CORRELATIVE SCIENCE PROCEDURE MANUAL

1. Purpose

This document describes the procedures required for the collection, shipping, and processing of biospecimens from patients pre-registered or registered on the ***A071601 PHASE II TRIAL OF BRAF/MEK INHIBITORS IN PAPILLARY CRANIOPHARYNGIOMAS***. This document also describes the procedures that will be followed subsequent to the receipt of biospecimens by the Alliance biorepository, 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 *A071601* biospecimen collection, processing, and submission; including staff at satellite institutions.

2. Scope

This document applies to all biospecimens collected specifically for ***A071601 PHASE II TRIAL OF BRAF/MEK INHIBITORS IN PAPILLARY CRANIOPHARYNGIOMAS*** 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
ABMAYO	Alliance Biorepository at Mayo Clinic
FFPE	Formalin fixed, paraffin embedded

4. Contact Information

4.1 For questions and problems related to protocol administration, eligibility, patient registration, and data submission, please contact the relevant contact listed on protocol page 1&2.

4.2 For information on using the BioMS system, please refer to the 'Help' links on the BioMS webpage to access the on-line user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org . For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org .

4.3 There is **NO study kit** provided for samples collection or submission.

4.4 For any other questions about biospecimen procurement and shipping procedures, please contact the Alliance Biorepository at Mayo Clinic

- Paraffin-embedded tissue: Helen Tollefson 507-266-0724 Tollefson.Helen@mayo.edu
- Non-paraffin-embedded samples: Roxann Neumann 507-538-0602 neumann.roxann@mayo.edu

5. Site Preparation

5.1 Please refer to the A071601 protocol document for any specific requirements related to patient eligibility, registration, and regulatory compliance.

5.2 Please ensure that you have appropriate log on credentials and can successfully access the BioMS application found at https://biomstest.wustl.edu/bioms/login. The BioMS application is used for logging the collection and shipment of biospecimens to the Alliance biorepository. For BioMS training, or assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org.

5.3 Please confirm that your institutional pathology department is willing to release one H&E and two unstained slides from the diagnostic surgical/biopsy tissue samples ≤ 28 days after pre-registration. Please also confirm that your pathology department is willing to release tumor tissue paraffin blocks, from surgical tissue prior to protocol treatment, and from surgical tissue at recurrence/progression; **OR** will be willing to submit seventeen 4-5 micron unstained slides (do not bake or coverslip slides) from such blocks. An institution whose pathology department is unwilling to comply with tumor block or slide submission should not enroll patients to this study.

5.4 Identify a reliable source of 4 degree refrigerators and cold pack (not frozen) for sample storage and shipment prior to shipment.

6. Collection Schema

Please refer to the specific protocol document (protocol section 6.2) for the precise biospecimen collection schedule. The following biospecimens are to be collected at each of the following time points. Please refer to individual collection kit instructions, biospecimen collection and processing methods, and specific shipping procedures below.

Time Point	Kit (Y/N)	Biospecimen	Quantity	Collection / Methodology	Shipping	Notes
≤ 28 days after Pre-Registration	N	Fixed tumor tissue	See section 8.1	Fixed tissue slides (8.1)	Ambient	1
Prior to Protocol Tx	N	Fixed tumor tissue	See section 8.1	Fixed tissue blocks or slides (8.1)	Ambient	2,3
Prior to Protocol TX	N	Whole blood for "buffy coat" (EDTA)	10ml	"Buffy Coat" (9.1)	Refrigerated Cold Pack	3
Prior to Protocol TX	N	Platelet Poor Plasma for nucleic acid (EDTA)	20ml	Plasma nucleic acid (9.2)	Frozen Dry Ice	3
Day1 of Cycle 3 (+/- 3days)	N	Platelet Poor Plasma for nucleic acid (EDTA)	20ml	Plasma nucleic acid (9.2)	Frozen Dry Ice	3
End of Protocol Tx	N	Platelet Poor Plasma for nucleic acid (EDTA)	20ml	Plasma nucleic acid (9.2)	Frozen Dry Ice	3
At Recurrence and Progression	N	Fixed tumor tissue	See section 8.1	Fixed tissue blocks or slides (8.1)	Ambient	2,3
At Recurrence and Progression	N	Platelet Poor Plasma for nucleic acid (EDTA)	20ml	Plasma nucleic acid (9.2)	Frozen Dry Ice	3

Notes:

1. One H&E and 2 unstained slides (charged, 4-5 micron thickness) from diagnostic tumor block should be submitted for all patients within 28 days after patient pre-registration. For patients with recurrent disease at pre-registration, one H&E and 2 unstained slides from the recurrent biopsy specimen block should be submitted in addition to the original diagnostic slides.
2. A paraffin tumor tissue block from the surgical resection/biopsy or 15 unstained slides (charged, 4-5 micron thickness) from such block should be submitted for all patients opted in for A071601 ST1 up to 6 months after study registration. For patients with recurrent disease at pre-registration, one tumor block (or 15 unstained slides) from the recurrent biopsy specimen block should be submitted in addition to the original diagnostic block/slides.

