JHM IRB - eForm A – Protocol

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1. Abstract

Neurofibromatosis type 1 (NF1) is one of the most common hereditary tumor predisposition syndromes. Patients with NF1 are at high risk of developing both benign tumors, such as plexiform neurofibromas (PN), and malignant tumors, such as malignant peripheral nerve sheath tumor (MPNST). There are currently very limited resources available to support laboratory and translational research of these tumor types. There are few PN cell lines, few PN xenografts, and there is no viable repository of primary PN samples resected from patients with NF1. There are MPNST cell lines, but few, and there is no functional biorepository of MPNST samples generally available to the medical community. With the support of a grant from the Neurofibromatosis Therapeutic Acceleration Program, we have built and maintain a biorepository of tumors and blood fractions from patients with NF1. Our goals also include the development of a series of PN and MPNST cell lines and xenografts, which we will also make available to the NF1 research community for the purpose of supporting translational research efforts aimed at developing novel treatments for these challenging tumors.

2. Objectives

The primary objective of this protocol is to develop a biorepository of tissue and serum from patients with NF1and to increase accessibility of these tissues to the global NF1 research community. The establishment of the biorepository will improve access to tissue as a core tool to support discovery of new therapeutics for patients with NF1, and increase the collaborative research opportunities within the community. This protocol also serves the purpose of aiding in the generation of PN and MPNST xenograft models to propagate primary human tissue for future research.

3. Background

Neurofibromatosis type 1 (NF1) is a common neurogenetic inherited syndrome caused by mutations in the *NF1* gene and characterized by a predisposition to the development

of nerve sheath tumors, including dermal neurofibromas, plexiform neurofibromas (PN), and malignant peripheral nerve sheath tumors (MPNST). Recent work has demonstrated that approximately 50% of patients with NF1 develop PN, and 55% of PN in childhood are symptomatic. Currently, surgery is the only effective treatment option for patients with symptomatic PN. Progress developing nonsurgical therapy for PN has been limited by a number of factors including 1) the lack of cell culture-based models of PN, 2) a limited number of animal models of PN, and 3) limited access of investigators to primary PN tissue from patients with NF1. Although progress is being made in the development and utilization of animal models and cell culture models, the limited availability of primary patient tissue remains unaddressed. Our plan is to establish a local, but shared, biospecimen repository for the purpose of 1) banking serum and tumor tissue from patients with NF1 undergoing surgical resection of cutaneous neurofibroma, PN, MPNST, or other cancers such as breast cancer, GIST, or gliomas, at Johns Hopkins Hospital, and 2) generating xenograft models to propagate primary human tissue.

The Johns Hopkins Comprehensive Neurofibromatosis Center is one of the largest and busiest clinical centers in the United States, with >1200 patients cared for routinely in the Center. Although surgery is relatively rare for PN, it is very common for cutaneous neurofibroma and for MPNST. This project will allow us to apply our expertise in bio-banking and xenograft creation and the strength of the clinical center for clinical correlative data and patient referral to improve access to tissue as a core tool to support discovery of new therapeutics for patients with NF1.

4. Study Procedures

Every patient with NF1 who presents to Johns Hopkins for the purpose of resection or biopsy of a tumor (cutaneous neurofibroma, PN, MPNST, breast, GIST, glioma, or other NF1-associated malignancy) will be offered the opportunity to enroll in a research protocol to allow for extra tissue resected at the time of surgery to be banked by Dr. Pratilas' laboratory. Tissue will be received for banking once an acceptable amount of tumor is retained by pathology for diagnostic purposes. The resection or biopsy will be performed as a part of a patient's clinical care to manage or diagnose his or her disease. Additional blood for banking will also be drawn at the time of the collection of clinical blood specimens. A total of 26ml of blood will be collected. Two 10ml EDTA tubes and a single 6ml plain red top will be collected. It is preferred that blood be collected within thirty days of surgery during one of the patient's already scheduled clinic visits or immediately pre- or post-operatively in the PACU.

<u>Multi-institution collaborative MPNST registry</u>
In addition, through a collaboration with other cancer centers (lead center Washington University, St. Louis), data will be included in a comprehensive database of clinical information from people with malignant peripheral nerve sheath tumors (MPNST), including information generated during genetic testing on specimens collected under banking studies. This database, "the MPNST registry", may be accessed by investigators

for future research projects designed to learn more about MPNST. Because this is a registry study, participants will not have any tests or procedures done solely as a result of enrollment. The study team is collecting information about tests or procedures that participants have had or will have regardless of participation.

For patients enrolling on the JHU NF1 biospecimen research study for the first time, they will be given the option of also participating in the collaborative MPSNT registry study, using an additional "yes/ no" check box on the ICF.

