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 A022004. 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), by Dartmouth Hitchcock Medical Center, and by Guardant Health, 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 A022004 biospecimen collection, processing, and submission, including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for A022004 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
H&E	Hematoxylin and eosin
TRF	Guardant Health A022004 REVEAL Clinical Trial Test Requisition Form

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: Rona D. Yaeger, MD yaegerr@mskcc.org Nursing Contact: Barbara Kleiber, RN Barbara.kleiber@osumc.edu Protocol Coordinator: Jamie Crawley jcrawley@bsd.uchicago.edu (where applicable) Data Manager: Joel Kyek kyek.joel@mayo.edu
Questions related to data submission, RAVE or patient follow-up:	Data Manager: Joel Kyek kyek.joel@mayo.edu
Questions regarding the protocol document and model informed consent:	Protocol Coordinator: Jamie Crawley jcrawley@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 specimens/specimen submissions:	**Tissue submission to Dartmouth Hitchcock Medical Center for real-time BRAF V600E mutation testing:** Amber Barrows 1-603-650-5498 or amber.j.barrows@hitchcock.org. **Blood submission to Guardant Health for *integral* ctDNA analysis:** 1-855-698-8887, option 1 or A022004@guardanthealth.com **All other biospecimens:** Alliance Biorepository at Washington University (WUSTL), alliance@email.wustl.edu
Questions regarding drug supply:	McKesson Specialty Pharmacy: crs_intake@mckesson.com
Questions regarding drug administration:	Pharmacy Contact: Myounghee Lee, PhD, PharmD Mlee1@umm.edu

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 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 A022004 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, Dartmouth Hitchcock Medical Center, and Guardant Health. 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 one (1) 5 micron H&E stained slide and two (2) 5 micron unstained slides to Dartmouth Hitchcock Medical Center for BRAF V600E mutation testing. The submission of these samples for BRAF mutation testing is required for all patients registered to this study, including those who are found to be ineligible, those who do not receive protocol therapy, and patients who have already been determined to have the BRAF V600E mutation locally. 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 pre-registered to A022004						
Within 14 days after pre-registration	N	Archival tissue for BRAF V600E testing	2 unstained slides (5 um) AND 1 H&E stained slide	Fixed tissue slides (9.2)	Ambient / Dartmouth Hitchcock Medical Center	1
Mandatory for <u>all</u> patients registered to A022004						
Within 7 days after registration/ Prior to randomization	Y-Guardant Kit	Whole blood (Streck BCT)	4 x 10 ml	Plasma for ctDNA-Guardant (10.1)	Ambient / Guardant	2
Within 14 days after randomization/ Prior to intiation of treatment	Y-ABWUSTL Kit	Whole blood (Streck BCT)	2 x 10 ml	Plasma for ctDNA-ABWUSTL (10.2)	Ambient / ABWUSTL	3
4 weeks after initiation of treatment (+/- 7 days)	Y-ABWUSTL Kit	Whole blood (Streck BCT)	2 x 10 ml	Plasma for ctDNA-ABWUSTL (10.2)	Ambient / ABWUSTL	3
6 months after randomization (+/- 7 days)	Y-Guardant Kit	Whole blood (Streck BCT)	4 x 10 ml	Plasma for ctDNA-Guardant (10.1)	Ambient / Guardant	4
6 months after randomization (+/- 7 days)	Y-ABWUSTL Kit	Whole blood (Streck BCT)	2 x 10 ml	Plasma for ctDNA-ABWUSTL (10.2)	Ambient / ABWUSTL	3
12 months after randomization (+/- 1 month)	Y-ABWUSTL Kit	Whole blood (Streck BCT)	2 x 10 ml	Plasma for ctDNA-ABWUSTL (10.2)	Ambient / ABWUSTL	3
24 months after randomization (+/- 1 month)	Y-ABWUSTL Kit	Whole blood (Streck BCT)	2 x 10 ml	Plasma for ctDNA-ABWUSTL (10.2)	Ambient / ABWUSTL	3
For patients consented to A022004 Biobanking						
Within 14 days after randomization/ Prior to initiation of treatment	N	Fixed tissue block	1	Fixed tissue block (9.3)	Ambient / ABWUSTL	5, 6

