

COMMENTARY

Germline Findings in Tumor-Only Sequencing: Points to Consider for Clinicians and Laboratories

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Abstract

Precision oncology holds great potential to improve patient therapies and outcomes. Tumor sequencing is rapidly moving into clinical care as our understanding of the cancer genome and the availability of targeted therapies increase. Analysis of the cancer genome is most informative when paired with germline genomic DNA to delineate inherited and somatic variants. Although tumor-only analysis remains the most common methodology for numerous reasons, it holds the potential to identify clinically significant germline variants. Here, we provide anticipatory guidance and points to consider for laboratories and clinicians regarding the potential for germline findings in tumor sequencing.

Precision oncology strategies that leverage the power of tumor genome sequencing to identify driver mutations predictive of response to targeted therapies are rapidly transforming cancer care (1–3). The use of the mutational profile of a tumor to guide treatment decision is not novel. Clinicians have utilized hormone receptor and HER2/neu expression status in breast cancer care and N-Myc amplification status in the treatment of neuroblastoma since the early 1990s (4–6). Until recently, clinical genomic testing of patient tumors has been limited in scope because of a lack of knowledge of relevant genomic biomarkers (7). Over the last several years, however, the scope of testing has rapidly expanded because of an increasing knowledge of the landscape of cancer genomes. Current tumor sequencing modalities range from single-gene assays and targeted mutation panels to genome-scale tests such as tumor whole-exome sequencing and whole-genome sequencing (WES/WGS) (8).

Genomic analyses of tumors have the ability to not only identify somatic (tumor-specific) variants with potential diagnostic, prognostic, and predictive therapeutic implications but also to detect germline variants with clinical implications for

both the patient and his or her family members (9,10). Most clinical tumor testing does not currently involve analysis of a matched germline sample in an effort to minimize cost and turnaround time. If only tumor DNA is sequenced, any variants identified can represent either somatic changes or germline variants retained in the tumor DNA (11). The sequencing of paired germline and tumor DNA from the patient can distinguish between these two possibilities and has been recently recommended to avoid misinterpretation of results (11,12). For analyses of specific, well-characterized, activating mutations in a limited number of oncogenes (eg, hotspot mutations in BRAF), the likelihood of identifying important germline findings from tumor-only sequencing is small. However, as the scale of sequencing increases to include tumor suppressor genes that are also germline cancer susceptibility genes (eg, TP53), this probability increases.

When considering genome-scale testing that includes a germline sample, it is well accepted that there is the potential to identify both phenotype-related and incidental (non-phenotype-related) germline variants of clinical significance. The American

College of Medical Genetics and Genomics (ACMG) has recommended that a minimum list of 56 genes, about half of which are cancer susceptibility genes, should be actively interrogated in germline DNA samples when performing clinical WES/WGS, even if the purpose of the germline testing is only to serve as a “control” for tumor sequencing in cancer patients (10). These ACMG guidelines, as currently written, do not extend to tumor-only analyses in which a germline sample is not sequenced.

It is important to recognize that germline variants can be identified and/or inferred from tumor sequencing results, even without direct analysis of germline DNA (11). This possibility is often unclear to both clinicians and patients and can result in medically actionable germline findings that are missed by the laboratory and clinician. Conversely, reporting such findings places providers and patients in situations in which they receive unanticipated germline findings. The National Human Genome Research Institute/National Cancer Institute—supported Clinical Sequencing Exploratory Research (CSER) Consortium—includes three sites serving both pediatric and adult oncology patient populations that are studying the implementation of genomics into clinical oncology and returning genomic results to oncologists and patients (www.cser-consortium.org). In this Commentary from the CSER Tumor Working Group, we provide examples of clinical scenarios in which a germline variant can be identified or inferred from a test solely utilizing somatic tissue. We also highlight issues for consideration by the laboratory and clinician when seeking consent from patients for tumor sequencing and utilizing the results of such testing for patient care.

