2, 3, etc.) and thickness (10 microns).

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

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

10.2.10 When placing the sections onto 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.



10.2.11 See figure above for proper mounting and labeling.

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

10.2.13 Use slide mailers provided in the biospecimen collection kit to submit slides. Alliance patient ID number, patient year of birth (YYYY), and date of tissue slide preparation (DDMMMYYYY) should be handwritten on the label adhered to the slide mailers. Slides should not be touching each other. Ensure that slides from only one patient are placed in one slide mailer.

10.2.14 Slide mailer should be placed into the biohazard bag(s) and sealed. Slides should be returned to Natera within the biospecimen collection kit. The gel pack provided within the kit should be frozen for a minimum of 24 hours prior to shipping the tissue.

10.2.15 A de-identified copy of the surgical pathology report and the A032103 Tumor Tissue Sample WES Requisition Form must be submitted along with the tumor tissue slides to Natera. A barcode label provided within the biospecimen collection kit should be affixed to the upper right corner of the requisition form. Failure to submit this form with the slides may delay turnaround time for central testing. The A032103 Tumor Tissue Sample WES Requisition Form can be located in **Appendix 1** of this manual or on the A032103 protocol-specific page on the CTSU and Alliance websites. A digital copy of the form should be sent at the time of shipment to AllianceMODERN-A032103@natera.com. This email should contain the following information:

Email Subject: A032103 Shipment Notification: Alliance patient ID number [enter Alliance patient ID number]

Body of Email: Sample Type (unstained slides); Visit (Pre-registration); Collection Date (DDMMMYYYY)

Attach a copy of the airway return label with tracking number

Attach a copy of the completed requisition form

10.2.16 Turnaround time for testing for the initial timepoint is 20 business days (30 calendar days), excluding US holidays, and begins when all required samples are received, data via the requisition form are received at Natera, and patient hold-required data points are resolved. Required materials for the initial timepoint are Tissue, 1 EDTA tube, and 2 Streck tubes.

10.2.17 Note: Resubmission of tissue in the event of specimen failure (i.e. adequate tissue is submitted, but testing unable to be performed) is acceptable. If resubmission is required, a request will come from Natera directly with further instructions. **Sites are encouraged to submit slides for Tumor Tissue WES as early as possible to allow the possibility for resubmission.**

10.2.18 If data clarification is required, sites will be contacted following procedures outlined in **Appendix 6**.

10.3 Fixed Tissue Block for Biobanking - ABWUSTL

10.3.1 A representative, archived tumor tissue block from a diagnostic biopsy should be submitted, if available. A tissue block from a site of tumor recurrence is also requested, if applicable.

10.3.2 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 endpoints have been met.

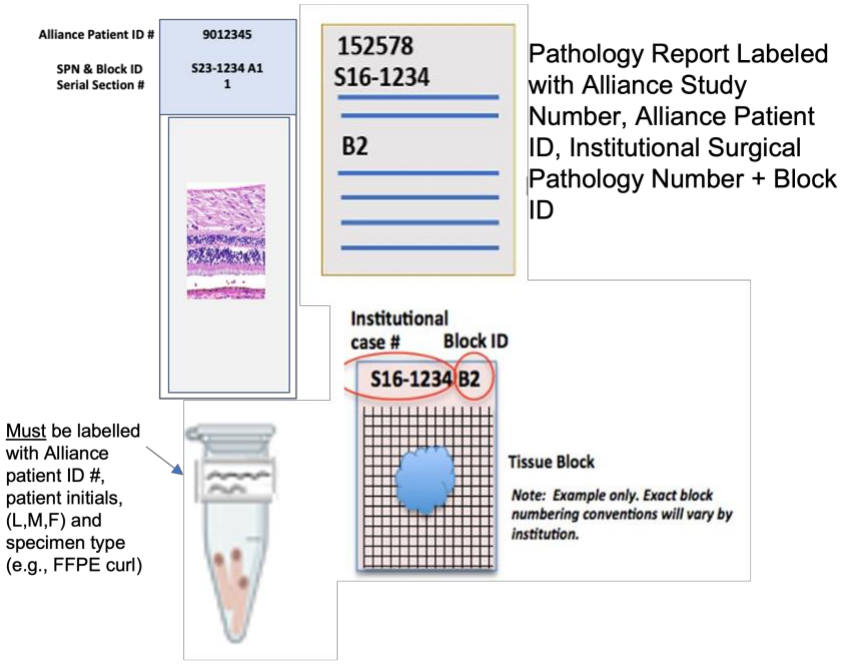
10.3.3 In the event that an institution will not release fixed tissue blocks, the institution may instead submit 1 H&E stained slide **AND** tissue scrolls from each of the requested blocks as an alternative (see **section 10.4**). **BLOCK SUBMISSION IS STRONGLY PREFERRED.**