Within 14 days after randomization/ Prior to initiation of treatment	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	5, 6
Within 14 days after randomization/ Prior to initiation of treatment	Y-ABWUSTL Kit	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.3)	Dry Ice / ABWUSTL	5, 7
Within 14 days after randomization/ Prior to initiation of treatment	Y-ABWUSTL Kit	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.4)	Dry Ice / ABWUSTL	5, 7
4 weeks after initiation of treatment (+/- 7 days)	Y-ABWUSTL Kit	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.3)	Dry Ice / ABWUSTL	5, 7
4 weeks after initiation of treatment (+/- 7 days)	Y-ABWUSTL Kit	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.4)	Dry Ice / ABWUSTL	5, 7
6 months after randomization (+/- 7 days)	Y-ABWUSTL Kit	Whole blood for plasma	6 x 1 ml aliquots	Frozen plasma (10.3)	Dry Ice / ABWUSTL	5, 7
6 months after randomization (+/- 7 days)	Y-ABWUSTL Kit	Whole blood for "buffy coat"	2 aliquots	"Buffy Coat" (10.4)	Dry Ice / ABWUSTL	5, 7

1. Sites will submit tissue directly to Dartmouth Hitchcock Medical Center for BRAF V600E mutation testing. Sites are required to submit 1 (5 um) H&E stained slide **AND** 2 (5 um) unstained slides for all patients registered to this study. Please see additional details in **section 9.2**.
2. Real-time central ctDNA testing will be conducted at Guardant Health. The submission of baseline samples for ctDNA testing is required for all patients registered to this study, including those who are found to be ineligible, and those who do not receive protocol therapy.
3. Whole blood (Streck BCT) for isolation of buffy coat and plasma at ABWUSTL.
4. Mandatory 6-month blood specimens for *integral* ctDNA testing only required for patients enrolled to the Phase II portion of this study.
5. Collection is optional for patients but requires all sites offer to patients to consent.
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 9.3**.

7. Peripheral blood (EDTA) 2 x 10 ml to be processed for plasma (6 x 1-1.5 ml aliquots) and "buffy coat", frozen on site and shipped on dry ice.

7. Biospecimen Collection Kits

7.1 <u>There are separate kits for the mandatory blood collection to Guardant Health and the mandatory blood collection to the Alliance Biorepository at Washington University in St. Louis. Please ensure the proper kits are ordered depending on where the blood samples are being sent.</u>

7.2 ABWUSTL Blood Collection

7.2.1 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.

7.2.2 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 (Streck BCT tubes, EDTA tubes) have expiration dates, do not request kits more than 90 days prior to their anticipated use. **<u>All kits must be requested by using the BioMS system.</u>**

7.2.3 Kit contents and specific instructions for use of the kit are provided in the kit box. **During warm weather months (i.e June – August), a refrigerated pack (not frozen) should be included in shipments to ABWUSTL to maintain ambient temperature. When shipping during other months of the year, a room temperature pack should be included in the shipment.**

7.2.4 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.

7.2.5 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).

7.2.6 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.

7.2.7 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.

7.2.8 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.

7.2.9 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 kit components are highly specialized (e.g. Streck BCT) and probably are not available at the institution.

7.2.10 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.

7.3 Guardant Health Blood Collection

7.3.1 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. Kits from Guardant Health include a pre-paid FedEx airbill for return shipping.

7.3.2 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 (Streck BCT 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.**

7.3.3 Kit contents and specific instructions for use of the kit are provided in the kit box. Shipments to Guardant Health should be held at ambient temperature and the included gel packs should not be refrigerated, even during warm weather months.

7.3.4 Guardant Health kits that have expired or missing components should be discarded according to institutional policy. Discarded kits should be recorded on the Guardant Health Investigational Kit Destruction Log (**Appendix 1**) to be maintained as part of the site's records.

7.3.5 It is recommended that sites have at least one additional kit on hand in the event that a biospecimen collection component (e.g Streck BCT tube) is missing, damaged, or expired. 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.

7.3.6 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.

7.3.7 **For samples sent to Guardant Health, please ensure all 4 tubes provided in the kit are filled with a minimum of 3 ml of blood. Otherwise, the samples will not be accepted.**

7.4 Dartmouth Hitchcock Medical Center and ABWUSTL Tissue Collection

7.4.1 **No kits are provided for submission of mandatory tissue to Dartmouth Hitchcock Medical Center for BRAF V600E mutation 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:**

7.4.2 Blocks and slides should be packaged to avoid breakage using a padded envelope or, preferably, a small Styrofoam container.

7.4.3 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.

7.4.4 Blocks, sections and slides may be shipped for standard overnight delivery according to institutional policies and using the preferred vendor.

7.5 Please see Section 11 – Biospecimen Shipping for specific instructions on shipping to ABWUSTL, Dartmouth Hitchcock Medical Center, and to Guardant Health.

