**Genomics of MPNST (GeM) Consortium**

**MPNST Pathology Review – Standard Operating Procedure**

Version 1.0

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| **Table of Contents** | **Page** |
| 1. Introduction | 2 |
| 2. Policy Statement | 2 |
| 3. Purpose | 2 |
| 4. Scope | 2 |
| 5. Pathology Reviewer Qualifications | 2 |
| 6. Role of the Pathology Reviewer | 3 |
| 7. Types of MPNST Pathology Reviews | 3 |
| 8. Diagnostic MPNST Pathology Review Guidelines | 4 |
| 9. Specimen Input Requirements | 5 |
| 10. Data and Paperwork Requirements | 5 |
| 11. Bio-specimen Special Handling and Logistics Including Shipping | 6 |
| 12. Receipt of Specimens and Next Steps for Coordinating Center | 9 |
| 13. Review Pathologists Used as Consultants | 10 |
| 14. Other Related Policy and Procedures | 10 |
| 15. References | 10 |
| 16. Policy Applications | 10 |
| 17. Policy Maintenance Responsibility | 10 |
| 18. Policy Authorization | 10 |
| 19. Version/Revision History | 11 |
| Appendix A: Specimen Shipment Checklist | 12 |
| Appendix B: Specimen Transmittal Form | 13 |
| Appendix C: MPNST Pathology Review Report Form | 15 |

1. **Introduction**

The Genomics of MPNST (GeM) Consortium’s MPNST Pathology Review will conduct comprehensive tests on Malignant Peripheral Nerve Sheath Tumors (MPNSTs) and related material retrospectively and prospectively collected by member institutions and contributed for use by the Consortium. The primary functions of the MPNST Pathology Review will be to confirm diagnoses and conduct case reviews, to determine specimen adequacy for extraction of genetic material for comprehensive molecular testing, and to advance our understanding of the anatomical, biochemical, and immunological markers that indicate malignancy and determine grade in these rare tumors.

1. **Policy Statement**

It is the policy of the GeM Consortium to require all eligible specimens submitted by member institutions for use by the Consortium be subject to an expert MPNST Pathology Review to ensure uniform study conduct, diagnostic criteria, and specimen-derived data collection. To support this policy, expert pathology reviewers will contribute to study protocol development and will participate in regular meetings to discuss study progress and opportunities for advancement of study objectives.

1. **Purpose**

The purpose of this policy is to outline the integral role of the MPNST Pathology Review in the GeM Consortium’s efforts, and to serve as a guide during study development and implementation for member institutions and study coordinators.

1. **Scope**

This policy applies to all GeM Consortium members and activities that require MPNST Pathology Review.

1. **Pathology Reviewer Qualifications**
* Evidence of scholarly activity and reputation in MPNST and/or neuropathology
* Current pathology appointment at GeM Consortium member institution

Boston Children’s Hospital serves as the coordinating center for the GeM Consortium, as will BCH Pathology serve as the coordinating site for the MPNST Pathology Review. Boston-area experts in MPNST pathology have convened to conduct this centralized review; no formal process was used to appoint individuals to this panel.

1. **Role of the Pathology Reviewer**
* Confirm diagnoses and conduct case review as necessary to determine eligibility for this study, including in consultation with pathologists from member institutions
* Determine specimen viability for extraction of genetic material for comprehensive molecular testing
* Advance our understanding of the anatomical, biochemical, and immunological markers that indicate malignancy and determine grade in MPNSTs
* Contribute to study protocol development, including design and necessary amendments, as necessary
* Participate in monthly meetings and maintain active role in centralized review of MPNSTs
* Author and publish original articles to contribute new knowledge to MPNST pathology literature
1. **Types of MPNST Pathology Reviews**

All central pathology review activities are contingent on 1) confirmation of sufficient allowances under the IRB protocol associated with each specimen submitted by member institutions for centralized review, 2) member institution adherence to specimen management and shipping guidelines included in this policy, and 3) provision of clinical data associated with each subject and/or specimen submitted for review (ex: subject NF-1 status).