Sequencing Findings That May Be Suggestive of Germline Variants

1. Well characterized genes and/or variants (eg, founder mutations) highly suggestive of hereditary cancer syndromes:

Tumor mutation panels often include genes that can undergo somatic “second hit” mutations in the presence of a germline variant (eg, *BRCA1*, *BRCA2*; the mismatch repair genes, *MLH1*, *MSH2*, and *MSH6*). In particular, if one of the identified variants is a highly recurrent or founder mutation (eg, *BRCA1* c.68_69delAG Ashkenazi Jewish founder mutation [also referred to as c.185delAG] or the recurrent inversion of *MSH2* seen in some families with Lynch syndrome), this is highly likely to be an inherited germline variant. It is important to remember that a negative or nonreported family cancer history does not eliminate the possibility that the variant is present in the germline (13).

2. Tumor mutation patterns identified that are suggestive of an underlying germline variant:

Certain characteristics of the tumor mutation data can suggest an underlying germline variant. For example, analysis of a tumor sample revealing thousands of somatic variants (referred to as a hypermutated tumor) may suggest an underlying variant in a DNA mismatch repair gene or the recently described *POLE* proofreading domain and the diagnosis of the associated inherited syndrome (14). Similarly, identifying chromothripsis (ie, massive chromosome rearrangements in one catastrophic event) in a pediatric medulloblastoma tumor specimen may suggest a germline *TP53* variant (15).

3. Identification of a genetic event that has been previously described as both a somatic and germline event in patients with cancer:

This is probably the most common scenario as there are a large number of genes that can undergo either somatic mutation or

be associated with germline cancer susceptibility syndromes, including *MSH2*, *ALK*, *RB1*, *TP53*, and *VHL*. In this situation, it is important to consider the clinical scenario including the specific tumor type, age of the patient, and family history. For example, a *VHL* variant in a renal cell carcinoma sample from a man age 60 years with unilateral disease is unlikely to be hereditary, whereas that same variant in a renal cell carcinoma tumor sample from a young adult with a cerebellar hemangioblastoma is highly likely to represent a germline variant underlying an associated diagnosis of von Hippel-Lindau syndrome. For many gene/tumor combinations, eg, missense variant in *ALK* in a neuroblastoma sample or *TP53* mutation in a sarcoma, analysis of a normal DNA sample is required to determine whether the reported variant is inherited or acquired. In the setting of tumor-only sequencing, this is the clinical situation most likely to lead to misidentifying a somatic variant as germline, or alternately, failing to identify a somatic mutation because it is assumed to be a rare germline variant.

Considerations for the Testing Laboratory

The possibility that tumor genomic testing may identify germline variants underscores the fact that clinicians and laboratories need to adequately prepare for such findings. Recent studies suggest that 3% to 10% of unselected tumor/normal pairs have a pathogenic germline cancer susceptibility variant (3,11,16,17). Given the collaboration necessary in variant analysis and clinical interpretation, we believe that both ordering clinicians and laboratories share the responsibility for identifying and managing the potential for germline findings and clinicians will need to appropriately prepare patients for this possibility.

We encourage laboratories performing panel or WES/WGS somatic analysis to consider the following points.

1. Careful construction of sequence analysis and data filtering algorithms with an eye to deliberate consideration of germline vs somatic variants.

Laboratories conducting tumor-only sequencing have a special challenge when deciding how to discriminate somatic from germline variants and subsequently how to identify and include clinically relevant variants on the test report. The decisions may be affected by the assay’s design (gene panel, WES, WGS), mutation calling pipelines, and mutation filtering or annotation strategy.

Many commercially available off-the-shelf testing assays that focus on mutational hot spots have concentrated sequencing information on specific exons with well-described somatic variants. Nevertheless, these gene panels as well as larger panels or whole-exome strategies may identify a potential germline variant. At present, individual laboratories may be using mutation callers such as GATK (18), VarScan2 (19), MuTect (20), or Strelka (21). When using these algorithms, the laboratory is responsible for determining and optimizing the sensitivity and specificity of their respective pipelines on reference materials. No standardized variant calling process exists, although this is an active area for development by the professional societies involving in clinical genetic/genomic testing (22,23). For example, one algorithm for assessing somatic vs germline variants utilizes the expected variant fraction for somatic variants from tumor admixture samples because the variant fraction can be predicted to be diluted by contaminating normal tissue in the tumor specimen, and this method has a reported 95% accuracy (24).