8. Biospecimen Labeling and Tracking

8.1 ABWUSTL

8.1.1 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.

8.1.2 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 Alliance patient ID number, institutional surgical pathology number, the block identifier, the section thickness, and serial section number (1, 2, etc.) if applicable.

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

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

8.1.5 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. 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.**

8.1.6 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. 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.

8.2 Guardant Health

8.2.1 Specimens shipping to **Guardant Health** should be labeled with Guardant Health's barcode labels which are provided in the Guardant Health biospecimen collection kits. Each barcode label must include the Alliance patient ID number and date and time of specimen collection. Four (4) of the barcode labels should be placed on each of the collected tubes. The fifth (5^{th}) barcode label should be placed on the Guardant Health A022004 REVEAL Clinical Trial Test Requisition Form (TRF, **Appendix 2**). The sixth (6^{th}) barcode label is provided as a backup.

8.2.2 All biospecimens that are collected and sent to Guardant Health 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. 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.**

8.3 Dartmouth Hitchcock Medical Center

8.3.1 Each slide containing tissue sections should be labeled with the Alliance patient ID number, institutional surgical pathology number, the block identifier, the section thickness, and serial section number (1, 2, etc.).

8.3.2 Label all slides with an indelible, solvent-resistant marker when they are at ambient temperature.

8.3.3 Do not affix any labels to slides. Label the collection containers directly with the marking pen.

8.3.4 All biospecimens that are collected and sent to Dartmouth Hitchcock Medical Center 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. 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.**

8.3.5 A de-identified copy of the surgical pathology report is **not** required to accompany the tissue submission to Dartmouth Hitchcock Medical Center.

8.3.6 The A022004 CLIA Laboratory BRAF V600E Mutation Testing Sample Submission Form must be submitted along with these slides to Dartmouth Hitchcock Medical Center. Failure to submit this form with the slides may delay turnaround time for central testing. The A022004 CLIA Laboratory BRAF V600E Mutation Testing Sample Submission Form can be located in **Appendix 3** of this manual or on the A022004 protocol-specific page on the CTSU and Alliance websites. A digital copy of the form and the shipment tracking number should be sent at the time of shipment to amber.j.barrows@hitchcock.org.

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 BRAF V600E Mutation Testing

9.2.1 A set of two (2) 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. 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 unstained slides	Section thickness	Slide type	Purpose
2	5 microns	Charged or Non-Charged	BRAF V600E mutation testing for eligibility

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

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

9.2.4 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 (5 microns).

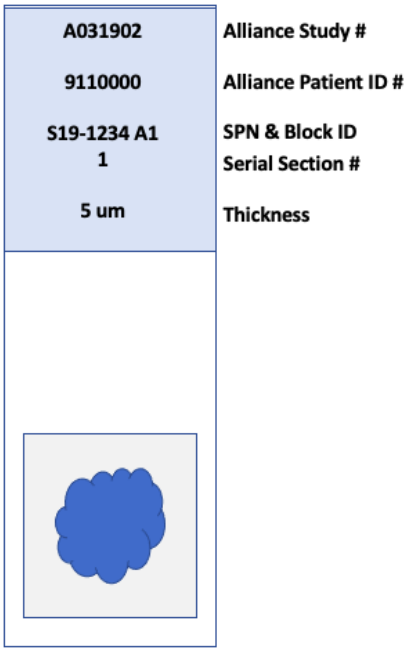
9.2.5 Do not label slides with adhesive labels. Write or print information on the textured surface of the slide with indelible, solvent-resistant ink.

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

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

9.2.8 When placing the sections onto the slides, ensure that the tissue is placed on the bottom third of the slide. Ensure that each serial section from the block is placed in the same orientation on each slide.

9.2.9 See figure below for proper mounting and labeling.



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

9.2.11 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.

9.2.12 The A022004 CLIA Laboratory BRAF V600E Mutation Testing Sample Submission Form must be submitted along with these slides to Dartmouth Hitchcock Medical Center. Failure to submit this form with the slides may delay turnaround time for central testing. The A022004 CLIA Laboratory BRAF V600E Mutation Testing Sample Submission Form can be located in **Appendix 3** of this manual or on the A022004 protocol-specific page on the CTSU and Alliance websites. A digital copy of the form and the shipment tracking number should be sent at the time of shipment to amber.j.barrows@hitchcock.org.

