**CORRELATIVE SCIENCE PROCEDURE MANUAL**

**AFT-42:** SAFETY, ACTIVITY, AND PHARMACOLOGY OF NIVOLUMAB IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER AND PRE-EXISTING AUTOIMMUNE DISEASE

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**AFT-42 Correlative Science Procedure Manual Signature Page**

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## 1. PURPOSE

This document describes the procedures required for the collection, shipping, and processing of biospecimens from all patients enrolled or registered on AFT-42. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the AFT Biorepository (AFB) (i.e., Siteman Cancer Center Tissue Procurement Core at Washington University) and Duke 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 AFT-42 study biospecimen collection, processing, and submission, including staff at satellite institutions.

## 2. SCOPE

This document applies to all biospecimens collected specifically for the AFT-42 study only. Please refer to the study 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 can be found on the AFT BioMS web site which is accessible via the AFT portal: (https://alliancefoundationtrials.org)

## 3. CONTACT INFORMATION

**3.1.** Please send any questions and problems related to protocol administration, eligibility, patient registration, and data submission, to the AFT Project Manager at: AFT42@alliancefoundationtrials.org or 1-617-525-8627.

**3.2.** For specific questions about kits or shipments, please contact the Siteman Cancer Center Tissue Procurement Core at: 1-314-454-7615 or tbank@wudosis.wustl.edu.

**3.3.** For questions about using the AFT BioMS web application for ordering kits, or registering and shipping biospecimens, please contact: 1-855-642-4667 or aftbiomshelp@email.wustl.edu

**3.4.** For any other questions about biospecimen procurement and shipping procedures, please contact the AFB Program Manager at: 1-314-747-4402 or afbhelp@email.wustl.edu

## 4. SITE PREPARATION

**4.1.** Please refer to the AFT-42 protocol for any specific requirements related to patient enrollment, registration, and regulatory compliance.

**4.2.** The AFT BioMS application is used for both requesting biospecimen collection kits and for logging the collection and shipment of biospecimens to the AFT Biorepository. For training and assistance in using the AFT BioMS application, please see the AFT BioMS web site which is accessible via the AFT portal: https://alliancefoundationtrials.org or contact the AFT BioMS Help desk at: aftbiomshelp@email.wustl.edu or 1-855-642-4667.

**4.3.** Prior to collection of biospecimens, a biospecimen collection kit must be at the collection site. Please see section 6 for requesting biospecimen collection kits. Please allow at least 10 working days to receive the collection kit.

**4.4.** Please confirm that your institutional pathology department will release a tumor tissue paraffin block or will be willing to submit tissue section slides from such a block, at the required time

point designated in this document and in the study protocol. An institution whose pathology department is unwilling to comply with tumor block or slide submission should not enroll patients to this study.

## 5. COLLECTION SCHEMA

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

| Time Point | Biospecimen | Quantity | Collection / Processing | Shipping | Recipient | Notes |
|---|---|---|---|---|---|---|
| Screening | Fixed tissue block | 1 | Fixed tissue block (8.2) | Ambient | AFB | 1 |
| Screening | Unstained tumor tissue | 10 | Fixed tissue slides (8.3) | Ambient | AFB | 1 |
| | | | | | | |
| Cycle 1, Day 1 | Whole blood for serum | 2 x 1 ml serum aliquots | Frozen serum for PK Assessment (9.1) | Dry Ice | AFB | 2 |
| Cycle 1, Day 1 | Whole blood (ACD tube) | 3 x 8.5 ml | Whole blood for Immune Cell Biomarker Studies (9.2) | Ambient | Duke | 3 |
| | | | | | | |
| Cycle 1, Day 14 | Whole blood for serum | 1 x 1 ml serum aliquot | Frozen serum for PK Assessment (9.1) | Dry Ice | AFB | |
| | | | | | | |
| Cycle 3, Day 1 | Whole blood for serum | 1 x 1 ml serum aliquot | Frozen serum for PK Assessment (9.1) | Dry Ice | AFB | 4 |
| Cycle 3, Day 1 | Whole blood (ACD tube) | 3 x 8.5 ml | Whole blood for Immune Cell Biomarker Studies (9.2) | Ambient | Duke | 3 |
| | | | | | | |
| Cycle 5, Day 1 | Whole blood for serum | 2 x 1 ml serum aliquots | Frozen serum for PK Assessment (9.1) | Dry Ice | AFB | 5, 6 |
| Cycle 5, Day 14 | Whole blood for serum | 1 x 1 ml aliquot | Frozen serum for PK Assessment (9.1) | Dry Ice | AFB | 6 |
| | | | | | | |
| Cycle 9, Day 1 | Whole blood for serum | 1 x 1 ml serum aliquot | Frozen serum for PK Assessment (9.1) | Dry Ice | AFB | 4 |
| | | | | | | |
| Cycle 13, Day 1 | Whole blood for serum | 1 x 1 ml serum aliquot | Frozen serum for PK Assessment (9.1) | Dry Ice | AFB | 4 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Progression | Whole blood (ACD tube) | 3 x 8.5 ml | Whole blood for Immune Cell Biomarker Studies (9.2) | Ambient | Duke | 3 |
| | | | | | | |
| EOT | Whole blood for serum | 1 x 1 ml aliquot | Frozen serum for PK Assessment (9.1) | Dry Ice | AFB | 4 |