Next, laboratories may choose to filter and annotate variants from tumor-only testing using a catalog of hot spot mutations and curated databases for somatic and germline variants and

polymorphisms (see Table 1). These databases aid laboratories in rapidly identifying variants with known clinical significance and frequency in the population, but are limited by the massive effort needed to keep these resources current. Finally, laboratories must decide whether and how they plan to disclose potential germline variants in their reports. This should be done in conjunction with American Board of Medical Genetics and Genomics and Association of Molecular Pathology certified molecular diagnosticians and pathologists with the necessary experience and expertise in germline analysis who can properly assess whether a germline variant is clinically significant (pathogenic or likely pathogenic) or, more likely, a variant of uncertain significance as defined by the recent ACMG variant classification guidelines (25). The recently published American Society of Clinical Oncology (ASCO) Policy Statement Update on genetic and genomic testing for cancer susceptibility similarly highlighted the need for appropriate expertise in germline reporting for any laboratory reporting these results (26).

2. Planning for downstream testing of potential germline variants.

Laboratories must decide what downstream testing will be performed, and how it will be performed, when potential germline findings are identified (12). Does the lab that carried out the tumor analysis also perform this germline test, and if so how should a germline sample from the patient be collected and the germline test ordered? Alternatively, the tumor analysis laboratory may partner with a reference diagnostic laboratory to perform the single-site germline variant testing based on the somatic result or potentially comprehensive analysis of the gene in question. In some circumstances, the results of this germline analysis might result in reconsideration of the tumor sequencing report. The laboratory should consider maintaining a database of options for internal and, when necessary, external confirmatory testing and clearly outline these options for confirmatory testing in the sequencing report. Recommendation for

referral to a genetic counselor, medical geneticist, or medical oncologist with appropriate expertise should also be considered. The potential for medical, legal, and ethical complications exists in instances of misclassification and/or misreporting of somatic vs germline variants (11).

3. Development of a standardized approach to the reporting of potential germline findings.

Laboratories should consider highlighting results that are suggestive of germline variants and including enough information to allow for germline testing by a second laboratory, if not done by the initial testing lab. Consider including in the reports a precise description of the variant (eg, the specific nucleotide change and the genome build utilized), adequate information to inform the ordering clinician of the potential implications of a germline finding and a plan for confirmation as appropriate. An example of such reporting language might be:

“Somatic tumor analysis identified a variant in *BRCA1* (c.68_69delAG previously known as c.185delAG). This type of variant often represents a germline variant and may have implications for the patient or the patient’s family members. Laboratory staff members are available to answer additional questions and can be reached at the number below. Germline testing from a normal sample from this patient should be considered. Genetic counseling can aid in determining whether additional genetic testing is indicated. Board- certified genetic counselors and medical geneticists can be found at www.nsgc.org or www.cancer.gov/cancertopics/genetics/directory.”

Laboratories may also include resources to educate clinicians on the results and next steps as part of their laboratory’s educational materials and provide these with the report or on the laboratory’s website.

Table 1. Subset of algorithms currently in use to assess tumor only sequence analysis*

Filter type	Examples	Advantage	Disadvantage
Hotspots	Commercial off-the- shelf somatic hot spot panels	Focuses clinical reporting on common actionable or clinically relevant somatic mutations Mutations are more likely billable for clinical labs and patients	May not be current
Cancer databases	CiViC Mycancergenome (mycancergenome.org) CanDL (candl.osu.edu)	Focuses clinical reporting on common actionable or clinically relevant somatic mutations	May be missing clinically relevant driver mutations from incomplete database May not be current
Germline databases	HGMD ClinVar IARC LOVD	Triggers clinical genetics referral for well-known germline mutations associated with clinical significance	May contain erroneous (false-positive) variant-phenotype associations
Polymorphisms databases	dbSNP HapMap 1000 Genomes Exome variant server ExAC	Removes many common polymorphisms	Databases may contain clinically relevant mutations that are filtered out
Sorting somatic versus germline mutations	Uses variant fraction in tumor admixture (24)	Focuses on somatic events with 95% accuracy	Not definitive for reporting clinically relevant germline results Not 100% accurate

* HGMD = Human Gene Mutation Database; IARC = International Agency for Research on Cancer; LOVD = Leiden Open Variant Database.

