TESTS THAT MAY HELP INFORM A PERSONAL TREATMENT PLAN

Molecular Profiling for Identification and Treatment

	TEST TYPE*	PURPOSE	WHEN**	SAMPLE REQUIRED†	LEAD TIME FOR RESULTS	PROS	CONS^
Hereditary Profiling	**Germline genetic testing (normal inherited DNA)**	Identifies genetic changes in non-tumor cells which can be associated with increased risks of cancer (for example, hereditary genetic sequence changes, deletions/amplifications), which could reveal other cancer risks and indicate a more aggressive treatment plan. Does not look at genomic profile of the tumor. Germline testing is primarily done with targeted gene panel sequencing. Examples of inherited predispositions with linkages to osteosarcoma include Li-Fraumeni syndrome and mutations of the BRCA genes.	Can be done at any time, typically only need to do it once.	Blood or saliva sample	~1 month	• Non-invasive and easy • Can help determine need for close surveillance to prevent other cancers • Likely offered at your hospital.	• May not provide insight into specific treatment options, other than identifying if the patient is at higher risk for other cancers and thus more aggressive treatment recommended. Many tests come back with uncertain results, which can be stressful and confusing. • Note this can add stress to an already stressful time including the stress of how and if to inform family members who may not want to know they may carry a cancer risk gene.
Tumor Profiling	**Whole genome sequencing (WGS)**	Identifies **somatic** (acquired, not inherited) variations in the **entire genome**, nearly all of the approximately 3 billion nucleotides of an individual's complete DNA sequence, including **exons** (the protein coding region of any gene) and **introns** (non-coding sections of DNA in between genes). Next generation sequencing (NGS) is a high throughput, low cost, speedy technology that is typically used to generate genetic information for WGS.	• At biopsy • At any resection	Fresh frozen tissue from the tumor. Blood or buccal swab to benchmark against healthy cells/DNA.	~1-2 months	• Can detect alterations in any part of the genome: single nucleotide variants, insertions/deletions, copy number changes, and large structural variants.	• Since it analyzes the entire genome, it is more expensive and takes longer • Since WGS sequencing is not as deep as WES, results may not be as sensitive. • May be difficult to find an institution that will do this - currently only accessible by participating in a research study.
	Whole exome sequencing (WES)	Identifies **somatic (acquired, not inherited) variations in only exons** (the protein coding region of any gene) across all genes. While the exome (all the exons) represents ~1-2% of the whole genome, most known mutations that cause disease are in the exons.	• At biopsy • At any resection	Formalin-Fixed Paraffin-Embedded (FFPE) tissue, fresh tissue, or fresh frozen tissue from the tumor.	~1-2 months	• Faster turnaround time vs. WGS • Lower cost vs. WGS • Since sequencing is deeper and more sensitive than WGS, WES may pick up lower frequency events.	• It is possible that some clinically significant mutations may be missed by this approach due to inefficient capture of certain exons or introns.
	Targeted gene panel sequencing	Can be used for either germline testing of hereditary mutations or somatic mutations in tumor tissue. When used on tumor tissue, identifies **possible somatic DNA and sometimes RNA mutations** (sequence changes, deletions/duplications) in the cancer cells in a **select number of genes, vs. the entire genome, or the entire exome**. Focused panels contain a select set of genes or gene regions that have known or suspected associations with certain types of cancer.	• At biopsy • At any resection	Depending on test type, FFPE or blood for tumor DNA. Sometimes blood/saliva is needed to benchmark against healthy cells/DNA.	~1 month	• Common type of test that is easily accessible. • Better sensitivity than WES or WGS • Faster turnaround time • Lower cost • Produces a smaller, more manageable data set compared to broader approaches such as WGS, making analysis easier.	• Targeted panels have not been designed specifically for osteosarcoma so genes relevant to osteosarcoma may not be included in the panel. • Newly discovered genes may not be included. • May not capture translocations or measure copy number alterations.
	RNA Sequencing (RNAseq)	Detects gene **fusions** (when part of one gene connects to part of another gene), and/or **expression** (how much RNA is made from the gene).	• At biopsy • At any resection	Fresh frozen tissue from the tumor preferred; FFPE possible	~1 month	• Fusions are common in pediatric cancers including osteosarcomas. Finding TP53 fusions can help to make the diagnosis. Expression data provides insight into which genes might be switched on or off, versus just identifying mutations in the genetic code.	• Targeted panel RNAseq is available from some academic and commercial institutions, but it may be difficult to find a commercial or research institution that will do whole transcriptome RNAseq.
	Single gene testing	Identifies genetic changes in one gene. These tests are typically used to confirm (or rule out) a specific diagnosis, particularly when there are many variants in the gene that can cause the suspected condition. (e.g. TP53 mutations and/or MYC rearrangement using fluorescence in situ hybridization (FISH) methodology).	• At biopsy • At any resection	Fresh frozen tissue from the tumor preferred; FFPE possible	~2 weeks	• Fast way to identify specific gene mutations • Can be used to rule out or prioritize certain therapeutic strategies	• Since it analyzes only changes in a single gene, other mutations may be overlooked
	Immunohistochemical Staining (IHC Staining)	Determines level of protein expression in the tumor cells. Provides a negative or positive result for a specific protein. This is typically used to determine the presence or absence of a specific mutation and can be a key criteria for acceptance to a clinical trial or determining whether a targeted therapy would likely be efficacious.	• At biopsy • At any resection • If determining eligibility for a specific clinical trial targeting a specific protein	FFPE from the tumor	~2 weeks	• Identifies specific biomarkers in the tumor that may be targeted. • Likely available at the hospital/research institution where the trial or targeted therapy would be undertaken.	• You must identify a specific protein to test; there isn't a test that includes a panel of proteins that can be tested all at once.

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**These tests may help identify any mutations or pathways that may be targetable with a drug, and might help prioritize a drug list for functional drug testing. However, these identified mutations may not necessarily be tumor drivers, nor have available drugs.*

Note, there are two other main technologies that are used in popular at home genetic products. One is genotyping, the other sequencing. Genotyping looks for specific variants in ~<1% of the genome. This can be an effective method for identifying variants, however, it requires a pre-defined list of variants to search for, which limits analysis to those on the list. If you have a variant that is not on this list, it will not be picked up.

Typically the test types in this table include genetic counseling with in-depth interpretations of the results. This Is not provided with at home tests.

***While germline testing just needs to be performed once (perhaps upon diagnosis), all of these tests can be done any time there is new tissue available from a biopsy or resection. Tumors can mutate and change over time so it can be helpful to test at each relapse. Since FFPE can be archived, any test that uses FFPE can be done at any time. You may also arrange for fresh tumor tissue to be cryopreserved so it can be accessed at a later date.*

†For tissue samples, resection specimens post-chemo should be the last possible choice for sample selection because often most of the tumor cells are dead from chemo. FFPE is a Formalin-Fixed Paraffin-Embedded tissue specimen.

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