Failure to confirm eligible diagnosis or to complete a centralized review for any reason will result in exclusion of the specimen from the study, and investigators at the member institution will be notified by the site coordinator and/or the MPNST Pathology Review team.

**Retrospective Pathology Review:**

GeM Consortium member institutions will volunteer retrospectively collected MPNST and related specimens stored in tissue banks under protocols independent of the present study or included as an amendment to the GeM Consortium’s study protocol. Retrospective specimens are of two types: 1) frozen MPNST and normal tissue and blood samples (from banks); paraffin slides and scrolls (from pathology department), and 2) paraffin slides and scrolls only (from pathology department) to be used for validation studies. The MPNST Pathology Review team will confirm the providing institution’s diagnosis for research purposes and viability of the specimen for comprehensive molecular testing, and in turn the specimen’s eligibility for inclusion in the present study. Ineligible specimens will be excluded and returned to the member (or “treating”) institution.

Retrospectively banked specimens will be collected by the Consortium for research purposes only; though central pathology results will be made available to the member institution, there will be no return of results to patients or care providers.

**Prospective Pathology Review:**

GeM Consortium member institutions will volunteer prospectively collected MPNST and related specimens covered under protocols independent of the present study or under the GeM Consortium protocol. Prospective collection of fresh frozen tissue with a paired normal sample should be prioritized by Consortium member institutions, in addition to preparation of FFPE specimens for MPNST Pathology review.

In some instances, BCH Pathology will provide the primary clinical diagnosis for patients at local institutions, while also determining specimen eligibility for this study. Return of results will be dictated by the attending pathologist and treating physician. Confirmation of established eligibility for the study will be made available to the site research coordinator as soon as possible for consent purposes.

The MPNST Pathology Review will confirm the member (or “treating”) institution’s diagnosis for research purposes and viability of the specimen for comprehensive molecular testing, and in turn the specimen’s eligibility for inclusion in the present study. Ineligible specimens will be excluded and returned to the member institution. Relapse tumor and normal tissue may be submitted for research purposes, but the extent of histopathological classification and biomarker determination will be determined by the expert pathologists.

1. **Diagnostic MPNST Pathology Review Guidelines**

Boston-area MPNST pathology experts will complete the MPNST Pathology Review for all prospectively collected primary diagnostic tumors and relapse specimens, as well as specimens collected retrospectively or under separate IRB protocols and submitted for use by the Consortium. The MPNST Pathology Review will utilize a histopathological and morphological classification system to confirm diagnoses and differentiate grade of MPNSTs and related tumors. If eligible diagnosis is not confirmed, further biomarker testing and classification will not be performed. Confirmed NF1-related and sporadic MPNST will undergo histologic classification and biomarker determination.

In addition to hosting the MPNST Pathology Review, BCH Pathology will extract DNA and RNA from viable specimens for comprehensive molecular testing, including Whole Genome Sequencing (WGS). Though not part of the centralized review process, specimen management and shipping requirements are included here for logistical purposes.

*Please see specimen logistics and shipping section that follows for information on submission of specimens (paraffin-embedded tumor blocks and slides and/or fresh frozen tissue) and, if applicable, corresponding blood samples for all distinct time-points from diagnosis through relapse and/or loss to follow-up.*

This study strongly encourages bio-banking of residual paraffin embedded, fresh frozen tumor, and peripheral blood samples by the member institution when specimens are submitted for central review.

1. **Specimen Input Requirements for MPNST Pathology Review**

|  |  |  |
| --- | --- | --- |
| **Procedure** | **Sample Type/Material** | **Required Input** |
| **MPNST Pathology Review** |  |  |
|  | FFPE Sections/Slides | 1 H&E from each block |
|  |  | 5 unstained slides from most representative block |
|  | Frozen Tissue | >1 gm optimal, but please send any available frozen tumor |
| **Nucleic Acid Extraction** |  |  |
|  | FFPE Sections/Slides | 5 unstained slides from most representative block |
|  | Frozen Tissue | 20 mg |
|  | Blood | One tube |
|  | Buffy Coat | 1-2 x 106 cells |

**Note:** Needle biopsies are NOT sufficient for histologic classification and biomarker determination, and will not be accepted for use by the Consortium.