3. "Prior to Protocol Tx", "Day1 of Cycle 3", "End of Protocol Tx" and "At recurrence and progression" biospecimen collection are optional for patients and requires additional patient consent for A071601-ST1. Please see protocol –specific consent documents.

7. Biospecimen Labeling and Tracking

7.1 All blood biospecimens MUST be labeled with the study patient ID # assigned at pre-registration, patient initials (Last, First, Middle), the date and time of collection, and the specimen type.

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 surgical pathology number and the block identifier. **Please do NOT use sticky labels on slides.** Provide a **de-identified** copy of the surgical pathology report, labeled only with the participant study ID number, corresponding to the blocks or slides submitted. **A copy of de-identified pathology report is required for each and all tissue submission.** 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, and photocopying the report. Please make sure to keep the pathology accession numbers.

7.3 Label all content and vials with the indelible marker provide, when they are at ambient temperature.

7.4 Do NOT affix any labels to vials, slides, tubes. Label the collection containers directly with the marking pen.

7.5 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. <u>This manifest must be printed out and must accompany all biospecimen shipments</u>. 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. Tissue Collection Methods

A copy of de-identified pathology report should be included in each and all tissue submissions.

8.1 Central Pathology and BRAF testing

For **all** patients pre-registered to A071601, submission of ONE H&E slide, TWO unstained slides (**charged**, 4-5micron) cut from representative, diagnostic pathology, formalin fixed paraffin embedded tumor tissue block (diagnostic biopsy and/or surgery) is required $\leq$ 28 days after patient pre-registration. This submission is MANDATORY. For patients with recurrent disease at pre-registration, one H&E and 2 unstained slides from the recurrent biopsy specimen block should be submitted in addition to the original diagnostic slides. Patients should not be enrolled to the study if the institution is unwilling to provide fixed, diagnostic material. For this Mandatory submission, please download and complete the "Central Pathology and BRAF Results Form" from the Alliance or CTSU websites. The completed result form is **REQUIRED** to be submitted for central review. See protocol Section 4.4 , 4.5 and 6.2.1 for more information.

8.2 Tissue for A071601-ST1

For patients who consent to A071601 ST1, a representative diagnostic block should be submitted prior to initiation of protocol treatment. For patients with recurrent disease at pre-registration, the recurrent biopsy specimen block should be submitted in addition to the original diagnostic slides.

For recurrence or progression after registration to A071601, a tissue block from recurrent disease and progression disease should also be submitted.

In the event that an institution will not release a tumor tissue block for ST1, the institution may instead submitted **fifteen**, 4-5 micron tissue sections, mounted and unstained to charged glass slides, do not coverslip or bake slides.

8.3 Tissue for future research

For patients who consent to biobanking for future research, any tissue that is submitted will be stored in the Alliance biorepository until 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.

9. Blood Collection Methods

9.1 EDTA Purple top collection for "Buffy Coat"

9.1.1 Collect ~10 mL of venous blood in EDTA lavender top using standard venous phlebotomy. Both K2 and K3 EDTA tubes are acceptable. If 10ml EDTA tubes are not available, two 5ml or three 3ml EDTA tubes will also suffice.

9.1.2 The tubes should be inverted approximately 8-10 times to mix the EDTA. Place the tube in a Ziploc/or sealed plastic bag with absorbent material. Then place the Ziploc or sealed plastic bag into the refrigerated transport bag.

9.1.3 Refrigerate sample until shipping. Ship the same day as the blood is drawn on a cold pack by overnight courier service to the appropriate Alliance biorepository at Mayo Clinic. Please see **Section 10** for shipping instructions.

9.2 Platelet Poor Plasma (PPP) Nucleic Acid Processing

9.2.1 Collect ~20 ml of blood into EDTA tubes using standard venous phlebotomy. Invert EDTA tubes 10 times. Either K2 or K3 EDTA tubes are acceptable. If 10ml EDTA tubes are not available, 5ml or 3ml EDTA tubes can be used to obtain total volume of ~20ml blood.