The study team will identify participants with diagnoses of MPNST who were enrolled prior to the protocol dated November 26, 2018. Once identified, the study team will contact these participants (or their parent or representative) through mail or telephone to determine if they are also willing to be included in the MPNST registry study. If so, participants will be re-consented using the most current IRB-approved consent form.

Collection of research samples from a deceased patient

In the event of death of a patient with NF1, consent for research autopsy will be obtained, when possible, prior to the patient's death. Research autopsy consent will be done by the autopsy pathologist, Dr. Jody Hooper, or her designee. Consent for research use and banking of post-mortem samples for the Nerve Sheath Tumor Bank (J1649) will also be obtained from the patient when possible.

Next of kin consent for samples derived from deceased patients: In the event of death of a patient with NF1 when research authorization has not been obtained from the patient prior to death or when death is unexpected, tumor samples from deceased NF1 patients treated at Johns Hopkins may be obtained through consent of next of kin. Next of kin will be contacted either in person or by telephone with information regarding collection of extra tissue resected at the time of autopsy to be banked by Dr. Pratilas' laboratory. The Investigator or consent designee will review the next of kin consent form and offer the opportunity to ask questions. If the family member is interested, the next of kin consent form will be mailed. Once received the next of kin will sign the consent form and mail it to the PI, Christine Pratilas, MD using the address listed on the first page of the consent form. Upon receiving the signed consent form, the Investigator or consent designee will telephone the next of kin to offer the opportunity to ask additional questions or provide comments. Once all questions are answered, the Investigator or consent designee will sign the next of kin Informed Consent Form (ICF). This consent process will be documented on a separate form. Tissue will be received for banking once an acceptable amount of tumor is retained by pathology.

After obtaining written informed consent, patient-reported pertinent medical and NF1 history and other clinical information will be collected. Once a sufficient amount of viable sample is received for banking, each sample will be given a unique identifier and logged into a secure database maintained by JHU SOM and partnered with the necessary clinical information linked to the specimen's barcode.

Processing of Blood: Blood will be centrifuged and white blood cells separated from plasma. Serum, plasma and buffy coat will be divided and frozen at -80^{o}C. DNA will be isolated from white blood cells and stored under appropriate conditions.

Processing of Tumor Tissue: All tumor tissue removed during the course of surgery will be evaluated first by pathology. An initial frozen section will allow for a preliminary diagnosis, assess for tissue adequacy and confirm the clinical impression of nerve sheath tumor or NF1-associated tumor in an NF1 patient. Next, pathology will ensure that there is sufficient tissue for diagnosis, tissue representation and future clinical molecular testing. At least one section per centimeter of tumor will be placed in formalin and embedded in paraffin blocks for permanent pathologic evaluation, including routing hematoxylin-eosin stained sections and immunohistochemistry. In addition, at least 1 cubic centimeter of tissue will be snap frozen in liquid nitrogen and stored at -80^{o} C in the clinical pathology repository for future molecular testing for clinical purposes as needed. Once these clinical needs are satisfied, the remaining tissue will be examined and selected for research purposes. Tissue that is available after being used to support ongoing research efforts at Johns Hopkins will be submitted to the biobank and made accessible to the entire NF1 research community.

Formalin-fixed, paraffin embedded tissue will be maintained as primary tissue blocks by the Department of Pathology, as is current clinical practice. The Biorepository database will include the surgical pathology specimen number so that the tissue block can be retrieved as needed in the future. Tissue that arrives in the lab will be divided and processed to allow for isolation and storage of RNA, DNA, protein, flash frozen tumor tissue, FFPE tissue and viably frozen tumor tissue (Cell Freezing Medium [Sigma C6295]). Each of these specimens will be divided and stored under appropriate conditions and with appropriate documentation.

Sample handling priority will be followed with emphasis on retaining a viable amount of tissue banked at Hopkins for use in research based at Johns Hopkins. Xenografts will be established for the propagation of the patient's tumor for research purposes under an ACUC-approved protocol. With patient consent for samples to be sent to investigators outside of Johns Hopkins, samples may be sent to members of the NF1 research community upon internal JH approval of a project's scientific merit.

A fragment of each tumor collected may be implanted in NOD/SCID/IL-2Rγ null (NSG) mice. Although the emphasis of NTAP is on plexiform neurofibroma, we will attempt to establish xenografts from a variety of nerve sheath tumors, including MPNST. The number of mice implanted will vary between 1 and 5 depending on the amount of tissue collected. Our experience, and that of others, indicates that minimizing the time between surgical resection and implantation into mice, and using the optimal mouse strain, dramatically impact the rate of xenograft establishment.