**Note:** When no frozen tumor is available for biomarker testing, paraffin embedded tissue blocks may be submitted for nucleic acid extraction. These specimens are submitted *in addition to* the routinely requested tumor slides for centralized pathology review. Blocks are preferable to slides as some assays require thick-cut tissue scrolls. It is important to communicate directly with the MPNST Pathology team to facilitate appropriate testing in this situation.

1. **Data and Paperwork Requirements**
* Clinical Data (can be submitted electronically to: NFresearch@childrens.harvard.edu)
	+ Pathology reports
	+ Operative reports
	+ Reports from any prior molecular testing
* Specimen Transmittal Form (hard copy to be included in specimen procurement kit)

Paperwork is to be submitted to the Coordinating Center with the pathology review specimen materials, and also uploaded in the GeM Consortium’s RedCap database, when applicable.

1. **Bio-specimen Special Handling and Logistics Including Shipping**

All specimens to be contributed for use by the Consortium should be handled and shipped in compliance with each member institution’s regulations. Submission of pathology material and paperwork shall be made directly from the member institution to the NF Research Initiative at the mailing address below.

The following applies to all specimens submitted for use by the Consortium, including retrospectively collected samples, diagnostic specimens, and specimens collected at time-points after diagnosis (ex: tumor relapse or progression).

Unless otherwise specified, the NFRI will supply each member institution with all required materials for specimen shipping (except dry ice), including a FedEx account number to cover the cost of shipping. Dual chamber procurement kits will allow collaborators to send FFPE specimens at room temperature with fresh frozen specimens on dry ice in separate chambers of the same container.

**FFPE Specimens: Instructions for Submission to the NF Research Initiative**

1. Verify you have all the necessary materials for shipment (See Appendix A).
2. Enter data into the Specimen Transmittal Form, including completion of the inventory form (see Appendix A), and print it out for inclusion in the shipments of all specimens. Please keep an electronic copy of the transmittal form for your records. Enter the Federal Express Tracking Number that is on the air bill included in the specimen procurement kit. The samples must be sent to the NF Research Initiative by Federal Express Overnight PRIORITY Mail. (See mailing address below).
3. *Specimens:*
	* *FFPE Slides:* Please include 1 H&E slide and 5 unstained slides from the most representative block for centralized pathology review, as well as 5 unstained slides for nucleic acid extraction (if frozen specimen unavailable). Label each glass slide with institution patient ID number and Surgical Pathology ID (SPID) number and block number from the corresponding pathology report (from treating institution). Please place all materials in appropriate packaging and keep at room temperature.
4. *Shipping:* Before specimens are placed into the specimen procurement kit, they first need to be placed in three separate layers of packaging:
	* Place the specimens in individual zip lock bags (keeping room temperature specimens separate from the frozen)
	* Place the individual bags in the biohazard diagnostic envelope with absorbent material (both are provided in the kit). Expel as much air as possible and seal the envelope.
	* Place the biohazard diagnostic envelope into the Tyvek envelope (also provided in the kit). Expel as much air as possible and seal the envelope.

If FFPE slides and/or blocks for central pathology review are available when frozen tissue samples are being shipped for extraction of genetic material, the slides/blocks may be shipped together in the provided dual-chamber specimen procurement kit. Please see below for submission instructions for frozen specimens.

Please ship tumor slides and paperwork for MPNST Pathology Review by Federal Express Priority Overnight (address below).