10.3.4 Include a **de-identified copy of the surgical pathology report** with all tissue submissions.

10.4 H&E Stained Slide and Fixed Tissue Scrolls for Biobanking - ABWUSTL

10.4.1 In cases where an institution is unwilling or unable to submit a tissue block, a single H&E stained slide for references and serial tissue sections (scrolls, ribbons, curls) from the same block may be submitted. **Sections mounted to unstained slides will NOT be accepted.**

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



10.4.3 Cut a ribbon (scroll) of 10 paraffin tissue sections at 10 microns. Place the ribbon of tissue directly into a single microcentrifuge tube or any other suitable container. Do not float the tissue ribbon or sections in a water bath. Label the tube of tissue following the guidelines outlined in **section 9**.

10.4.4 Include a **de-identified copy of the surgical pathology report** with all tissue submissions.

11. Blood Collection Methods

11.1 Whole Blood- EDTA Tube to be Sent to Natera for WES

11.1.1 The gel pack provided within the biospecimen collection kit should be refrigerated for a minimum of 24 hours prior to collection of specimen.

11.1.2 Blood tube should be labeled as indicated in **section 8.** Collect 6 ml of whole blood by standard venous phlebotomy technique, using a 21 or 22 gauge needle, into the provided purple top (EDTA) tube for WES at Natera. Vein collapse may require a second venipuncture with a fresh tube from another Natera collection kit (Do not mix the contents of different Natera collection kits or use collection materials from own stock). Following collection, gently invert tube 8-10 times to thoroughly mix blood with reagents. *If unable to use a 21 or 22 gauge needle, you should still collect and submit the sample, but Natera may be unable to process the sample.*

11.1.3 **Blood should be collected Monday-Friday only. Samples should be shipped within 24 hours of collection. If unable to send the same day (late Friday collection for example), the sample may be held until the following Monday and sent priority overnight at that time.** Store EDTA tube with the whole blood at 4 degrees Celsius (i.e. refrigerated) until shipping. Do not freeze tube.

11.1.4 EDTA tube should be placed into the absorbent sleeve provided within the biospecimen collection kit. The absorbent sleeve containing the tube should then be placed within the foil pouch. The foil pouch should be sealed inside the provided biohazard bag. The biohazard bag should be returned within the biospecimen collection kit with the pre-cooled gel pack to maintain proper temperature during shipment.

11.1.5 Note: The stability of the Whole Blood EDTA sample is 14 days from collection and must be received at Natera at least 1 day ahead of blood stability expiry and at least 2 days ahead of kit/tube expiry.

11.1.6 The A032103 Whole Blood Sample WES Requisition Form must be submitted along with the EDTA tube to Natera. Failure to submit this form with the blood may delay turnaround time for central testing. A barcode label provided within the biospecimen collection kit should be affixed to the upper right corner of the requisition form. The A032103 Whole Blood Sample WES Requisition Form can be located in **Appendix 2** of this manual or on the A032103 protocol-specific page on the CTSU and Alliance websites. A digital copy of the form should be sent at the time of shipment to AllianceMODERN-A032103@natera.com. This email should contain the following information:

Email Subject: A032103 Shipment Notification: Alliance patient ID number [enter Alliance patient ID number]

Body of Email: Sample Type (EDTA whole blood); Visit (Pre-registration); Collection Date (DDMMMYYYY)

Attach a copy of the airway return label with tracking number

Attach a copy of the completed requisition form

11.1.7 Turnaround time for testing for the initial timepoint is 20 business days (30 calendar days), excluding US holidays, and begins when all required samples are received, and data via the requisition form are received at Natera, and patient hold-required data points are resolved. Required materials for the initial timepoint are Tissue, 1 EDTA tube, and 2 Streck tubes.