9.2.13 Turnaround time for testing is 5 business days from time of receipt of tissue and all required documents (i.e. slides, query free A022004 CLIA Laboratory BRAF V600E Mutation Testing Sample Submission Form). Resubmission of tissue in the event of specimen failure (adequate tissue is submitted, but testing unable to be performed) is acceptable. If resubmission is required, a request will come from Dartmouth Hitchcock Medical Center directly with further instructions.

9.2.14 Sites will receive results via secure email to the email address provided on the A022004 CLIA Laboratory BRAF V600E Mutation Testing Sample Submission Form. It is very important that the email address be entered correctly on the BRAF V600E submission form to prevent delay.

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 original primary tumor diagnostic biopsy.

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**.

10. Blood Collection Methods

10.1 Plasma Nucleic Acid (Streck) Tube Processing to be Sent to Guardant Health

10.1.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the Streck tubes. A total of 40 ml of whole blood should be collected into the Streck tubes (4 x 10 ml) for ctDNA testing at Guardant Health. Following collection, invert tubes 10 times.

10.1.2 Store Streck BCT tubes with whole blood at room temperature. Do not freeze or refrigerate the tubes.

10.1.3 **<u>For blood submitted to Guardant Health, the tubes must be shipped on the day of collection and received at the lab within 24 hours of collection.</u>** Guardant Health is open for sample receipt Monday through Saturday, excluding major US holidays.

10.1.4 A physical copy of the A022004 REVEAL Clinical Trial Test Requisition Form must be submitted with the Streck BCT tubes to Guardant Health. The A022004 REVEAL Clinical Trial Test Requisition Form can be located in **Appendix 2** of this manual or on the A022004 protocol-specific page on the CTSU an Alliance websites.

10.1.4.1 All shaded boxes MUST be filled in. In row 1, please additionally add your site Prinicipal Investigator name. In the notes section, please additionally add the name of a reliable site point of contact and their email address.

10.1.5 One A022004 REVEAL Clinical Trial Test Requisition Form should be submitted for each patient / time point. A completed barcode label must be placed in the upper right corner of the form. The form must be signed by authorized site personnel (does not have to be the principal investigator). A photocopy of the form should be retained for the site's records, and the original should be submitted with the Streck BCT tubes to Guardant Health in the outer pocket of the specimen bag.

10.1.6 In the event a specimen is received with incomplete or inlegible documentation, the site will be contacted for resolution.

10.2 Plasma Nucleic Acid (Streck) Tube Processing to be Sent to ABWUSTL

10.2.1 Collect 10 ml of whole blood by standard venous phlebotomy technique into each of the Streck tubes. A total of 20 ml of whole blood should be collected into the Streck tubes (2 x 10 ml) for ctDNA isolation at ABWUSTL. Following collection, invert tubes 10 times.

10.2.2 **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.

10.3 Plasma Processing

10.3.1 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.

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

10.3.3 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 10.4).

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

10.3.5 Label 6 cryovials as instructed in **section 8**. Make certain each vial is labeled completely and identically.

10.3.6 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.

10.3.7 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.

10.4 "Buffy Coat" (White Blood Cell) Processing

10.4.1 Follow procedures in section 10.3 for collecting and processing plasma from EDTA tubes.

10.4.2 Label 2 cryovials as instructed in **section 8**.

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

10.4.4 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 Celsius 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.

11. Biospecimen Shipping

11.1 Overview

11.1.1 Please see the instructional 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. 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 Tissue sent to ABWUSTL must include a de-identified copy of the surgical pathology report labeled with the Alliance patient ID number.

11.1.4 Tissue sent to Dartmouth Hitchcock Medical Center should additionally be accompanied by the A022004 CLIA Laboratory BRAF V600E Mutation Testing Sample Submission Form.

11.1.5 Streck BCT tubes sent to Guardant Health must additionally include the A022004 REVEAL Clinical Trial Test Requisition Form. Please see section 10.1.4.1 for additional instructions.

11.1.6 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.

11.1.7 **Do not ship samples to ABWUSTL or Dartmouth Hitchcock Medical Center on Friday, Saturday, Sunday or the day before a nationally recognized holiday.**

11.1.8 Guardant Health is open for sample receipt Monday through Saturday, excluding major US holidays. For questions regarding shipping via FedEx, please contact Guardant Health Client Services via email at clientservices.trials@guardanthealth.com.