**Frozen Tumor Specimens and Blood: Instructions for Submission to the NF Research Initiative**

1. Verify you have all the necessary materials for shipment (See Appendix C).
2. Enter data into the Specimen Transmittal Form, including completion of the inventory form (see Appendix A), and print it out for inclusion in the shipments of all specimens. Enter the Federal Express Tracking Number that is on the air bill included in the specimen procurement kit. The samples must be sent to the NF Research Initiative by Federal Express Overnight PRIORITY Mail. (See mailing address below).
3. *Specimens*:
	* *Frozen Tissue:* At least 20 mg is optimal, but send any available frozen samples. Specimens should be snap frozen as soon as possible following surgery. The short interval to freezing is critical to preserve RNA and facilitate gene signature testing. This specimen must not be fixed. At least one specimen from the primary (if present) and metastatic areas (if present) should be cut into 3-5 mm slices and wrapped in the foil and snap frozen in vapor phase liquid nitrogen (do not submerge tissue in liquid nitrogen) or isopentane/dry ice. Label tumor samples with patient’s name, birth date, institution patient ID number, specimen type, site from which tissue was collected, collection date and time. Mark foils as “primary” or “metastatic”. Store at -20o C or preferably at -80o C until shipped. All snap frozen tissue should be placed in a specimen bag and must be shipped on dry ice (approximately 4 lbs. total). We strongly encourage pathologists who receive a large MPNST sample to send all tumor tissue not needed for clinical management. Do not freeze more than 1 gram of tissue in each piece of foil.
	* *Blood:* Obtain at least 1 mL of blood in EDTA or 10 mL in PAXgene tube. Label blood with patient’s name, birth date, specimen type, collection date and time.
		1. Note: If blood is not obtained before therapy then only collect blood in an EDTA tube. If blood is to be submitted after chemotherapy initiation, please ensure the WBC count is at least 2,000/mm3 to ensure sufficient DNA for testing and banking.
4. *Shipping:* Before specimens are placed into the specimen procurement kit, they first need to be placed in three separate layers of packaging:
	* Place the specimens in individual zip lock bags (keeping room temperature specimens separate from the frozen)
	* Place the individual bags in the biohazard diagnostic envelope with absorbent material (both are provided in the kit). Expel as much air as possible and seal the envelope.
	* Place the biohazard diagnostic envelope into the Tyvek envelope (also provided in the kit). Expel as much air as possible and seal the envelope.

Snap frozen tumor tissue and serum should be placed in one of the kit compartments with dry ice. Layer the bottom of the compartment with dry ice until it is approximately one-third full. Place the frozen specimens on top of the dry ice. Cover the specimens with dry ice until the compartment is almost completely full. Place the foam on top to secure specimens during shipment.

Peripheral blood specimens should be shipped in the other kit compartment at room temperature. Insulate ambient specimens with bubble wrap or similar material. Place the foam insert on top of the kit compartment to secure specimens during shipment.

**All shipments:**

Verify that specimens are labeled as described above per specimen type. This includes (but is not limited to) PATIENT NAME, birth date, specimen type, collection date and time. If informed consent is obtained for the research portion of this protocol, Protected Health Information (PHI) will not be given to the investigators when specimens are forwarded for research studies and banking.

Insert the complete Specimen Transmittal Form into the kit. Close the outer lid of the Specimen Procurement Box and secure with filament or other durable sealing tape. Complete the pre-printed Federal Express air-bill, insert it into the plastic pouch and attach the pouch to the top of the kit. Complete the dry ice label (UN 1845). Attach the dry ice and Exempt Human Specimen labels to the side of the kit.

Specimens should be shipped on Monday through Thursday for delivery on Tuesday through Friday. Special considerations will be made for holidays, to ensure receipt of shipments. Ship specimens via Federal Express Priority Overnight.

Arrange for Federal Express pick-up through your usual institutional procedure, but stress that pick-up is at your institutional address. Ship PRIORITY OVERNIGHT Delivery.