11.1.8 Note: Resubmission of blood in the event of specimen failure is acceptable. If resubmission is required, a request will come from Natera directly with further instructions. **Sites are encouraged to submit EDTA tubes for WES Analysis as early as possible to allow the possibility for resubmission.**

11.1.9 If data clarification is required, sites will be contacted following procedures outlined in **Appendix 6**.

11.2 Whole Blood- Streck BCT Tubes to be Sent to Natera for ctDNA

11.2.1 The gel pack provided within the biospecimen collection kit should be refrigerated for a minimum of 24 hours prior to collection of specimen.

11.2.2 Blood tubes should be labeled as indicated in **section 8.** Collect 10 ml of whole blood by standard venous phlebotomy technique, using a 21 or 22 gauge needle, into each of the Streck tubes. Allow 60-90 seconds to pass to ensure complete filling of each tube. A total of 20 ml of whole blood should be collected into the Streck tubes (2 x 10 ml) for ctDNA isolation at Natera. Vein collapse may require a second venipuncture with a fresh tube from a Natera collection kit. (Do not mix the contents of different Natera collection kits or use collection materials from own stock). Following collection, gently invert tubes 8-10 times to thoroughly mix blood with reagents. *If unable to use a 21 or 22 gauge needle, you should still collect and submit the sample, but Natera may be unable to process the sample.*

11.2.3 **Blood should be collected Monday-Friday only. Samples should be shipped within 24 hours of collection. If unable to send the same day (late Friday collection for example), the sample may be held until the following Monday and sent priority overnight at that time.** Store the Streck tubes with whole blood at room temperature. Do not freeze or refrigerate the tubes.

11.2.4 Streck tubes should be placed into the absorbent sleeve provided within the biospecimen collection kit. The absorbent sleeve containing the tube should then be placed within the foil pouch. The foil pouch should be sealed inside the provided biohazard bag. The biohazard bag should be returned within the biospecimen collection kit with the pre-cooled gel pack to maintain proper temperature during shipment.

11.2.5 The stability of the Whole Blood ctDNA sample is <8 days from collection and must be received at Natera at least 1 day ahead of blood stability expiry and at least 2 days ahead of kit/tube expiry.

11.2.6 The A032103 Whole Blood Sample ctDNA Requisition Form must be submitted along with the Streck tubes to Natera. Failure to submit this form with the blood may delay turnaround time for central testing. A barcode label provided within the biospecimen collection kit should be affixed to the upper right corner of the requisition form. The A032103 Whole Blood Sample ctDNA Requisition Form can be located in **Appendix 3** of this manual or on the A032103 protocol-specific page on the CTSU and Alliance websites. A digital copy of the form should be sent at the time of shipment to AllianceMODERN-A032103@natera.com. This email should contain the following information:

Email Subject: A032103 Shipment Notification: Alliance patient ID number [enter Alliance patient ID number]

Body of Email: Sample Type (Streck BCT); Visit; Collection Date (DDMMMYYYY)

Attach a copy of the airway return label with tracking number

Attach a copy of the completed requisition form

11.2.7 Turnaround time for testing for the initial timepoint is 20 business days (30 calendar days), excluding US holidays, and begins when all required samples are received, and data via the requisition form are received at Natera, and patient hold-required data points are resolved. Required materials for the initial timepoint are Tissue, 1 EDTA tube, and 2 Streck tubes.

11.2.8 Turnaround time for testing for subsequent timepoints is 10 business days (14 calendar days), excluding US holidays, and begins when all required materials and data via the requisition form are received at Natera, patient hold-required data points are resolved, and the result of the initial time point has been delivered. Required materials for subsequent timepoints are 2 Streck tubes.

11.2.9 Note: Resubmission of blood in the event of specimen failure is acceptable. If resubmission is required, a request will come from Natera directly with further instructions. **Sites are encouraged to submit Streck tubes for ctDNA analysis as early as possible to allow the possibility for resubmission.**

11.2.10 If data clarification is required, sites will be contacted following procedures outlined in **Appendix 6**.

11.3 Plasma Processing for Biobanking - ABWUSTL

11.3.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the purple top (EDTA) tubes. A total of 20 ml of whole blood should be collected into the EDTA tubes (2 x 10 ml). Following collection, invert tubes 10 times.