11.2 Shipping to Dartmouth Hitchcock Medical Center:

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

Ship to:
Amber Barrows
Clinical Genomics and Advanced Technology
Department of Pathology and Laboratory Medicine
4th Floor WTRB
Dartmouth Hitchcock Medical Center
1 Medical Center Drive
Lebanon, NH 03756
Phone: 603-650-5498

11.3 Shipping to ABWUSTL

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

Ship to:
Alliance Biorepository 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

11.4 Shipping to Guardant Health

11.4.1 Ship container using the pre-printed FedEx airbill that is provided in the study kit for PRIORITY OVERNIGHT DELIVERY according to IATA guidelines and standard institutional policies.

Ship to:
Guardant Health
ATTN: Biospecimen Management
505 Penobscot Drive
Redwood City, CA 94063
Phone: 1-855-698-8887, Option 1

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
2.1	Updated data manager in contact table	KL	12/15/2023
2.0	Updated Guardant forms Added contact information from protocol	PAA	07/28/2023
1.1	Added instructions for filling out Guardant TRF	KAL	04/13/2013
1.0	New	AAW	02/24/2023

Appendix 1: Guardant Health Investigational Kit Destruction Log

INVESTIGATIONAL KIT DESTRUCTION LOG

INVESTIGATOR NAME:			
SITE NUMBER:		**PROTOCOL ID #:**	
PROTOCOL NAME:			

Kit Information			Kit Destruction		
Kit-Track ID - Lot #	**Kit-Track ID - Barcode #**	**Kit Expiry Date dd-MMM-yyyy**	**Date Destroyed dd-MMM-yyyy**	**Confirmed By (Initials)**	**Reason for Destruction**

Comments:

Principal Investigator, Study Coordinator, or Designee (Signature) **Date**

Monitor (Print Name) **Date**

Appendix 2: Guardant Health A022004 REVEAL Clinical Trial Test Requisition Form

GUARDANTREVEAL™

clientservices.trials@guardanthealth.com | 855.698.8887
NPI 1184045619

TRIAL

– Required –
Place Barcode
Here

REVEAL Clinical Trial Test Requisition Form
(A022004)

All shaded boxes MUST be filled in

Study ID: A022004

1. SITE PRINICIPAL INVESTIGATOR

Site Principal Investigator Name

The study subject has signed an informed consent to participate in this study, which includes a REVEAL® test as a part of study participation.

X _____
Authorized Site Personnel Signature

2. INVESTIGATOR SITE INFORMATION (Must complete if not preprinted)

3. SUBJECT INFORMATION

Subject ID _____

Sex ☐ F ☐ M

4. SPECIMEN INFORMATION

Blood Collection Date (mmm/dd/yyyy)

Name of Person Collecting Blood Specimen

Email address for Person Collecting Blood Specimen

5. DIAGNOSIS: STAGE II / III Colorectal Carcinoma

Date of Surgery (mmm/dd/yyyy)

6. TIMEPOINT (MUST choose one)

☐ Baseline ☐ 6 Months

GH Internal Use only: ALL_03 (Processing) ALL_04_Retro (Biobank Samples)

NOTES

CAUTION – Investigational device.
Limited by Federal (or United States) law to investigational use.

LBL-000340 R2

Appendix 3: A022004 CLIA Laboratory BRAF V600E Mutation Testing Sample Submission Form

ALLIANCE A022004
Tumor Tissue for BRAF Testing at Dartmouth

The submission of these samples for BRAF V600E mutation testing is required for all patients pre-registered to this study.

This form must be filled out, printed, and submitted along with the slides to the lab at Dartmouth Hitchcock Medical Center. The form must be completed by typing; **do not handwrite. Failure to submit this form with the specimen may delay turnaround time for BRAF analysis.**

Patient Initials:_____ Alliance Patient ID:_____ Date of Birth (DD/MM/YYYY)_____

Site Name:_____Site CTEP ID: _____

Collection Date: _____ Courier Tracking Number: _____

Percentage of tumor cell content (If Available) _____

Diagnosis_____

Specimens being submitted: ☐ 1 H & E slide ☐ 2 Unstained slides, 4 - 6 microns.

Responsible CRA Name: _____

E-mail Address: _____

Phone Number:_____Emergency Contact Number*: _____

Alternate CRA Name: _____

E-mail Address: _____

Phone Number: _____ Emergency Contact Number*_____

**Please provide a pager or cell phone number for questions outside of regular business hours.*

Comments (unusual circumstances during collection/processing of samples):

Shipped by: _____ Shipment Date: _____

If there are any questions regarding specimen submission, please contact Amber Barrows, DHMC-CGAT Research Coordinator. Email: amber.j.barrows@hitchcock.org; Tel: 603-650-6821

ver: 11/9/21.