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 test that has been validated in the CLIA-certified and CAP-accredited COMPASS clinical genomics program of 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. Complex germline results will be discussed with the ordering provider **BEFORE** the results are released to the patient. Otherwise, all results will be in CRIS with a Genetic Consult note written by the genetic healthcare provider that saw 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 and detects most clinically relevant mutations, including single nucleotide variants, small insertions, and deletions, and copy number variation in 523 unique cancer genes. In addition, the data from the TSO500 assay is used to quantify tumor mutational burden and microsatellite instability. The TSO500 panel is also paired with whole RNA exome sequencing to report fusions involving 532 cancer-associated genes. However, the TSO500 and whole RNA exome sequencing are performed only on tumor samples and will not be able to differentiate if a variant is of germline or somatic origin.

Test Payment for Non-Center for Cancer Research Users

Given current budget constraints in Center for Cancer Research (CCR) the Laboratory of Pathology has been directed to make changes in the financial structure of the genomic tests. Non-CCR investigators are required to cover a portion of the costs for Next Generation Sequencing (NGS) tests performed by the Laboratory of Pathology. Effective October 1 2023, non-CCR users are required to provide payment for NGS tests, including TSO500, RNAseq, and whole exome sequencing (WES). To accomplish this, an upfront CAN transfer is needed to purchase the necessary reagents. The Laboratory of Pathology will require an initial payment corresponding to reagent costs that cover 50% of the prior year's test order volume placed by each investigator's service and will provide information on that amount to the investigator. **For non-CCR users without prior year activity, please contact Dr. Ken Aldape.**

At this time, the Laboratory of Pathology envisions a charge only to recoup reagent costs, but this is subject to change depending on the annual budget. To arrange for payment, please have your Administrative Officer contact Dr. Joe Chinquee chinquej@mail.nih.gov to coordinate a CAN transfer.

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 ~4-6 weeks and will be reported in CRIS to the ordering provider. The germline test takes ~4-6 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 ordering provider if indicated.

Germline results will be provided to the patient by the genetic healthcare provider that consented the patient or their designee. The ordering provider or their designee are responsible for providing the somatic results.

For tumor tissue samples which are inadequate for analysis (e.g., insufficient tumor), the germline test (which is being done to inform the somatic analysis) **will not** be performed. If both the tumor/germline samples are sequenced and the tumor sequence quality control failed, the tumor result **will not** be reported. However, if the germline sequence passed quality control, the germline result **will** be reported. The ordering provider or their designee is responsible for providing the patient with information on test cancellations due to samples that are inadequate for analysis.

