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HEAD AND NECK TISSUE BANK PROTOCOL

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Synopsis

Title	Head and Neck Tissue Bank
Short Title	HNTB
Protocol Date	10.19.2016
Study Duration	20+ years
Study Center(s)	Northwestern University/Northwestern Memorial Hospital
Objectives	To collect biospecimens from patients undergoing biopsy and/or surgery for lesions/tumors of the head and neck, and bank the specimens for future research
Number of Subjects	5000
Diagnosis and Main Inclusion Criteria	Adult patients (≥ 18 years) with a lesion/tumor of the head and neck requiring biopsy and/or surgery

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1.0 INTRODUCTION

1.1 BACKGROUND: Basic and translational research heavily relies on the use of human tumor tissue, to understand the mechanisms of tumor formation and identify new therapeutic targets in addition to biomarkers (measurable substance in body fluids or tissue) for predictive and prognostic purposes as well as for following disease response to treatment. Blood and other body fluids are extremely important for the development of biomarkers. The study of human tumor tissue and other biospecimens (any human-derived material), and ability to match the individual tumor characteristics with clinical outcome is crucial and profoundly fundamental for targeted and personalizing cancer therapy for patients. Molecular studies have shown great variation and heterogeneities in gene and protein expression between individual patient's tumors, even if these tumors belonged to the same site disease group and histology. The molecular characteristics of tumors not only can identify novel therapeutic approaches to the specific tumor type, but can also provide clinical information on response to therapy and survival. The study of adjacent healthy tissue as well as premalignant tissue is equally important in the understanding of carcinogenesis, early detection and prevention of cancer. Additionally, normal human tissue to serve as a control is necessary to differentiate neoplastic changes from normal variants.

1.2 RATIONALE: To facilitate meaningful research, there has to be a selection of biospecimens (any human-derived material) from a sufficient number of patients to allow appropriately powered analyses across samples, in addition to sufficient storage and preservation of these specimens to allow for the relevant molecular (genomic and proteomic) analysis. The Head and Neck Tissue Bank (HNTB) aims to collect, store, use and distribute high quality biospecimens, to support basic science, clinical, and translational research across Northwestern University and outside entities that partner with Northwestern Investigators (e.g. multi-institutional projects, pharmaceutical companies in clinical trials, etc.), with an ultimate goal to result in better cancer detection, diagnosis, treatment and prevention.

Tumor tissue from consenting patients will be obtained during biopsy or surgery performed for routine clinical care, and will be banked as frozen tissue for molecular analysis, paraffin-embedded tissue for histopathology and dissociated tumor cells for cytometric analysis (such as flow cytometry) and cell culture. Fresh tissue taken during routine biopsy or surgery may also be used to develop primary cell cultures and xenografts in mice, to improve the experimental models of head and neck malignancies. Such models, closely mimic real tumors in patients or premalignant conditions, and are optimal for evaluating cancer pathogenesis, genes or proteins driving the tumor, factors contributing to an aggressive biology of the tumor and resistance to treatment, as well as identifying new targets for treatment. Blood and other types of biospecimens will be stored frozen for molecular analysis and biomarker studies.

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Although non-neoplastic control tissues would be highly desirable for research purposes, they are understandably very rare to nonexistent in the surgical setting. However, at times there may be the opportunity to collect other biospecimens (e.g. blood, saliva) from normal healthy control subjects. If such an opportunity arises, informed consent and specimen collection will be done as usual, with the same degree of confidentiality and protection of PHI as is provided for cancer patients.

In addition to tissue acquisition, we will obtain additional consent from patients on certain optional elements. These will include update of patient personal and clinical information, as well as contacting the patient to offer participation in two other studies per year. Another optional element for patients participating in the HNTB would be to give approval to release their genetic and health information (with no direct identifiers) into scientific databases.

2.0 OBJECTIVES of the HEAD AND NECK TISSUE BANK

1. To bank human tumor tissue from patients treated at Northwestern Memorial Hospital for head and neck malignancies (either at initial diagnosis or recurrent/metastatic disease), obtained when patients undergo routine biopsy and/or surgery, for use in research.
2. To bank human pre-malignant tissue for research purposes from patients seen at Northwestern Memorial Hospital for symptoms and abnormal areas of the head and neck, and diagnosed with head and neck pre-malignant conditions by biopsy and/or surgery.
3. To bank peripheral blood and other types of biospecimens such as urine and saliva and stored as frozen for future research, from patients treated at Northwestern Memorial Hospital for head and neck malignancies or pre-malignant conditions.

