SOP#: ADGC-5  Tumor/Normal Whole Exome Sequencing: Consenting, Ordering, and Obtaining Results

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POLICY
Patient consent is required prior to ordering the Tumor/Normal Whole Exome Sequence test and the fully signed consent document must be uploaded into the patient’s CRIS medical record prior to the test being performed. Patient consent for the Tumor/Normal Whole Exome Sequence testing must be obtained by a genetic healthcare provider.

The Tumor/Normal Whole Exome Sequencing Policy applies to all NIH intramural investigators ordering the Tumor/Normal Whole Exome Sequencing CLIA approved test performed in the Laboratory of Pathology.

PURPOSE
To provide instructions for the process of consent, determining the somatic and germline samples to submit, entering the order, submission of samples, and obtaining results for Tumor/Normal Whole Exome Sequence testing.

BACKGROUND
A major limitation of tumor-only genomic analysis is the inability to discern whether a variant is somatic or germline [1]. The purpose of performing the tumor (somatic)/normal (germline) paired analysis is to subtract the germline variants from the somatic variants using bioinformatics resulting in a somatic-specific genomic tumor profile [2].

Somatic Whole Exome Sequencing
The somatic whole exome sequencing will detect and report all clinically or pathologically actionable variants, copy number alterations, microsatellite instability (MSI), and tumor mutational burden.

Somatic whole exome sequencing will NOT detect all genomic changes in a tumor. Whole exome sequencing will not typically detect: complex structural rearrangements, large deletions or duplications, mitochondrial variants, epigenetic changes such as methylation, histone modifiers and readers, chromatin remodelers, microRNAs, and other components of chromatin,
mosaicism, uniparental disomy, variants in repetitive or high GC-rich regions, or variants in genes with an associated pseudogene or other highly homologous sequences.

**Germline Whole Exome Sequencing**

The germline whole exome sequencing will identify and report **ONLY** a list of clinically significant cancer genes (>150). **Supplement A: List of Genes for Germline Reporting** provides the current list of germline genes being reported by the Laboratory of Pathology at NIH. Only two non-cancer genes on the current American College of Medical Genetics and Genomics (ACMG) list of incidental and secondary genomic findings will be reported. Only pathogenic, likely pathogenic, and variants of uncertain clinical significance will be reported. Variants classified as likely benign or benign will **NOT** be reported. Germline results may or may not have current clinical relevance for the patient depending on the state of their disease. But germline findings could have relevance to biologic family members. All germline results will be discussed with the treating oncologist **BEFORE** the results are released to the patient.

**TSO500**

If a patient declines germline testing, the patient is eligible for TSO500 panel sequencing and will be referred back to their oncologist to discuss this option. The TSO500 is a next-generation sequencing assay that analyzes cancer-relevant genes from both DNA and RNA in one integrated workflow. With simultaneous analysis of both DNA and RNA, various types of biomarkers relevant to a given tumor type (single nucleotide variants, indels, fusions, splice variants, tumor mutational burden, and microsatellite instability) can be assessed from the same sample. The TSO500 panel includes 523 genes for DNA variant detection and 55 genes for fusion and splice variant detection. However, TSO500 is performed only on tumor samples and will not be able to differentiate if a variant is of germline or somatic origin.

**Test Reports**

This test will generate both a somatic and a separate germline clinical report. The test will begin **ONLY** after consent has been obtained and is uploaded in CRIS and the tissue and blood samples are received by the Laboratory of Pathology. The somatic test takes ~4 weeks and will be reported in CRIS to the ordering provider. The germline test takes ~4 weeks and will be reported in CRIS. Germline results will be available to Clinical Cancer Genetics Program prior to CRIS reporting to facilitate discussion with the treating oncologist.

**Consent**

Tumor/Normal Whole Exome Sequencing requires the patient to receive the germline results which could include incidental or secondary findings [3]. If the patient declines germline testing, this specific test cannot be performed. In this case, the patient would not be able to undergo this test and will be offered instead the tumor-only TSO 500 test.