Considerations for the Ordering Physician

As clinicians (including oncologists, surgeons, pathologists, and radiation oncologists) increasingly utilize tumor sequencing tests such as gene panels and WES/WGS testing, they will need to recognize that somatic sequencing can identify actionable germline cancer susceptibility variants. For WES/WGS scale testing of matched tumor/normal samples, clinicians should be prepared for the approximately 5% of patients who will have secondary/incidental findings, results that are not anticipated based on the reported medical/family history (27). Clinicians who are ordering and reporting tumor sequencing tests should consider the following points.

1. Clinicians will need to ensure that patients are informed of the possibility that somatic sequencing may reveal germline information (12). While documentation of formal informed consent may not be feasible in all circumstances, it is essential that providers engage patients in a discussion about the possibility of identifying a germline variant with implications for the patient or family, including secondary/incidental findings. The discussion should include summary information about the types of results that might be found, including both pathogenic variants and variants of uncertain significance, and should address the fact that results may have implications for family members. If there is a strong family history suggestive of a hereditary condition, cancer or otherwise, a formal referral for genetic counseling should be considered as somatic testing does not offer a comprehensive evaluation of the germline DNA. Patients should also understand it is not always easy to “opt-out” of receiving germline findings (whether cancer specific or incidental), especially when the results may be an integral part of the molecular tumor interpretation. At a minimum, clinicians should consider documenting these patient conversations in the associated clinical note.
2. Clinicians should ascertain their patients’ wishes regarding to whom any germline results should be disclosed in the circumstance that the patient is not available to receive them (eg, too ill, changed caregivers, or deceased). It is often helpful to the clinician and the family to have this conversation at the time that the genomic testing is being considered given the varying timeframe of return of genomic results. Providers will want to abide by state laws when considering disclosure to alternate individuals and may want to include information about the patient’s preference for an alternative recipient of the results in their clinic notes. Written documentation of the patient’s preferred proxy may be required.
3. Physicians will need to plan in advance how they will disclose and explain potential germline results to patients. The disclosure conversation may include information on the potential implications of the results, the importance of confirmatory testing from a normal sample, and the availability of genetic counselors or geneticists who can help contextualize the results. The ordering physician should consider who will disclose the result, if not the provider him or herself, and in what setting. For example, a tumor report that includes the potential for germline findings is likely to be difficult to convey through an office staff member not familiar with this area or by phone to an ill cancer patient. Depending on prior professional experience with germline cancer susceptibility testing, the clinician may consider in advance if any additional health professionals, eg, genetic counselors or clinical geneticists associ-

ated with the oncology team, can or should be included in the disclosure visit as well as encouraging a close family member to accompany the patient. Clinicians may need to prepare the patient for the logistical aspects of validating a potential germline finding, including insurance prior authorization and sample collection. Of note, in our clinical experience, germline testing for the variant in question in at-risk family members may not be feasible until the index patient with cancer has had testing of a normal (typically blood) sample confirming that the variant is germline and not a cancer-specific finding. Clinicians should also consider providing feedback—with patient permission—to the somatic testing laboratory with regard to the results of follow-up testing for the potential germline finding in order to improve overall reporting.

4. We encourage physicians to rely on their professional resources, the genetic testing lab, colleagues, fellow oncologists, and experienced genetic counselors/geneticists when reading and interpreting a somatic tumor report. Being familiar with the report structure and variant calling vocabulary will avoid “overcalling” somatic variants as germline (or vice versa) and inadvertently providing patients with misleading information.

The promise of precision oncology is vast. Clinicians and laboratories should not shy away from the use of genomic technologies in the care of their patients because of the potential to uncover unanticipated information. Instead, oncology providers, including both ordering clinicians and testing laboratories, should acknowledge the fact that tumor-only testing may reveal actionable germline information and actively implement solutions that maximize the clinical utility of this germline information while minimizing patient misunderstanding and harm. Raising the possibility of unanticipated information to the awareness of laboratory directors, clinicians, and health care providers, as summarized here, will hopefully encourage conversation and future educational initiatives among the oncology community, such as a very recent update by ASCO on cancer susceptibility testing guidelines, in an effort to increase uptake and utilization of precision oncology tests.

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Notes

List of Resources for patients and physicians: National Society of Genetic Counselors: www.nsgc.org; National Cancer Institute Cancer Genetics Services Directory: www.cancer.gov/cancertopics/genetics/directory; GeneTests Clinic Directory: <https://www.genetests.org/clinics/>.

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