NOTES:

1. Submission of a representative, archived tumor tissue block **OR** 10 unstained slides from such a block is encouraged. An archival block submission is strongly preferred.
2. On cycle 1, day 1, 1 ml of serum will be collected pre-dose and an additional 1 ml will be collected at 30 minutes.
3. Whole blood in ACD tube will be shipped directly to Duke.
4. On cycle 3 day 1, cycle 9 day 1, cycle 13 day 1, and at EOT, 1 ml of serum will be collected pre-dose.
5. On cycle 5 day 1, 1 ml of serum will be collected pre-dose and an additional 1 ml will be collected at 30 minutes.
6. Serum aliquots for all cycle 5 time points should be stored at -70 to -90 degrees Celsius following collection. Three aliquots collected during cycle 5 will be batch shipped to the AFB following cycle 5 day 14 collection.

## 6. BIOSPECIMEN COLLECTION KITS

**6.1.** To facilitate the proper collection and shipping of all 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. A pre-paid FedEx waybill is provided for shipping the kit with the biospecimens back to the AFB at Washington University in St. Louis or to Duke University via priority overnight shipping.

**6.2.** 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 many of the collection materials (vacutainer tubes) have expiration dates, do not request kits more than 90 days prior to their anticipated use. **All kits must be requested using the AFT BioMS system.**

**6.3.** Kit contents and specific instructions for use of the kit are provided in the kit box and are available for download on the AFT BioMS webpage which is accessible via the AFT portal: https://alliancefoundationtrials.org. **Please return any used collection materials with the kit**.

**6.4.** 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.

**6.5.** Please return all components of the kit, regardless of whether they have been used or not. Kits and kit components are recycled when possible, minimizing the kit cost.

**6.6.** Well in advance of collecting biospecimens, inspect the biospecimen collection kit to ensure that all components are present and not expired, particularly if the kit has been onsite for longer than 30 days.

**6.7.** Note that individual kit components that are expired, damaged, or missing cannot be replaced. The remedy is to order a complete, new kit. (Please note in your request that you are replacing an expired or damaged kit).

**6.8.** Please return all kits that have expired or missing components. Return the ENTIRE kit using the pre-paid FedEx waybill provided. 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 out-going and in coming kits are tracked, and sites that have requested many more kits than they have returned will be charged for non-returned kits.

**6.9.** If a biospecimen collection component (e.g. vacutainer collection tube) is missing, damaged, or expired, the institution may substitute a like-kind collection tube from their own supply. However, note that while some kit components are generic (EDTA tubes) others are highly specialized and probably are not available at the institution.

6.9.1. Note that protocol requirements are based on blood volumes, not tube sizes. If the protocol requires the collection of 8 cc of whole blood, generally a 10 cc tube is provided in the kit for convenience. If desirable or necessary to collect 8 cc in 3 x 3 cc tubes (for example), that is permissible.

**6.10.** Please see **Section 10 – Biospecimen Shipping** for specific instructions on packaging biospecimens into the shipping kit for shipment to AFB or to Duke University.

## 7. BIOSPECIMEN LABELING AND TRACKING

**7.1.** All research biospecimens (vacutainer tubes, cryovials) MUST be labeled with the participant study number and patient initials (Last, First, Middle). Blood tubes and cryovials should additionally be labeled with the date and time of collection.

**7.2.** 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 are being submitted instead of the block, each tissue section slide should be labeled with the participant study number and the block identifier. Provide a **de-identified** copy of the surgical pathology report, labeled only with the participant study number, corresponding to the blocks or slides submitted. See section 8 for additional details.

**7.3.** Label all containers and vials with an indelible, solvent-resistant marker when they are at ambient temperature. Do not try to label frozen vials.