3.0 SELECTION OF SUBJECTS

3.1 Inclusion criteria

- ☐ Age 18 years and older.
- ☐ Patients with lesions affecting the epithelial surfaces of the upper aerodigestive (including but not limited to lips and oral cavity, nose, sinuses, pharynx, larynx, cervical esophagus)
- ☐ Patients with lesions arising from cutaneous surfaces of the head and neck.
- ☐ Patients with lesions arising from the salivary glands.
- ☐ Patients with lesions arising from the thyroid gland.
- ☐ Patients with lesions arising from connective tissues of the head and neck.
- ☐ Patients with pre-malignant or high risk lesions of the head and neck.
- ☐ Healthy individuals without evidence of head and neck malignancy, referred to the head and neck team for evaluation of symptoms related to head and neck (with or without lesions in the head and neck area), and are willing to provide biospecimens to the tissue bank, to use as control.

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- ❑ Participant and/or individual with Medical Power of Attorney (MPA) for participant, demonstrates understanding of the purpose of obtaining tissue and other biospecimens for the tissue bank, and is able to sign informed consent.

3.2. Exclusion criteria

- ❑ Participant does not have the competency to sign informed consent and MPA is either absent or not competent to consent for participant.

3.3. Subject Registration

Patients presenting for evaluation by the head and neck team at the Northwestern Memorial Hospital (NMH), Northwestern Medicine Developmental Therapeutics Institute (NMDTI), for lesions in the head and neck area and are scheduled to undergo biopsy and/or surgery, will be screened for inclusion in the head and neck tissue bank project. Patients who meet the inclusion criteria will be consented for participation in the tissue bank either during clinic consultation or at the time of the procedure (biopsy and/or surgery). Consent will be obtained by a physician (attending or resident), advanced practice nurse or physician assistant who is part of the clinical treatment team.

Patients referred to the head and neck team for evaluation of symptoms related to head and neck (with or without presence of any lesions), will be asked if they would be willing to participate in the head and neck tissue bank project and provide any type of biospecimen they wish to use as control. Consent will be obtained by a physician (attending or resident), advanced practice nurse or physician assistant who is part of the clinical treatment team.

4.0 SELECTION OF SPECIMENS

4.1 Type of Specimens

- Fresh Frozen Tissue (from malignant lesions, normal area adjacent to tumor - at least 3cm far away from the tumor lesion, premalignant lesions).
- Formalin-fixed Paraffin-embedded tissues.
- Blood (whole blood, Plasma, Serum, Buffy coat).
- Saliva.
- Urine.

4.2 Specimen Processing and Usage for Research

- **Processing:** Standardizing methods for collection, processing, long-term storage, retrieval, and distribution of specimens across collection sites are essential to ensure

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biospecimen quality and enable consistent analysis of DNA, RNA, proteins and metabolites [1].

- **Usage for Research:** The samples will be used in studies and assays for genomics (DNA, RNA), proteins (proteomics), and metabolites (metabolomics) [2]

5.0 METHODS AND PROCEDURES

5.1 Tissue Bank Set Up

Governmental and professional agencies and organizations provide some guidelines for standard operating procedures for biorepositories and banking of human tissue. These include the Food and Drug Administration (FDA), the Centers For Disease Control (CDC), the American Association of Tissue Banks (AATB), the American Association of Blood Banks, The International Red Cross, International Society for Biological Repositories (ISBER), BioBank Central and the International Ethical Guidelines for Biomedical Research Involving Human Subjects. The design and set up of the Head and Neck Tissue Bank will be according to the international standard operation procedure (SOP), following the necessary steps as outlined below [3]:

- (a) Approval by the local hospital Ethical Committee.
- (b) Obtaining the informed consent from patients before blood, urine, saliva and/or tissue sample collection.
- (c) Sites for sample collection will be the operation rooms, clinics, inpatient floor, as well as intervention radiology department.
- (d) Storage of samples at very low temperature as quickly as possible after collection to ensure that the integrity of protein, nucleic acids and metabolites is well preserved.
- (e) Tissues will ideally be put in liquid nitrogen within 30 min after excision. Tissue samples will be transferred to the laboratory and put in -80°C freezers for long-term storage.
- (f) Formalin-fixed Paraffin-embedded tissues and specimens fixed in 10% formalin for H&E staining will be stored in room temperature in the pathology department.
- (g) A detailed clinical information of patients from our electronic medical record or outside facility will be collected along with the tissue or other samples.
- (h) In order to preserve confidentiality and the privacy of patients, the data will be supervised by an authorized administrator. De-identified dataset will be created and all biospecimens will be given a serial number. The dataset will include tissue database with links to clinicopathological data.