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

11.3.3 Carefully remove the plasma layer from each vacutainer tube (~3—5 ml in volume per 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 11.4**).

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

11.3.5 Label 6 cryovials as instructed in **section 9**. Make certain each vial is labeled completely and identically. Please use 2 ml cryovials with threaded caps. Do not use flip-top tubes or cryovials larger than 2 ml.

11.3.6 Carefully remove 6 ml of plasma (without touching the pellet) and divide into six (6) 2 ml labeled cryovials. Each aliquot should be between 1—1.5 ml in volume.

11.3.7 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. Frozen plasma should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

11.4 "Buffy Coat" (White Blood Cell) Processing for Biobanking – ABWUSTL

11.4.1 Follow procedures in **section 11.3** for collecting and processing plasma from EDTA tubes.

11.4.2 Label 2 cryovials as instructed in **section 9**. Please use 2 ml cryovials with threaded caps. Do not use flip-top tubes or cryovials larger than 2 ml.

11.4.3 After removing the plasma, carefully remove the white, "buffy coat" white blood cell layer, avoiding the red blood cell mass as much as possible.

11.4.4 Transfer the buffy coat layer (approximately 0.2 – 0.5 ml) from EDTA tubes 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 C until ready for shipment on dry ice. Frozen buffy coat should be shipped to the Biorepository within 30 days of collection. Batch shipment is allowed.

12. Biospecimen Shipping

12.1 Overview

12.1.1 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.

12.1.2 Specimens being submitted to Natera should also be accompanied by the appropriate requisition form (**see Appendix 1-3)**.

12.1.3 All biospecimens should be shipped within the timeframes indicated above in **section 10 and 11.** 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.

12.1.4 **Do not ship specimens to ABWUSTL on Friday, Saturday, Sunday or the day before a nationally recognized holiday.**

12.1.5 Natera is open for sample receipt Monday through Saturday, excluding major US holidays. If shipping specimens for Saturday delivery, please indicate Saturday delivery on the FedEx waybill. Do not ship specimens on Saturday, Sunday or the day before a nationally recognized holiday.

12.2 Shipping to Natera

Ship container for PRIORITY OVERNIGHT DELIVERY using pre-paid FedEx waybill provided in the biospecimen collection kit.

Ship to:
ATTN: Accessioning- Signatera Natera Inc.
13011A McCallen Pass Suite 100
Austin, TX 78753
Phone: 1-877-869-3052

12.3 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: 1-314-454-7615

13. ABWUSTL Biospecimen Receipt and Quality Assurance Measures

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

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

13.3 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.

13.4 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.

13.5 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.

13.6 Aliquoted biofluids will be stored under liquid nitrogen vapor.

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

14. Document History

Version	Description and Justification of Change	Author	Effective Date
1.0	New	PAA, KAL	01/09/2024

Appendix 1:

Tumor Tissue Sample - WES Requisition Form

natera | Signatera™
Residual disease test (MRD)

Please place collection kit barcode here:

Only Signatera Collection Kit contents may be used during sample collection. DO NOT USE EXPIRED KITS

COMPLETE ALL INFORMATION TO AVOID DELAYS

For data entry errors, draw a single line through the error, initial and date (DDMMMYYYY). Overwritten entries will be queried.

Protocol ID: Alliance A032103 **Tumor Tissue Sample – WES**

1. SUBJECT INFORMATION

Alliance Patient ID

Year of Birth (YYYY)

Male ☐ Female ☐ Biological Sex (Check one box)

2. SITE INFORMATION

Site Name

CTEP Site Code

Shipped by: First Name (PLEASE PRINT)

Shipped by: Last Name (PLEASE PRINT)

3. CANCER DIAGNOSIS

Cancer Type ☐ Urothelial carcinoma of the bladder ☐ ☐

Cancer Subtype ☐ ☐ ☐ ☐

4. SAMPLE GUIDELINES

Tumor Tissue Sample – WES Sites must submit 10 unstained slides (charged and unbaked) at 10-microns each INCLUDING one contiguous H&E slide. (If 10 unstained slides cannot be submitted, a minimum of 6 unstained slides is required.)