 **Send specimens to:**

 NF Research Initiative

 ℅ **CONTACT NAME HERE**

 Center for Life Sciences – Chris Walsh Lab

 3 Black Fan Street, CLS15

 Boston, MA 02115

 Phone: (617) 919-6277

 Email: NFresearch@childrens.harvard.edu

Be sure to specify PRIORITY OVERNIGHT DELIVERY

Saturday, Sunday and holiday deliveries are not accepted. If specimens are obtained on a Friday or on the day before a holiday, please keep tissue frozen and blood in a refrigerator and ship for arrival the next available business day.

1. **Receipt of Specimens and Next Steps for Coordinating Center**

The NFRI research coordinator will complete the following steps for each specimen shipment before specimens are distributed for pathology review or extraction:

* Check for completion of Specimen Transmittal Form, and request any missing information from the source institution
* Inventory specimens in each shipment by comparing the Specimen Transmittal Form with the labeled specimens included in the procurement kit, and troubleshoot any discrepancies with the source institution
* Record the source institution specimen/subject identification numbers associated with each specimen received in the NFRI’s Subject Registry spreadsheet
* Assign a unique NFRI identification number to correspond with each specimen, which will henceforth replace any source institution identifiers, and record in the NFRI’s Subject Registry spreadsheet

**Specimens Received for Centralized Pathology Review**

* FFPE specimens will be stored at room temperature in the NFRI office
* The research coordinator will email Dr. Al-Ibraheemi to notify her of the received shipment and arrange an appropriate time to transfer specimens to BCH pathology
* The research coordinator will denote specimen location in the NFRI Registry spreadsheet at any time point when the physical location of the specimen changes

**Specimens Received for Nucleic Acid Extraction**

* Frozen specimens will remain in storage in the Walsh Lab in the CLS building
* The research coordinator will email Monica Callichio, manager of the BCH Molecular Lab, to notify her of batch of specimens to be sent for nucleic acid extraction and arrange an appropriate time to transfer specimens
* The research coordinator will complete and print a LaMPP Request Form for each specimen being sent for extraction
* The research coordinator will denote specimen location in the NFRI Registry spreadsheet at any time point when the physical location of the specimen changes
1. **Review Pathologists Used as Consultants**

In some instances, a MPNST Pathology Team pathologist may serve as personal consult for primary diagnosis at a local institution. Member institution pathologists may request personal consultation by a MPNST Pathology Team pathologist to clarify diagnostic criteria or specimen eligibility for the study. Neither of these instances will be considered to be a centralized review for the Consortium until confirmation of eligibility for this study.

1. **Other Related Policy and Procedures**

*In progress: DFCI/HCC IRB protocol for Genomics of MPNST (GeM) Consortium - including Manual of Procedures*

1. **References**

Hum Pathol. 2017 May 24. pii: S0046-8177(17)30167-3. doi: 10.1016/j.humpath.2017.05.010

1. **Policy Applications**

This policy applies to all GeM member institutions, including clinicians and associated research personnel, who will be following these guidelines for specimen submission, who have oversight and supervisory responsibilities to adhere to this policy, and who have the authority to update this policy as necessary.

1. **Policy Maintenance Responsibility**

Expert pathologists from the MPNST Pathology Review team and the BCH study staff (as GeM site coordinator) will be responsible for maintenance of this policy.

1. **Policy Authorization**

This policy will be reviewed and authorized by the GeM Consortium’s Steering Committee.

1. **Version/Revision History and Inquiry**

Reassessment of this policy will occur every 12 months, with interim revisions as necessary.

|  |  |  |
| --- | --- | --- |
| **Approval Date** | **Version** | **Version/Revision Summary** |
|  | 1.0 |  |
|  |  |  |
|  |  |  |

**MPNST Pathology Review inquiry should be addressed to:**

NF Research Initiative

 ℅ Dr. Alyaa Al-Ibraheemi

 Boston Children’s Hospital - Pathology Department

 300 Longwood Avenue, Bader 113

 Boston, MA 02115

 Phone: (617) 919-2422

 Email: Alyaa.Al-Ibraheemi@childrens.harvard.edu

**Appendix A: Specimen Shipment Checklists**

*These checklists will be completed prior to shipments and will serve as reference forms to help guide individuals placing shipments. Checklist 1will be filled out by the Coordinating Center and Checklist 2 will be filled out by Member Institutions.*