9.2.2 1st Centrifugation step: centrifuge the two EDTA tubes at 1500g for 15 minutes at ambient temperature. After centrifugation, three different fractions are distinguishable: the upper clear plasma layer, the intermediate buffy coat layer containing concentrated leukocytes and the bottom layer of red cells. Using a transfer pipette, draw off the plasma layers from the two centrifuged tubes, minimizing removal of the intermediate buffy coat layers, and transfer the plasma to a 15 mL conical tube.

9.2.3 2nd centrifugation step: centrifuge the 15 mL conical tube at 1500g for 15 min at ambient temperature. After centrifugation, transfer ~1.5mL of the upper clear platelet poor plasma layer into each 2mL cryovial tubes (up to 8 vials) *. **NOTE: Be careful to not disturb the pellet below the PPP layer.**

9.2.4 Acceptable Cryovial Choices: Some examples of acceptable 2.0 mL cryovials are: Nalgene (Cat #5012-0020), Fisher (Cat #05-669-57), Corning (Cat #430488), VWR (Cat #16001-102). **NOTE: DO Not use pop top or hinged tops cryovials.**

9.2.5 Freeze samples at -80 °C until shipping. The samples should be placed in a Ziploc/sealed plastic bag and shipped on dry ice within 30 days as the blood is drawn by overnight courier service to the Alliance BAP Freezer. If –80°C is not available, temporary storage on dry ice or at –20°C prior to shipment is acceptable for up to approximately 48 hours.

10. Biospecimen Shipping

10.1 Blood specimens shipping

10.1.1 ”Buffy coat” from whole blood should be shipped on the same day that they are collected (Monday – Friday) on refrigerated cold packs by courier service to the appropriate Alliance biorepository at Mayo Clinic, and must be received by the Alliance biorepository at Mayo Clinic within 24 hours of collection. Platelet Poor Plasma for nucleic acid should be placed in a Ziploc bag with absorbent material and then placed into a biohazard bag and shipped on dry ice within 30 days from collection and must be received by the Alliance biorepository at Mayo Clinic within 24 hours of shipment. If collected biospecimens cannot be shipped as described above (e.g. Saturday or Holiday collections for the whole blood for “buffy coat”), please contact the Alliance biorepository at Mayo Clinic BAP freezer manager Roxann Neumann at 507-538-0602 for further instructions, at least 24 hours prior to anticipated collection.

10.1.2 Follow packaging instructions to ensure shipment compliance with IATA Packaging Instructions.

Each set of blood samples should come with the labels clearly marked and with its own BioMS packing list.

For the PPP plasma for nucleic acid, please place the Ziploc/sealed plastic bags containing absorbent material. cryovials then placed into a biohazard bag. Keep PPP samples frozen at -80^{o}C while making transportation arrangements. Carefully place the biohazard bag on top of sufficient amount of dry ice in a box. Place a copy of the BioMS packing list in the box. Please seal the box well to prevent evaporation. Ship the frozen shipper to Alliance Biorepository at Mayo BAP (Section 10.4) NOTE: Be sure to mark the weight of the dry ice is on the shipping label or FedEx will refuse.

Place the EDTA purple top tube in a Ziploc/sealed plastic bag with absorbent material. Place this Ziploc bag into the biohazard bag. Place a copy of the BioMS packing list in the pocket of the biohazard bag. Keep samples refrigerated while making transportation arrangements. Place the refrigerated cold pack at the bottom of the return shipping box

and place several layers of paper toweling over the cold pack. Place the refrigerated biohazard bag on top of the paper toweling. Place several more layers of paper toweling on the refrigerated samples and then place the bag with ambient samples on top. Ship the refrigerated shipper to Alliance Biorepository at Mayo BAP (Section 10.4)

NOTE: Specimens must be completely packaged in shipping containers prior to courier pickup:

- **Refrigerated Specimens (April through October)** — Put a **frozen** cold pack in the bottom of the Styrofoam® container. Place three to four paper towels (for insulation) over the cold pack. Place the "refrigerated" specimen bag on top of the paper towels. Seal the container with packaging tape.

NOTE: Freeze and store cold packs in a -20°C freezer, not on dry ice. Dry ice freezes the cold packs at a temperature that may cause samples to freeze. If freezing on dry ice is your only option, remove the cold pack from the dry ice 3 hours before use.

- **Refrigerated Specimens (November through March)** — Put a **refrigerated** cold pack in the bottom of the Styrofoam® container. Place three to four paper towels (for insulation) over the cold pack. Place the "refrigerated specimen" transport bag on top of the paper towels. Seal the container with packaging tape.