Access to banked specimens and xenografts will be available to the entire NF1 community, based on the following parameters: 1) An application for use of banked

material will be filed. This application will be reviewed by the co-investigators (JHU internal review board) and must include justification for the type and number of requested samples. In the event of a conflict of interest (either a co-investigator is the applicant, or the proposed use presents a conflict for one of the co-investigators), the conflicted individual will be recused from the discussion and replaced by a member of the NTAP Scientific Advisory Board. 2) Approved applications will result in provision of the requested samples, with the condition that the physician/scientists involved in acquisition of those particular tissues (the surgeon who did the resection, the oncologist who cared for the patient, etc.) will be offered the opportunity to collaborate on the research project. Completed applications will be reviewed within two weeks, and if approved, samples will be distributed within two weeks of MTA approval, provided relevant regulatory requirements are met. This procedure will ensure that samples are widely available (anyone can apply for materials), rapidly available, and are distributed based on the scientific merit of the proposed use.

Short Questionnaire: If a participant has MPNST, the study team will ask the participant to complete a short questionnaire, which will include questions about demographic information, contact information, diagnosis and family history, and willingness to participate in different types of future research. Once completed, the investigator will be prompted to enter information from the participant's medical record into the database, such as additional information regarding diagnosis, information about treatments received, information about how the participant's MPNST has responded to treatment, imaging information, and the results of any analyses performed on specimens collected under tissue banks, including the results of genetic research.

The study team may contact participants at future time points either by phone or at a routine clinic visit (possibly two to three times per year, depending on the scheduled doctor visits and resources available). The purpose of this contact is to collect information about health status and quality of life. If the study team cannot reach the participant, this information may be obtained by reviewing medical records or by accessing public records. The National Death Index (NDI) may be utilized to confirm vital status if necessary. The NDI is a centralized database of death record information on file in state vital statistics office maintained by the Centers for Disease Control and Prevention. Social security number (SSN) is used to search the NDI. Although the study team is collecting SSN as part of participation in this study in order to meet NCI requirements, participants may opt out of allowing the study team to use their SSN to search the NDI.

Patients may withdraw consent at any time. Once consent is withdrawn, no future samples will be collected. Samples collected prior to withdrawal of consent will still be maintained in the biobank along with all pertinent clinical data.

5. Inclusion/Exclusion Criteria

Any patient with a diagnosis of Neurofibromatosis type 1 (NF1) is eligible to participate in this trial.

Tumors may also be obtained and banked from deceased patients with a diagnosis of NF1 if informed consent is obtained from the patient prior to death, or from the decedent's next of kin.

6. Drugs/ Substances/ Devices

N/A

7. Study Statistics

N/A

8. Risks

The risks associated with having a blood sample taken are minimal. As blood collection for this trial will be performed at the time blood is being collected for clinical tests, no additional discomfort beyond the initial needle stick and potential bruising and tenderness from the blood collection is likely.

This study uses only leftover tumor sample from tumors which will be removed as part of your medical care. No extra tumor will be collected for this research trial, and thus there will be no additional biopsy procedures or discomfort beyond what is necessary for the patient's clinical care.

The greatest risk to the patient is the collection of information from their health records. Efforts will be made to keep personal information confidential. The number of people whom have access to the identifying information of the patient will be limited to staff within Dr. Pratilas' laboratory, the clinicians working with the patients for clinical care, co-investigators, and collaborating institutions. Collaborating researchers may be doing research in areas similar to this research or in other unrelated areas. These researchers may be at Washington University, St. Louis (lead site for collaborative MPNST clinical registry), at other research centers and collaborating institutions, or industry sponsors of research. The study may also share research data with large data repositories for broad sharing with the research community. If individual research data is placed in one of these repositories, only qualified researchers who have received prior approval from individuals that monitor the use of the data, will be able to look at information.

Participants may change their mind and request the study team not store and use their data for future research. In this case, the data will no longer be used for research

purposes. However, if some research with data had already been completed, the information from that research may still be used.

Tissue will be stored in a manner that will permit future genetic analysis and DNA sequencing. These evaluations are not a part of the current protocol, and will only be performed after obtaining appropriate approvals. Nevertheless, since samples provided to the biorepository might ultimately be evaluated by gene sequencing, it is important to note that no data from such evaluations will be reported back to the patient whose tumor was sequenced, nor will it be reported to a treating physician, nor will it be made a part of the patient's medical record.

9. Benefits

The goal of this trial will be to create a resource for the NF1 research community as a whole. The collection and storing of samples will improve access to tissue as a core tool to support discovery of new therapeutics for NF1 patients.

10. Payment and Remuneration

N/A

11. Costs

N/A