5.2 Patient selection

All patients presenting to the head and neck team at Northwestern Memorial Hospital (NMH), Northwestern Medicine Developmental Therapeutics Institute (NMDTI) for evaluation, will be screened for inclusion in the head and neck tissue bank project. Patients who meet the inclusion criteria will be consented for participation in the tissue bank either during clinic consultation, hospital encounter or at the time of the procedure (biopsy and/or surgery for those patients undergoing these procedures). Consent will be obtained by a physician (attending or resident), head and neck nurse, physician assistant, or study coordinator who is part of the clinical treatment team.

5.3 Collection, Processing and Storage of Specimens

All collection and processing steps will be conducted under a well-defined quality assurance program in order to preserve all bio-specimens in optimal condition appropriate for the intended laboratory analyses [4-6].

5.3.1 Fresh Frozen Tissue

Patients will undergo biopsy and/or surgery for resection of head and neck lesions, according to standard clinical practice. Once the tissue specimen is out, it will be immediately received by an NMH personnel assigned to tissue collection, the specimen will then be taken to the NMH pathology Gross Room for evaluation by a pathologist or pathologist's assistant. After adequate tissue specimen is sent to pathology for clinical diagnosis, the pathologist's assistant, will release any remaining suitable tissue for banking to the Pathology Core Facility (PCF) biorepository technician (authorized by the HNTB team). All tissue banking specimens will be flash frozen in liquid nitrogen in cryogenic vials by the PCF technician. Each cryogenic vial will have a unique bar code. Samples will then be transported in a liquid nitrogen flask or on dry ice, to the Biorepository located at Olson 8-501, stored in -80 °C or colder storage freezer and entered into the BSI-II inventory management system.

5.3.2 Formalin-Fixed Paraffin-Embedded Tissue.

Patients will undergo biopsy and/or surgery for resection of head and neck lesions, according to standard clinical practice. Once the tissue specimen is out, it will be immediately received by an NMH personnel assigned to tissue collection, the specimen will then be taken to the NMH pathology Gross Room for evaluation by a pathologist or pathologist's assistant. After adequate tissue specimen is sent to pathology for clinical diagnosis, the pathologist's assistant will release any remaining suitable tissue for banking. The specimen will be placed in 10% neutral buffered formalin and then submitted to the PCF Histology Lab for processing into a paraffin embedded block. Once the blocks are created, all research blocks will be stored in room temperature in the

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Biorepository located at Olson 8-501 and entered into the BSI-II inventory management system. All research blocks will be made readily available in the event NMH Pathology requires additional tissue for clinical diagnosis.

5.3.3. Blood Samples

In the clinic or inpatient setting, collection of blood specimens will be carried out by trained nursing staff and/or phlebotomists to avoid causing study participant discomfort, or compromising the quality or quantity of the sample [7]. Blood will be drawn from peripheral vein, or peripherally inserted central catheter (PICC line), or central venous catheter placed for treatment indicated by standard clinical practices. Each patient will have an additional 30-40ml collected at the time of routine blood drawn, as determined by the clinical treating team based on standard practices. Patients undergoing surgery, can choose to have blood collected in the operating room instead, drawn by the anesthesiologist while the patient is under anesthesia, from an arterial line, central venous line, or peripheral iv line placed as part of routine anesthesia care. The blood samples will be received by the (PCF) technician (authorized by the HNTB team) and taken to the Lab for processing and storage as per SOP's. Blood in the EDTA tube will be centrifuged for separation of blood cells (buffy coat) and plasma, which will be individually collected and frozen separately at -80°C or colder. Another blood specimen will be centrifuged for separation and collection of serum, which will be divided into three aliquots and stored at -80°C or colder. The locations of all these samples will be recorded in the BSI-II inventory management system.