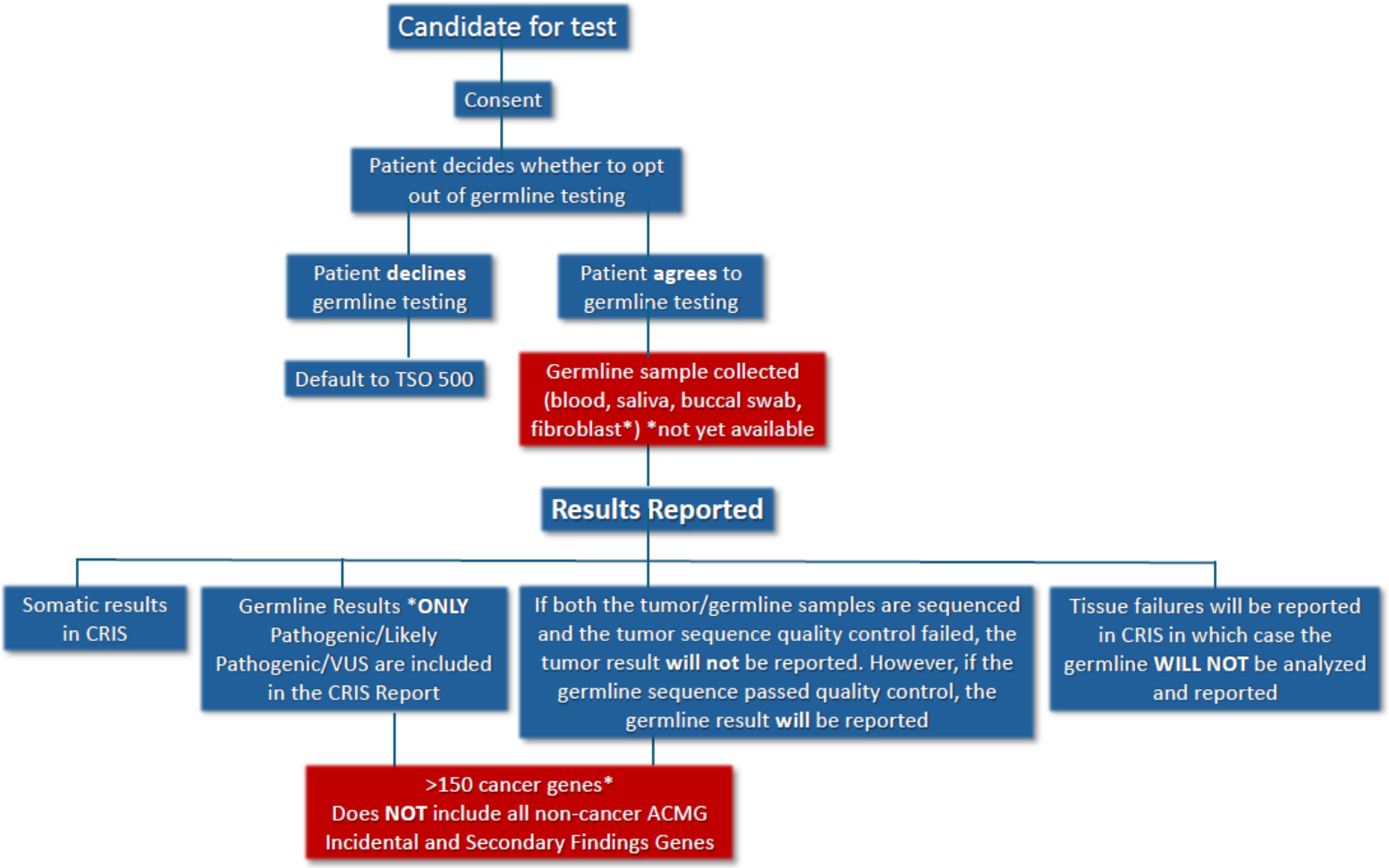
Consent

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. Both English and Spanish versions are available in iMed Consent.

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



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

- **T/N WES Lead Genetic Counselor**
  Yi Liu, MS, LCGC
  301-222-3228, yi.liu2@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"

iMed Consent Resources via CRIS Education Resources > Communication with Other Staff and Patients > iMed Consent

PROCEDURES

STEP 1: Obtaining Consent

- All patients must meet with a genetic healthcare provider for consent **PRIOR to collection of the germline sample**.
    - 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, confirmation that the patient has been notified to expect a contact to schedule a consent appointment (virtual or in-person), and who to notify when consent has been obtained. **If using email, you must send encrypted.**
            - Please submit a completed Tumor/Normal Whole Exome Sequencing Test Referral Checklist specific to the type of patient referred (general or hematologic) **Supplement D**.
        - Confirm the patient has recent pertinent medical history (History of Present Illness [HPI] and Past Medical History [PMH]) documented in CRIS. ***Patients cannot be seen without a recent history (HPI and PMI) documented in CRIS.**
            - 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 using iMed, either virtually or in-person using the approved procedure with 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 using iMed either virtually 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.
- If the iMed system is not available, please use the paper consent accessible 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 **or** saliva (including buccal swab). Saliva is collected using an Oragene DX Saliva Kit and buccal swabs are collected using the ORA collect DX buccal swab kit delivered to 3S247
- Specific instructions for entering the order can be found on the Laboratory of Pathology website at: https://ccrod.cancer.gov/confluence/display/CCRLP/CRIS+Order+Instructions

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 saliva, or buccal swab sample. Email or call (see above **Resources**, Request for Consent) to determine eligibility. The Genetic Counselor will determine the optimal sample for germline collection that can be submitted for the analysis for any patient with a prior transplant (organ or bone marrow) and for patients with a current hematologic malignancy.

- **Tumor Sample Submission Parameters**
- **Germline Sample Submission Parameters**
- **Saliva Sample Submission Parameters**
  - Saliva samples should be collected using the Oragene DX saliva kit. Instructions for the collection of saliva are located at the DNA Genotek website.
- **Buccal Swab Submission Parameters**
  - Buccal swabs samples should be collected using the ORA collect DX buccal swab kit. Instructions for the collection of buccal swabs are located at the DNA Genotek website.
- **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**
    - All tumor results including failed tests (e.g., insufficient tumor) will be released in CRIS to the ordering provider. The ordering provider or their designee are responsible for retrieving the tumor results and notifying the patient.
- **Germline Sample**
    - All germline results will be preliminarily available to the 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 if indicated. The genetic counselor that consented the patient or their designee are responsible for retrieving the germline results and notifying the patient.

STEP 6: Repeating the Analysis at the Time of Disease Progression or Other Indication

- **Tumor Sample**
    - 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**
    - 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 Principal Investigator of the study must provide a detailed justification of the need for the BAM files in the request memo.

- **Somatic and germline data**
    - 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
    - Requests will be reviewed first to confirm the regulatory approval is in place as is patient consent.
    - Requests will be reviewed weekly and the submitter will be notified via email of the approval, disapproval, or requirement for further information.
    - Once approved, the request will be forwarded to the Laboratory of Pathology for processing.
    - 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**
    - 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

1. Mandelker, D. and O. Ceyhan-Birsoy, *Evolving Significance of Tumor-Normal Sequencing in Cancer Care.* Trends Cancer, 2020. 6(1): p. 31-39.
2. Mandelker, D. and L. Zhang, *The emerging significance of secondary germline testing in cancer genomics.* J Pathol, 2018. 244(5): p. 610-615.
3. Presidential Commission for the Study of Bioethical Issues, *Anticipate and Communicate: Ethical Management of Incidental and Secondary Findings in the Clinical, Research, and Direct-to-Consumer Contexts*. 2013.
4. Li, M.M., et al., *Points to consider for reporting of germline variation in patients undergoing tumor testing: a statement of the American College of Medical Genetics and Genomics (ACMG).* Genet Med, 2020. 22(7): p. 1142-1148.