Therefore, prior to undergoing this test, the patient must provide written consent in the form of a Tumor/Normal Whole Exome Sequencing procedure consent [4]. A copy of both the English and Spanish versions of the consent can be found in **Supplement B**. This consent currently requires an encounter with a genetic healthcare provider (Geneticist, Genetic Counselor,
Genetic Nurse) for education and genetic counseling about this test along with documentation of their decision.

Following the consent, the patient will be provided with a copy of the Tumor/Normal Whole Exome Sequencing companion education sheet in Supplement C.

**Workflow**

![Workflow Diagram]

**RESOURCES**

**Clinical Cancer Genetics Program**

- **Request for Consent**
  
  Call 240-760-7350 OR
  
  Email TumorNormalWES@mail.nih.gov

- **Clinical Cancer Genetics Program**
  
  Kathleen Calzone, PhD, RN, AGN-BC, FAAN
  
  240-760-6178, calzonek@mail.nih.gov

- **Clinical Cancer Geneticist**
  
  Chimene Kesserwan, MD, FCAP, FACMG
  
  301-222-3925, chimene.kesserwan@nih.gov
Health Information Management Department Forms website

- Form NIH-3012 “Consent for Clinical Somatic/Germline Testing”
- Form NIH-3012 SP “Consent for Clinical Somatic/Germline Testing (Spanish)”
- Form NIH-527-1 “Authorization for the Release of Genetic Test Results”
- Form NIH-527-1-SP “Authorization for the Release of Genetic Test Results (Spanish)”

PROCEDURES

STEP 1: Obtaining Consent

- All patients must meet with a genetic healthcare provider for consent.
  - Email or call (see above Resources, Request for Consent) to request a genetic healthcare provider to meet with the patient to obtain consent.
    - Please include patient’s name, MRN, and who to notify when consent has been obtained. If using email, you must send encrypted.
    - The Genetic Counselor following consent will provide the team with information about whether the patient did or did not consent to germline results which will inform what order to place.

- Consents will be done either virtually using iMED or in-person using the approved procedure with printed consent form NIH-3012, also available in Spanish. See above Resources for links to the consent documents. For other languages a translator will be required. This is a procedure consent so no short form is used.

- Form NIH-527-1 Authorization for the Release of Genetic Test Results must be completed either virtually using iMED or in-person as part of the consent process. See above Resources for links to this release consent. If completed on paper, send the signed document to HIMD for uploading into CRIS.

- All patients undergoing consent are also provided with a copy of the companion education sheet found in Supplement C.

- Form NIH-3012 Consent for Clinical Somatic/Germline Testing is also obtained via the iMED system. See Supplement D for directions on the iMED system.

- If the iMED system is not available, please use the paper consent assessable via the link under Resources above. Send the signed consent to HIMD for uploading into CRIS.

- The completed consent will be housed in the CRIS Consents tab under Procedure.
STEP 2: Entering the Order for the Tumor/Normal Whole Exome Sequence Test

- The provider entering the order can ONLY enter the order once consent has been obtained because the patient’s decision on the consent will dictate the type of order. If a patient DOES NOT agree to reporting of the germline findings, this test CANNOT be performed, and the only option is the TSO500 panel.
- For patients that consent to germline reporting, two separate orders are required. One order for the tissue, one order for the blood. See Supplement E for screen shots of the order sets.
  - Molecular Pathology, Tissue:
    - Step 1-Select COMPASS, from “Test Requested” dropdown list.
    - Step 2-Select Specimen Source AND check Whole Exome Sequencing/RNAseq.
    - Step 3-Review your order, confirm that consent has been obtained, and proceed to entering the order for the blood sample.
  - Molecular Pathology, Blood:
    - Select COMPASS Germline Mutation Control from “Test Requested” dropdown list.
    - This is the order set for the blood draw at NIH Outpatient Phlebotomy

STEP 3: Determine the Tumor and Germline Sample(s) to Submit

NOTE: -any patient with a prior hematologic malignancy may NOT be able to submit a blood or saliva sample. Email or call (see above Resources, Request for Consent) to determine eligibility. Skin fibroblasts may be needed. ONLY skin fibroblasts can be submitted for the analysis for any patient with a prior transplant (organ or bone marrow) and for most patients with a current hematologic malignancy.
- Tumor Sample Submission Parameters
- Germline Sample Submission Parameters
- Saliva Sample Submission Parameters
- Fibroblast Sample Submission Parameters
  - This capacity is currently NOT available. Once available this SOP will be revised to include sample submission details.