Requirements for FFPE Tissue Samples provided on Slides:

1. The Slide Holder must be labeled with two identifiers: Barcode Label plus Alliance Patient ID or Block ID
2. Individual Slides inside the Slide Holder must be labeled with at least one identifier: Alliance Patient ID or Block ID

5. PATHOLOGY INFORMATION (include Pathology De-Identified Report)

Anatomical site/organ biopsy: ☐ ☐ Other (please specify)

Tissue collection method: ☐ Surgical resection ☐ Other (please specify)

D D / M M M / Y Y Y Y Date of procedure to obtain sample Complete month field in English (Example: 01 JAN 2001)

Material submitted (Select all that apply): ☐ One contiguous H&E slide ☐ Unstained slides

Original Block ID/Number:

6. UNSTAINED SLIDES INFORMATION

Number of unstained slides submitted:

D D / M M M / Y Y Y Y Slide Preparation Date Complete month field in English (Example: 01 JAN 2001)

Slide thickness (microns): ☐ 10 Other (please specify)

7. VISIT INFORMATION

☐ Pre-registration

Appendix 2:

Whole Blood Sample - WES Requisition Form

natera | Signatera™ Residual disease test (MRD)


123456-2-X

Please place collection kit barcode here:

Only Signatera Collection Kit contents may be used during sample collection. DO NOT USE EXPIRED KITS

COMPLETE ALL INFORMATION TO AVOID DELAYS

For data entry errors, draw a single line through the error, initial and date (DDMMMYYYY). Overwritten entries will be queried.

Protocol ID: **Alliance A032103**

Whole Blood Sample – WES

1. SUBJECT INFORMATION

Alliance Patient ID

Year of Birth (YYYY)

Male ☐ Female ☐

Biological Sex (Check one box)

2. SITE INFORMATION

Site Name

CTEP Site Code

Shipped by: First Name (PLEASE PRINT)

Shipped by: Last Name (PLEASE PRINT)

3. COLLECTION INFORMATION

D D / M M M / Y Y Y Y

Collection Date

Complete month field in English (Example: 01 JAN 2001)

24 Hour Clock

:

Collection Time (Record midnight as 00:00)

4. SAMPLE GUIDELINES

Whole Blood – WES

One 6mL Lavender EDTA Tube. Please fill the tube completely.

Do not provide empty or uncollected tube to Natera.

5. VISIT INFORMATION

☐ Pre-registration

Appendix 3:

Whole Blood Sample - ctDNA Requisition Form

natera | Signatera™
Residual disease test (MRD)


123456-2-X

Please place collection kit barcode here:

Only Signatera Collection Kit contents may be used during sample collection. DO NOT USE EXPIRED KITS

COMPLETE ALL INFORMATION TO AVOID DELAYS

For data entry errors, draw a single line through the error, initial and date (DDMMMYYYY). Overwritten entries will be queried.

Protocol ID: Alliance A032103

Whole Blood Sample - ctDNA

1. SUBJECT INFORMATION

☐☐☐☐☐☐☐ Alliance Patient ID

☐☐☐☐ Year of Birth (YYYY)

Male ☐ Female ☐ Biological Sex (Check one box)

2. SITE INFORMATION

Site Name

☐☐☐☐☐ CTEP Site Code

Shipped by: First Name (PLEASE PRINT)

Shipped by: Last Name (PLEASE PRINT)

3. COLLECTION INFORMATION

D D / M M M / Y Y Y Y Collection Date
Complete month field in English (Example: 01 JAN 2001)

24 Hour Clock ☐☐:☐☐ Collection Time (Record midnight as 00:00)

4. SAMPLE GUIDELINES

Whole blood – ctDNA

Two 10mL Cell Free DNA Streck Tubes. Please fill tubes completely. Do not provide empty or uncollected tubes to Natera.

5. VISIT INFORMATION

Please check visit timepoint (one box only):

☐ **Pre-registration**

Cohort A, Arm 1

Year 1: ☐ Month 3 ☐ Month 6 ☐ Month 9 ☐ Month 12
Year 2: ☐ Month 18 ☐ Month 24

Cohort A, Arm 2

Year 1: ☐ Month 3 ☐ Month 6 ☐ Month 9 ☐ Month 12
Year 2: ☐ Month 18 ☐ Month 24

Cohort B, Arm 3

Year 1: ☐ Cycle 1, Day 1 ☐ Month 3 ☐ Month 6 ☐ Month 9 ☐ Month 12
Year 2: ☐ Month 15 ☐ Month 18 ☐ Month 21 ☐ Month 24
Year 3: ☐ Month 30 ☐ Month 36