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

**7.5.** All biospecimens that are collected and sent to the AFT biorepository (AFB) and to Duke University **must be logged and tracked in AFT BioMS**. The AFT BioMS system is a web-based application that tracks the collection and shipping of biospecimens. Once individual biospecimens are logged and ‘shipped’ in the AFT 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 AFT BioMS system and for further information about training, access, and use, please contacts the AFT BioMS Help desk at: 1-855-642-4667 or aftbiomshelp@email.wustl.edu. Note that the AFT BioMS system is similar to but independent

of the NCI BioMS application, which is used to manage biospecimens collected on NCI NCTN Alliance trials.

**7.6.** In the event that AFT BioMS cannot be accessed, please complete an AFT BioMS specimen manifest form, which can found here- https://cbmiapps.wustl.edu/confluence/display/AB/Forms

## 8. TISSUE COLLECTION METHODS

### 8.1. Overview

8.1.1. Please refer to protocol-specific instructions for procedures related to actual tissue procurement from individual participants.

8.1.2. FFPE cell pellets, alcohol-fixed specimens, fine needle aspirates, ascites fluid, pleural fluid, bone metastases, or cytological specimens will not be accepted. Core needle or excisional biopsies, or resected tissue is requested.

### 8.2. Diagnostic Pathology Fixed Tissue Blocks

8.2.1. This protocol requests submission of ONE representative, diagnostic pathology, formalin fixed paraffin embedded (FFPE) tumor tissue block, if available.

8.2.2. Any clinical surgical pathology block that is submitted for research studies will not be exhausted or rendered unsuitable for future diagnostic use. Any clinical surgical pathology block that is submitted will be returned within ten working days of written request, when needed for clinical management or clinical trial enrollment for a specific patient. Otherwise, all blocks will be returned to the submitting institution when the correlative science study end points have been met.

8.2.3. In the event that an institution will not release a tumor tissue block, the institution may instead submit tissue sections, mounted and unstained to glass slides.

### 8.3. Unstained Slides from Diagnostic Fixed Tissue Blocks

8.3.1. In cases where institutions are unable or unwilling to submit the requested tissue block, a set of 10 unstained tissue slides may be sent as an alternative. Please follow the procedures below for submitting unstained tissue slides. If your pathology department is unwilling or unable to follow these tissue-sectioning instructions, please consider submission of a tissue block, which can be cut at the biorepository and returned to your institution at a later date.

| # of slides | Section Thickness | Slide Type | Purpose |
|---|---|---|---|
| 10 | 5 micron | Positively Charged | Correlative Studies |

8.3.2. Serial, tissue sections should be cut fresh from the appropriate FFPE tissue block.

8.3.3. Cut sections at 5 micron thickness as indicated onto positively charged slides.

8.3.4. Ensure that each slide is labeled with the patient study number, the institutional block ID, and the slide serial section number (1, 2, 3, etc.).

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

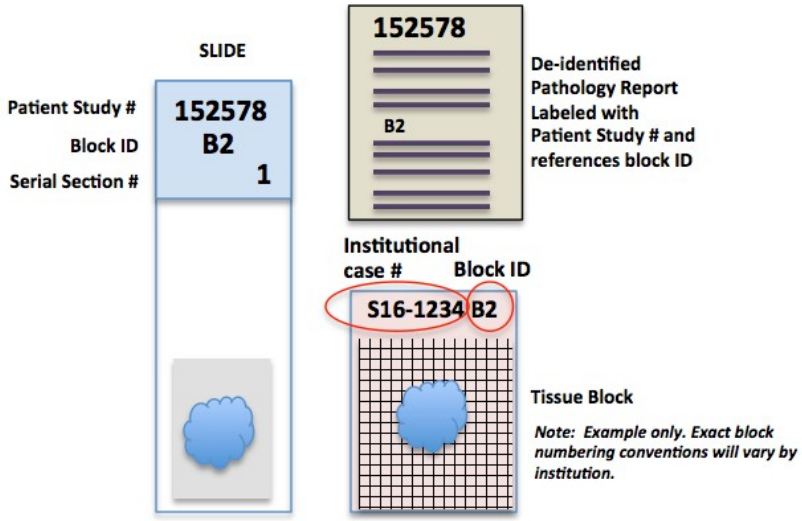
8.3.6. No adhesives or other additives should be used in the water bath.

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

8.3.8. 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.

8.3.9. See figure below for proper mounting and labeling

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




8.3.11. Use the slide boxes provided in the kit for shipping slides. Slides should not be touching each other. Ensure that slides from only one patient are placed in one slide mailer.

8.3.12. Include a copy of a de-identified pathology report, labeled only with the patient study number with all slide submissions.

## 9. BLOOD COLLECTION METHODS

### 9.1. Serum Processing for PK Assessment

9.1.1. Using an indelible, solvent resistant marker, label cryovial with participant study number, sample type (i.e., “serum”), patient initials, and date and time of collection.

