

## TESTS THAT MAY HELP INFORM A PERSONAL TREATMENT PLAN

TEST TYPE	PURPOSE	WHEN*	SAMPLE REQUIRED†	LEAD TIME FOR RESULTS	PROS	CONS^	
understanding tumor characteristics and potential drivers	<b>Germline genetic testing (normal inherited DNA)</b>	Identifies <b>inherited</b> predispositions to cancer (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.	Can be done at any time, only need to do it once.	blood or saliva sample	~1 month	<ul style="list-style-type: none"> <li>• Non-invasive and easy</li> <li>• Can help determine need for close surveillance to prevent other cancers</li> <li>• Likely offered at your hospital.</li> </ul>	<ul style="list-style-type: none"> <li>• Does 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.</li> <li>• Note this can add stress to an already stressful time including the stress of informing family members who may not want to know they may carry a cancer risk gene.</li> </ul>
	<b>Whole genome sequencing (WGS)</b>	Identifies <b>somatic</b> (acquired, not inherited) variations in the <b>entire genome</b> , nearly all of the approximately 3 billion nucleotides of an individual's complete DNA sequence, including <b>exons</b> (the protein coding region of any gene) and <b>introns</b> (non-coding sections of DNA in between genes).	<ul style="list-style-type: none"> <li>• At biopsy</li> <li>• At any resection</li> </ul>	Fresh frozen tissue. Blood or buccal swab to benchmark against healthy cells/DNA.	~1-2 months	<ul style="list-style-type: none"> <li>• Can detect alterations in any part of the genome: single nucleotide variants, insertions/deletions, copy number changes, and large structural variants.</li> </ul>	<ul style="list-style-type: none"> <li>• Since it analyzes the entire genome, it is more expensive and takes longer</li> <li>• Since WGS sequencing is not as deep as WES, results may not be as sensitive.</li> <li>• May be difficult to find an institution that will do this - currently only accessible by participating in a research study.</li> </ul>
	<b>Whole exome sequencing (WES)</b>	Identifies <b>somatic (acquired, not inherited) variations in only exons</b> (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.	<ul style="list-style-type: none"> <li>• At biopsy</li> <li>• At any resection</li> </ul>	FFPE, fresh tissue, or fresh frozen tissue	~1-2 months	<ul style="list-style-type: none"> <li>• Faster turnaround time vs. WGS</li> <li>• Lower cost vs. WGS</li> <li>• Since sequencing is deeper and more sensitive than WGS, WES may pick up lower frequency events.</li> </ul>	<ul style="list-style-type: none"> <li>• It is possible that some clinically significant mutations may be missed by this approach due to inefficient capture of certain exons or introns.</li> </ul>
	<b>Targeted gene panel sequencing</b>	Can be used for either germline testing of hereditary mutations or somatic mutations in tumor tissue. When used on tumor tissue, identifies <b>possible somatic DNA and sometimes RNA mutations</b> (sequence changes, deletions/duplications) in the cancer cells in a <b>select number of genes, vs. the entire genome, or the entire exome</b> . Focused panels contain a select set of genes or gene regions that have known or suspected associations with certain types of cancer.	<ul style="list-style-type: none"> <li>• At biopsy</li> <li>• At any resection</li> </ul>	Depending on test type, FFPE or blood for tumor DNA. Sometimes blood/saliva is needed to benchmark against healthy cells/DNA.	~1 month	<ul style="list-style-type: none"> <li>• Very common type of test that is easily accessible.</li> <li>• Better clinical sensitivity</li> <li>• Faster turnaround time</li> <li>• Lower cost</li> <li>• Produces a smaller, more manageable data set compared to broader approaches such as WGS, making analysis easier.</li> </ul>	<ul style="list-style-type: none"> <li>• Targeted panels have not been designed specifically for osteosarcoma so genes relevant to osteosarcoma may not be included in the panel.</li> <li>• Newly discovered genes may not be included.</li> <li>• Does not capture translocations or measure copy numbers.</li> </ul>
	<b>RNA Sequencing (RNAseq)</b>	In a select number of genes, identifies possible <b>RNA rearrangements</b> (2 genes changing places), <b>fusions</b> (when part of the DNA from one chromosome moves to another chromosome), and <b>expression</b> (how much RNA is made from the gene) that are analyzed versus just looking at DNA mutations.	<ul style="list-style-type: none"> <li>• At biopsy</li> <li>• At any resection</li> </ul>	Fresh frozen tissue preferred; FFPE possible	~1 month	<ul style="list-style-type: none"> <li>• Provides insight into which genes might be switched on or off (gene expression), versus just identifying mutations in the genetic code.</li> </ul>	<ul style="list-style-type: none"> <li>• Targeted panel RNAseq is available from some commercial institutions, but it may be difficult to find a commercial or research institution that will do whole transcriptome RNAseq.</li> </ul>
	<b>Immunohistochemical Staining (IHC Staining)</b>	Determines level of <b>protein expression</b> in the tumor cells. Provides a negative or positive result for a specific protein.	<ul style="list-style-type: none"> <li>• At biopsy</li> <li>• At any resection</li> <li>• If determining eligibility for a specific clinical trial targeting a specific protein</li> </ul>	FFPE, fresh tissue or fresh frozen tissue depending on the test	~2 weeks	<ul style="list-style-type: none"> <li>• Identifies specific biomarkers in the tumor that may be targeted.</li> </ul>	<ul style="list-style-type: none"> <li>• 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.</li> </ul>

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.

\*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.

Note, there are two main technologies that are used in popular genetic products. One is genotyping, the other sequencing. Genotyping is what is used by 23&me. 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.