**Checklist 1:** Specimen Collection Kit

|  |  |  |
| --- | --- | --- |
| 1. | Dual compartment shipper |  |
| 2. | Kit/shipping instructions |  |
| 3. | Two sets of biohazard and Tyvek diagnostic envelopes |  |
| 4. | Zip lock bags |  |
| 5. | Bubble wrap  |  |
| 6. | Transmittal Form(s) |  |
| 7. | Included labels for specimen shipment (shipping, dry ice, Exempt Human Specimen) |  |
| 8. | Sealing tape |  |
| 9. | Shipping label (for out shipping) |  |

**Checklist 2:** Specimens to BCH Pathology Department

|  |  |  |
| --- | --- | --- |
| 1. | Dual compartment shipper |  |
| 2. | Properly labeled frozen and ambient specimens |  |
| 3. | Two sets of biohazard and Tyvek diagnostic envelopes |  |
| 4. | Zip lock bags (one bag per specimen type/time point) |  |
| 5. | Bubble wrap (ambient specimen insulation) |  |
| 6. | Dry ice (fresh frozen specimens) |  |
| 7. | Completed Transmittal Form(s) |  |
| 8. | Appropriate labels on box (shipping, dry ice, Exempt Human Specimen) |  |

**Appendix B: Specimen Transmittal Form**

*To be completed by the Member Institution and included in the box with specimens for shipment.*

**1.** **Contact**

Please provide the following contact information:

|  |  |
| --- | --- |
| Institution | Name: |
| Specimens packaged and shipped by | Name:Position:Department:Email:Phone: |

**2.** **IRB**

Please provide the following IRB-related information:

|  |  |
| --- | --- |
| IRB protocol(s) associated with specimens included in this shipment | Institution:Protocol Number:Title: Principal Investigator: |
|  | Institution:Protocol Number:Title: Principal Investigator: |
| *Does the Coordinating Center have the most recent version of the IRB protocol?* | ☐ Yes☐ No (Stop! *See below*) |
| *If no, please submit the most recent version of the IRB protocol to the Coordinating Center for review* ***prior*** *to shipping specimens.*IRB submission: <https://app.smartsheet.com/b/form/fda3e91f6270494fb6923fe7ca44e281> |

**4.** **Shipment Inventory**

Please provide the following information on each specimen included in this shipment, using one row to represent each specimen/sample and grouping specimens by Subject ID# (i.e., list specimens from the same patient consecutively)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **IRB Protocol** | **Institution** **Subject ID #** | **Institution Specimen ID #** | **Specimen Type**FF/FFPE/Blood | **Pathology Report Available** | **Operative Report Available** | **Reports from Molecular Testing** | **NFRI ID #***Please leave blank* |
| 1 |  |  |  |  |  |  |  |  |
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| 15 |  |  |  |  |  |  |  |  |

**Appendix C: MPNST Pathology Review Report Form**

*This form will be completed by the expert pathologist after centralized review of diagnostic material is performed.*

|  |  |  |  |
| --- | --- | --- | --- |
| **Question (Element)** | **Valid Value** | **Edit Checks** | **Public ID** |
| **SPECIMEN INFORMATION** |  |  |  |
| Institutional surgical pathology number |  | QUERY if field is blank |  |
| Specimen Collection Date |  | QUERY if field is blank |  |
| **FINAL DIAGNOSIS:** |  |  |  |
| Do you agree with the institutional assessment? | * Yes
* No
 | QUERY if field is blankIf no, send email notification |  |
| Description: |  | QUERY if blank and “No” aboveQUERY if answered and “Yes” above |  |
| **COMMENTS** |  |  |  |
| Comments: |  |  |  |