9.1.2. If using a non-refrigerated centrifuge, pre-chill the rotor or swinging buckets for at least 1 hour prior to sample processing in a refrigerator set at 2 to 8 degrees. **Do not place in the freezer.**

9.1.3. Collect 3 ml whole blood by standard venous phlebotomy technique into the pre- labeled red top (plain glass with clot activator) tube. Do not collect whole blood into a “tiger top” / “SST” / “gel tube.” Invert tube 10 times.

9.1.4. Allow blood to clot for 30 min at room temperature with tube in upright position.

9.1.5. Spin blood in vacutainer tube using pre-chilled rotors / swinging buckets in non-refrigerated centrifuge or at 4 degrees in a refrigerated clinical centrifuge at 1700 x G for 10 min.

9.1.6. Carefully remove 1 ml of serum (without touching the clot layer) into labeled, 1 ml cryovial.

9.1.7. Freeze serum containing cryovial upright on dry ice or a -70 to -90 degree Celsius ultralow freezer. Store at -70 to -90 degrees Celsius until ready for shipment on dry ice. If -70 to -90 degree Celsius ultralow freezer is not available, serum may be stored in a -20 degree Celsius freezer. The blood samples must be processed to serum and placed in freezer within 1 hour of collection.

### 9.2. Whole blood for Immune Cell Biomarker Studies (ACD Tube - no processing)

9.2.1. Collect 8.5 ml of blood into each of the ACD tubes using standard venous phlebotomy. Invert tubes 10 times.

9.2.2. Store ACD tubes with whole blood at ambient temperature until shipping. Do not freeze or refrigerate the tubes. **Blood should be collected Monday—Thursday only. The tubes must be shipped on the same day they are collected and must be received by Duke University within 24 hours of collection (e.g. Friday—Saturday collections or Holiday collections are not allowed).** Ensure that the ACD tubes are shipped at ambient temperature to avoid freezing.

## 10. BIOSPECIMEN SHIPPING

### 10.1. Overview

10.1.1. All biospecimens should be shipped on the same day that they are collected (Monday – Thursday). Biospecimens must be received by the AFB or Duke University within 24 hours of collection. If collected biospecimens cannot be shipped on the same day that they are collected (e.g. Friday – Saturday or Holiday collections), please contact the AFB Program Manager at 1-314- 747-4402 for further instructions, at least 24 hours prior to anticipated collection.

10.1.2. Please see the Directions for Use (DFU) document that is included in each kit, for specific directions on how to package and ship biospecimens. The DFU document is also available for download on the AFT BioMS webpage which is accessible via the AFT portal: (https://alliancefoundationtrials.org).

10.1.3. Place the original, completed copy of the AFT BioMS packing manifest and a copy of the de-identified surgical pathology report, labeled with the patient study number in the kit. **Do not send specimens without a completed AFT BioMS packing manifest or substitute "AFT BioMS Downtime Form." Biospecimens cannot be accepted without this completed form.**

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

**10.2. Shipping to AFB**

10.2.1. Ship container for PRIORITY OVERNIGHT DELIVERY using the pre-paid FedEx waybill provided within the specimen collection kit. Ship to:

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

**10.3. Shipping to Duke**

10.3.1. Ship container for PRIORITY OVERNIGHT DELIVERY using the pre-paid FedEx waybill provided within the specimen collection kit. Notice of shipment and FedEx tracking information should be emailed to Substrate Services Core: surgerysubstrate@dm.duke.edu. Ship to:

Attn: Substrate Services Core and Research Support
Duke University Medical Center
203 Research Drive
MSRB I, Room 459
Durham, NC 27710
Phone: (919) 684-2294

## 11. AFB BIOSPECIMEN RECEIPT AND QUALITY ASSURANCE MEASURES

**11.1.** Upon receipt at AFB, all biospecimens will be accessioned into the TPC informatics system, OpenSpecimen.

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

**11.3.** 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.

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

**11.5.** 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 AFB Program Manager, who will contact the submitting site for reconciliation.

**11.6.** Aliquoted biofluids will be stored under liquid nitrogen vapor.

**11.7.** All biospecimens will remain in storage until additional processing or review is requested in writing by the appropriate protocol principal investigator.

## 12. DOCUMENT HISTORY

| VERSION | DESCRIPTION AND JUSTIFICATION OF CHANGE | AUTHOR | EFFECTIVE DATE |
|---|---|---|---|
| 2.0 | Reformatted to AFT Template<br>Updated Collection Schema<br>Updated shipping address for Duke<br>Updated contact information for AFT PM | PAA | 15-June-2018 |
| 1.0 | New | PAA | 09-Jun-2017 |