10.2 Tissue shipping

Because paraffin tissue blocks or slides cut from such blocks may be requisitioned and received from the surgical pathology department at a different time than the day of procurement for other biospecimens, paraffin blocks or cut slides may be sent independently of other biospecimens, using the following guidelines:

10.2.1 There is no "kit" for this study.

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

10.2.3 During warm weather months, paraffin slides and blocks 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 blocks and slides.

10.2.4 Blocks and slides may be shipped ship for standard overnight delivery according to institutional policies and using the preferred vendor.

10.2.5 Slides for mandatory pathology review and BRAF IHC must be submitted within 28 days after patient pre-registration to A071601. Blocks or slides collected for A071601-ST1 could be batch shipped. **Paraffin block or alternatives should be sent up 6 months after registration (for pre-tx specimen) and up to 6 months after progression (for progression/recurrent specimens).**

10.3 Do not send specimens without a completed BioMS Packing Manifest or substitute. Biospecimens cannot be accepted without this completed form.

10.4 There are **THREE** different shipping destinations for different specimens for this study

Ship container for PRIORITY OVERNIGHT DELIVERY according to institutional policies and using the preferred vendor. Here are the THREE destinations

Slides for MANDATORY central pathology review and BRAF IHC Testing should be sent to the following address:

Sandro Santagata Lab
C/O Fiona Watkinson
Center for Neuro-Oncology
Dana-Farber Cancer Institute
450 Brookline Ave. JF 215A
Boston, MA 02215
Phone: 617-632-5482
Email: ssantagata@partners.org
FionaJ_Watkinson@DFCI.harvard.edu

Ship specimens to DFCI on Monday through **Thursday** only. Do not ship specimens on Fridays or Saturdays or the day before national holidays.

Paraffin blocks or slides from patients who consent to participate in A071601-ST1 should be sent to the following address:

Alliance Biorepository at Mayo Clinic FFPE Tissue
Attn: PC Office
RO-FF-03-24-CC/NW Clinic
200 First Street SW
Rochester, MN 55905

Ship specimens to Mayo Clinic on Monday through **Friday** only. Do not ship specimens on Saturdays or the day before national holidays.

Whole blood from patients who consent to participate in A071601-ST1 should be sent to the following address:

Alliance Biorepository at Mayo Clinic BAP Freezer
Stabile SL-16
150 Third Street SW
Rochester, MN 55902

Ship specimens to Mayo Clinic on Monday through **Friday** only. Do not ship specimens on Saturdays or the day before national holidays.

11. Biospecimen Receipt and Quality Assurance Measures

11.1 Tissues for mandatory histology review and BRAF IHC assay will be sent to Dana Farber Institute and conducted by Dr. Sandro Santagata (study pathologist) and his team.

11.2 Except the biospecimens for mandatory histology and BRAF review by Dr. Sandro Santagata, all biospecimens will be shipped to and received by the Alliance Biorepository at the Mayo Clinic, a CAP-accredited biorepository.

11.3 All biospecimens sent to the Alliance biorepository will be accessioned into the TPC informatics system BioMS, at **ABMAYO** Research Laboratory Information Management System (RLIMS) which interfaces with BioMS.

11.4 All biospecimens received at the Alliance biorepository will be logged, associated, and tracked by the unique patient system generated number.

11.5 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 submitting site for reconciliation.

11.6 Upon receipt, any biospecimens 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.

11.7 Fixed tissue biospecimens will be processed using TPC standard operating procedures.

11.8 Tissue submitted to Dr. Sandro Santagata' s group will be kept under Dr. Santagata's custody and will be forwarded to the Alliance biorepository (in batches if needed) upon the completion of the study. All biospecimens submitted to the Alliance biorepository will remain in storage until exhausted. In the case of patient withdraws consent, the specimens will be disposed/returned according to Alliance Policy and Procedure Chapter 11.

12. Document History

Version	Description and Justification of Change	Author	Effective Date
1.0	New (Master template)	MAW	08/04/2017

Signature Page

Signatures of the following individuals indicate review and acceptance of the attached document version:

Name	Role	Signature	Date
Roxann Neumann	Alliance Biorepository at Mayo Clinic BAP freezer manager		08/03/2017
Helen Tollefson	Alliance Biorepository at May Clinic pathology coordinator		08/03/2017
Yujia Wen	Director, Alliance TRP Operations		08/03/2017
Priscilla Brastianos	A071601 Study Chair		08/03/2017