5.3.4 Saliva

Patients will have saliva collected at the time of routine clinic visit by nursing staff authorized by the HNTB. Patients will be instructed to refrain from eating and drinking for 1 hour prior to saliva collection. They will be given distilled drinking water and asked to rinse their mouth out well for 1 minute. Subsequently, the patient will be instructed to either expectorate or swallow the water. Five minutes after the rinse, the subject will spit approximately 5ml of saliva into a chilled container/tube [8]. The container/tube will be received by the PCF technician, placed on wet ice until processing. The saliva sample will be centrifuged to separate the supernatant and cell pellet, which will be individually collected and frozen separately at -80°C or colder. Their location will be recorded in the BSI-II inventory management system.

5.3.5 Urine

Patients will have urine collected at the time of routine clinic visit or while inpatients, by trained nursing staff using aseptic technique for all patients that have catheters. Urine (30-50ml) will be collected from the catheter draining port. All other patients will be instructed to provide a clean catch, midstream urine sample in a standard urine collection cup. Urine samples will be received

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by the PCF technician and taken to the lab for processing and storage at -80°C or colder [9]. Urine sample locations will be recorded in the BSI-II inventory management system.

5.4 Specimen Requests and Distribution

To obtain materials from the HNTB, investigators will complete and submit a form that describes the project, the number and type of specimens requested, and documentation of IRB approval for their specific project. All requests are promptly reviewed by the HNTB Principal Investigator, PCF biorepository coordinator for HNTB and/or PCF biorepository lab manager for HNTB. Once their IRB approval is verified, requests deemed easily met (e.g. a small number of unstained slides from preexisting paraffin blocks) will be fulfilled promptly. More complicated requests (e.g. large number of fresh tissues, or something that would exhaust the bank's collection of a specific tumor type) will be discussed with the HNTB Committee Members. This multidisciplinary committee consists of: 1) Maria Matsangou MD, Principal Investigator (Department of Medicine, Division of Hematology-Oncology), 2) Sandeep Samant MD, Chief of Head and Neck Surgery, Department of Otolaryngology, 3) Ajit S Paintal, MD, Department of Pathology, 4) Demirkan B. Gursel M.Sc. Ph.D., Scientific Director of the Pathology Core Facility.

6.0 STATISTICAL PLAN

There is no finite estimate for tissue collection, as the goal of the HNTB is to collect and bank as many biospecimens as possible indefinitely, or for as long as the HNTB can be sustained and funded. We estimate that the HNTB will run for more than 10 years and collect specimens from more than 1500 patients. Based on the current Head and Neck operative volume and patients requiring biopsy for lesions at the head and neck, we anticipate enrollment and tissue collection from approximately 100-150 patients per year. A statistical plan is not applicable as this is not an outcomes study.

7.0 DATA COLLECTION AND RECORD KEEPING

Written informed consent will be obtained from patients (or their assigned MPA) prior to undergoing any procedures or providing any type of biospecimen at Northwestern Memorial Hospital or NMDTI. The original signed consent for each case will be kept in a secure file cabinet in the Pathology Core Facility (PCF) as well as in digital form on a secure server. Additionally, consent to participate in the study will be scanned in the patient's medical record and a copy will also be logged in eNOTIS.

Tissue, blood, and other biospecimens will be processed and stored as described above. All specimens will be labeled with unique barcodes and identified with a coded patient identifier.

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Each individual specimen will be logged in the BSI-II database. Basic patient demographic data may also be logged in the database for each specimen including patient age, patient gender, date of specimen procurement, histopathological diagnosis, primary or recurrent tumor, and tumor location. This information in addition to other clinical information, clinical variables and other data elements will be stored in a separate secure database system, the Head and Neck Cancer Registry (HNCR).

When specimens are accessed for research under IRB approved protocols, only the de-identified information in the BSI-II database will be available to investigators. If more clinical information is necessary, clinical data can be requested from the Head and Neck Cancer Registry (HNCR). For studies requesting information beyond what is recorded in the registry, clinical data can be additionally requested from the EDW. Authorized HNTB personnel will provide authorized HNCR, or EDW personnel the decoded patient list to obtain the approved clinical data. The HNCR or the EDW will then provide de-identified results matched to the tissue code to the requesting investigator.

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