Cohort B, Arm 4 – Surveillance

Year 1: ☐ Day 1 ☐ Month 3 ☐ Month 6 ☐ Month 9 ☐ Month 12
Year 2: ☐ Month 15 ☐ Month 18 ☐ Month 21 ☐ Month 24
Year 3: ☐ Month 30 ☐ Month 36

Appendix 4:

Natera Signatera Kit Order Form

Alliance MODERN A032103 Signatera Kit Order Form

Protocol ID: Alliance MODERN A032103

Instructions:

To order collection kits, complete this form in full and email to CTKits@Natera.com
Use email subject convention: "Kit Order Request: Alliance MODERN A032103 <Insert Site #>".
Please reach out to CTKits@Natera.com if you have any questions regarding this form or the kit order.
All required fields are notated with *

Basic Details:

Is this an initial kit order (Y/N) _____

Date _____ Name _____

Site Name* _____ CTEP Site Code* _____
(5 character site ID, ex: AM999)

Natera Account ID _____
(Natera assigned and "N/A" for new sites/new location.
This ID will be provided to sites after an initial kit order is placed)

Shipping Address:

Has the Shipping Contact / Address been updated since the last order was placed? (Y/N) _____

Shipping Contact* _____ Shipping Contact Email * _____
(First & Last Name) *(Email address to receive the kit order confirmation)*

Address Line 1* _____

Address Line 2 _____
(Bldg/Suite #)

City* _____ State* _____ Zip Code* _____

Country* _____

Kit Order:

Do NOT use expired kits to collect samples.
Enter the required quantity for each kit type listed below.
Kits should be used according to expiration date with those soonest to expire used first.

Do not leave quantity blank, enter "0" if this kit is not needed.
Any quantity of kits that exceeds 5 kits per kit type will not be processed without Alliance sponsor approval.

Collection Kit Type	Quantity*
Signatera Blood Kit - ctDNA (Streck tubes)	
Signatera Blood Kit - WES (EDTA tubes)	
Signatera Tissue Kit - WES (Tissue)	

Please Note:
- Tissue and blood collection kits for shipment to Natera will include a pre-paid, pre-addressed shipping label.
- Please allow up to three calendar weeks for the kits to arrive.
- There is no automatic resupply of kits for this study. Please ensure ample time is allotted to receive a resupply order.
- Ensure that you have a primary and back-up kit onsite for any upcoming visit when placing kit orders.

Appendix 5:

Natera Kit Destruction Log

Collection Kit Type*	**Kit Barcode**	**Expiration Date**	**Date Discarded**	**CTEP Site Code** (where the kit was discarded)	**Site Name** (where the kit was discarded)

*Kit Types: "Signatera Blood Collection Kit – ctDNA (Streck tubes)" | "Signatera Blood Collection Kit – WES (EDTA tube)" | "Tissue Collection Kit – WES"

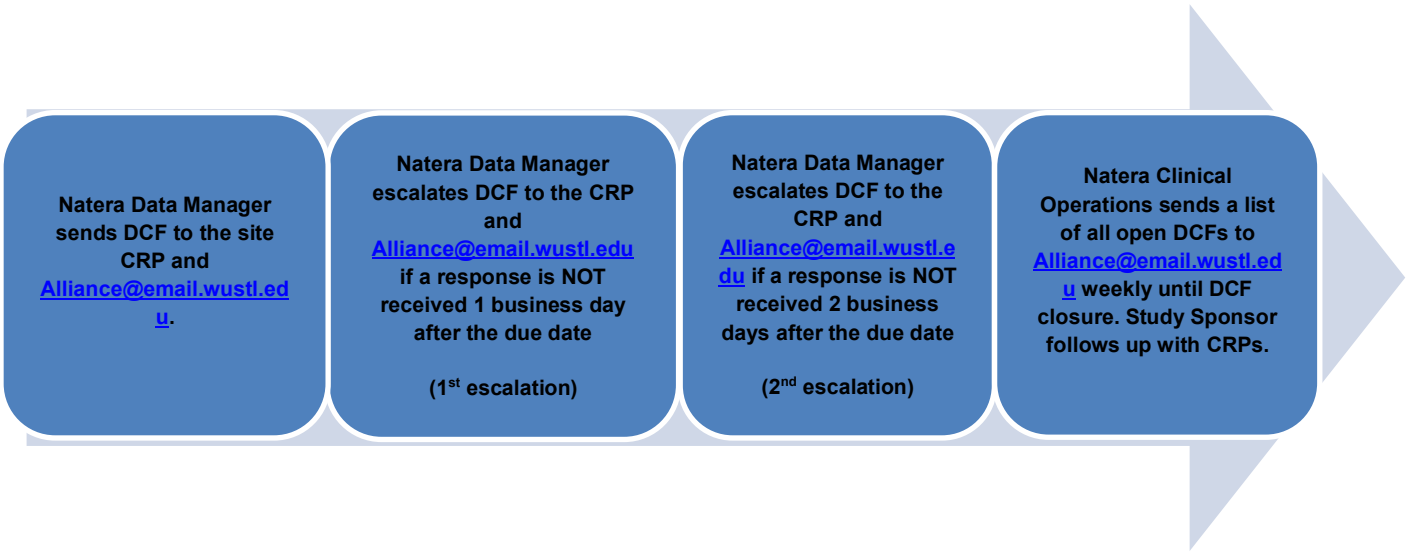
Appendix 6:

Natera Data Clarification Procedure

Data Clarification Procedure

All e-mails, memoranda, or queries that Natera generates to send to clinical sites should contain the appropriate project identifier (Alliance A032103 MODERN) the date, and the subject identifier. Alliance@email.wustl.edu is copied on site communications sent by Natera to sites.

Queries to sites (CRPs) are sent using the Data Clarification Form (DCF) via the Virtru e-mail encryption method or any other approved method used for the study (see template DCF in Appendix A). Please refer to figure below on the DCF Escalation pathway for the Natera team.



The Subject Line for site queries has the following format:

[ACTION REQUIRED] Study ABC_Site ID_Subject ID_DCF-ID_Date-of-Query

Example: [ACTION REQUIRED] Alliance A032103 MODERN_Site 12345_Subject ID 1234567_DCF-001.0001_08Jan2020

Sites are expected to review each DCF, complete the requested information, sign, and scan the completed form back to AllianceMODERN-A032103@natera.com within 3 business days. Natera recommends, but does not require, sites to return the completed form via Virtru encrypted e-mail or another secure method to resolve sample holds for processing and to minimize the risk of unauthorized access. To maintain subject confidentiality, direct subject identifiers must not be included on this form.

If the DCF is unanswered by the due date, Natera sends a reminder email 1 business day after the due date for a response within 1 business day with the following format:

> [URGENT ACTION REQUIRED] Study ABC_Site ID_Subject ID_DCF-ID_Date of Original Query
>
> Example: [URGENT ACTION REQUIRED] Alliance A032103 MODERN_Site 12345_Subject ID 1234567_DCF-001.0001_08Jan2020

Urgent follow-up queries are sent every business day until the DCF is resolved and is escalated to Alliance@email.wustl.edu after 3 attempts. DCF queries still pending follow-up may remain open and continue resolution post reporting for non-critical data clarification fields that do not impact sample processing or results release.

NOTE: the Subject ID in both the DCFs and the Subject Line of the DCF email notification will contain the Alliance Patient ID; however, the field on the DCF and the Subject Line of the email will still read “Subject ID” as seen in the examples above and in the form in the Appendix. The form itself is standard across all Natera studies, and the Subject Line is auto-generated.

8.2 Data Clarification Form Closure Procedure

A non-critical DCF is a query that does not stop Signatera testing and is not a field included in the Signatera Results Report headers. If a response from the Site is not obtained within 30 calendar days from the date the non-critical DCF was issued and after the follow-up and escalation procedure, Natera Data Management closes the DCF and indicates that a response from the Site has not been received after 4 attempts.

A critical DCF is a query that stops Signatera testing and is a field included in the Signatera Result Report headers. These DCFs are escalated to Alliance@email.wustl.edu per the DCF follow-up and escalation process. Alliance@email.wustl.edu must follow up with the site until DCF resolution. However, if a response from the Site is not obtained within 14 calendar days from the date the critical DCF was issued, Alliance A032103 MODERN provides a signed memo to initiate DCF closure.