STEP 4: Obtaining Patient’s Pedigree

- For patients that DO NOT have a pedigree on file in CRIS, the patient will be asked to complete the NCI family history form Supplement F prior to the results becoming available, which will provide information for constructing a pedigree. The NCI family history form will be sent to the patient following consent using secure email with a request to return the completed form within 2 weeks.
• Upon receipt of the family history form, a genetic counselor/genetic counselor assistant will construct a pedigree which will allow for appropriate interpretation of germline genetic findings

STEP 5: Obtaining Results
• Tumor Sample
  o All tumor results will be released in CRIS.
• Germline Sample
  o All germline results will be preliminarily released to Clinical Cancer Genetics Program prior to releasing in CRIS. This will provide the genetics team the time needed to discuss the findings with the clinical oncologist and if time allows notify the patient of the findings.

STEP 6: Repeating the Analysis at the Time of Disease Progression or Other Indication
• Tumor Sample
  o Repeat all tumor specific procedures starting with Step 2. A second consent will NOT be required as the patient has already obtained their germline findings.
• Germline Sample
  o No additional germline sample collection is required. The germline from the prior analysis will be used for any additional somatic testing.

STEP 7: Requesting Somatic and Germline for Research Purposes
Data will be made available for research purposes with the required regulatory approval (IRB or exemption approval) and as applicable patient consent. The approval must correspond to protocol or exemption submission and approval that specifies that access is provided to the Tumor/Normal Whole Exome Sequencing clinical test data, which will be provided via the Clinical Oncogenomics database.

Data that can be provided include all annotated variants (somatic and germline), fusions, CNVs. Tumor Signature, HLA, Neoantigen, and Circos. Any of these data has a download link. Also the VCF file has a download link.

Handling and transferring of BAM files are a substantial burden for the Laboratory of Pathology. If the Oncogenomics analysis is not sufficient and specific research project requires BAM files, the Principle Investigator of the study must provide a detailed justification of the need for the BAM files in the request memo.
• Somatic and germline data
  o Submit a written request in the form of a memo to TumorNormalWES@mail.nih.gov that includes the following information:
    ▪ Data type; somatic, germline or both somatic and germline
    ▪ What members of the team require access to the data
    ▪ Protocol or exemption number that corresponds to the regulatory approval to gain access to this clinical data
    ▪ Specific test, Tumor/Normal Whole Exome Sequencing
    ▪ Patient(s) Medical Record Number(s)
    ▪ Justification for BAM files if applicable
    ▪ Mechanism for transfer of the BAM files if applicable
    ▪ Primary contact for questions associated with the submission
  o Requests will be reviewed first to confirm the regulatory approval is in place as is patient consent.
  o Requests will be reviewed weekly and the submitter will be notified via email of the approval, disapproval, or requirement for further information.
  o Once approved, the request will be forwarded to the Laboratory of Pathology for processing.
  o Once a submission has been approved, the Laboratory of Pathology will organize a sub-project under COMPASS in the Oncogenomics portal specific to this request and the individuals specified as requiring access to the data.
    ▪ For changes (additions or deletions) in previously approved team members that require access, please submit a written request to TumorNormalWES@mail.nih.gov.

• If a Somatic or Germline Actionable Finding is Identified Through Research Secondary Analysis
  o CLIA confirmation will be required for any finding PRIOR to using this information for clinical care
    ▪ A new sample may not be required. Submit information on the finding to Liqiang Xi to investigate and advise if existing CLIA samples and/or data are sufficient for issuing an amended report.
    ▪ For Germline findings, genetic education, counseling in addition to the CLIA confirmation will be required before acting on the finding. Please submit a consult request to TumorNormalWES@mail.nih.gov.
REFERENCES


