Reference Guide

Evidence-based Categorization and Interpretation of Sequence Variants in Cancer

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Introduction

Unlike interpretation of germline sequence variations, which focuses on pathogenicity of a variant for a specific disease or disease causality, interpretation of somatic variants should be focused on their impact on clinical care.

Use this convenient reference guide when categorizing somatic variants. All recommendations are based on joint consensus guidelines published by the Association for Molecular Pathology (AMP) in the Journal of Molecular Diagnostics along with best practices from the PierianDx Interpretation Services Laboratory.[1,2]

Evidence-based categorization of somatic variants can be broken down into 5 distinct steps:

1. Collect the evidence
2. Determine the clinical impact
3. Evaluate the strength of the clinical evidence
4. Classify the variant
5. Draft the interpretation

The rest of this guide outlines each step.

Step 1: Collect the Evidence

Clinical evidence for a genetic alteration is of various types. Consider the mutation type and information sources available.

Evaluate the Mutation Type

- ❏ What type of mutation is it (e.g., activating mutation, loss of function mutation, missense, nonsense, indel, splicing, CNV, fusion)?
- ❏ Is it present or absent in various variant databases (e.g., somatic and germline variant databases)?
- ❏ What is the variant allele fraction (VAF)? Could it be a germline variant?

- ❑ What is the minor allele frequency (MAF) or population frequency of the variant? Is it ≥1% or <1%?
- ❑ Has the variant been functionally characterized? If so, what is the source of information? Functional study, population study, other, or prediction algorithm (reference only)?
- ❑ Is the variant important in any particular biological pathway?

Consider the Clinical Impact Information Source

- ❑ FDA-approved therapies
- ❑ Clinical practice guidelines
- ❑ Well-powered studies with consensus
- ❑ Investigational therapies, including clinical trials
- ❑ Small studies with and without consensus
- ❑ Case reports
- ❑ Preclinical studies

Step 2: Determine the Clinical Impact

After you have collected all different types of evidence from all different sources for every variant in the patient, then you must assess the clinical impact of each variant. It could be one of the following:

- ❑ Diagnostic
- ❑ Prognostic
- ❑ Therapeutic
- ❑ Preventive

Step 3: Evaluate the Strength of the Clinical Evidence

After you assess the clinical impact of each variant and whether it's diagnostic, prognostic, therapeutic, or preventive, then you must evaluate the strength of the clinical or experimental evidence that led you to that conclusion. The joint consensus guidelines from

AMP recommend classifying this evidence into one of 4 levels, depending on the source from which you gathered the clinical evidence:

Level A	Level B	Level C	Level D
FDA-approved therapies Professional guidelines	Well-powered studies with consensus from experts	Multiple small studies with some consensus Clinical trials	Preclinical studies Small studies A few case reports without consensus

Step 4: Classify the Variant

After you have collected all different types of evidence from all different appropriate sources, evaluated the clinical impact of this evidence in the context of patient's disease and other diseases, and evaluated the strength of the clinical evidence based on these sources, then you must categorize the somatic variant into one of four joint consensus recommended (AMP/ASCO/CAP) classification tiers:

Tier	Classification (*Therapeutic, Prognostic, Diagnostic*)	Evidence Level
I	**Variants of Strong Clinical Significance**	A B
II	**Variants of Potential Clinical Significance**	C D
III	**Variants of Unknown Clinical Significance**	
IV	**Benign or Likely Benign Variants**	

What about Polymorphisms (Tier IV alterations)?

In general, there is no existing published evidence of cancer association for these variants. These are benign or likely benign variants, and they're observed at significant allele frequency in the general or specific population databases. The joint consensus guidelines recommend not reporting these Tier IV alterations in the report.

Per joint consensus recommendations, consider the following whenever evaluating Tier IV classification for a variant:

- ❏ Don’t overlook variants which have >1% population frequency (MAF), because these may be of clinical significance for your patient. Examples include pathogenic variants in genes associated with cancer predisposition syndrome and activating mutations with predictive or prognostic impact. A perfect example is KDR Q472H that we see frequently in the patient population. This known polymorphism exists with very high population frequencies, but it is also an activating mutation. There's recent literature that establishes that this is a pathogenic germline variant in melanoma, and melanoma patients with this alteration may benefit from anti-angiogenesis treatment.

- ❏ Use population databases carefully to exclude polymorphisms. The joint consensus guidelines caution the use of population databases to exclude polymorphisms because there are several well-known cancer-associated and targetable somatic alterations that are included as germline variants in population databases. This happens because the individuals who participated in the sequencing studies that populated data into these databases were assumed to be healthy or free of supplemental disease at the time of participation in the study.

- ❏ There is no standardized cutoff for MAF that could be used for eliminating polymorphic or benign variants. In the absence of the paired normal tissue, the joint consensus guidelines recommend using 1 percent as a primary cutoff. Aggregate global mass is commonly used in laboratories, and you may also consider using specific MAFs based on the ethnic background of the patient, if that is known to you.

What about Germline Pathogenic Variants?

Tumor sequencing with or without matched normal tissues may reveal variants that are of germline origin. For these variants, the joint consensus guidelines provide the following recommendations:

- ❏ Report germline variants with known evidence of clinical impact and germline pathogenic variants in genes that are associated with hereditary cancer syndrome that has an established guideline for clinical surveillance. The guidelines also recommend that cancer genetic counseling should be provided for the patient in conjunction with these findings.

- ❏ In general, distinguish somatic variants from inherited germline variants. Clinical laboratories must have policies that address detection, disclosure, non-disclosure, interpretation, and reporting of germline variants.

- ❏ Follow the American College of Medical Genetics and Genomics (ACMG) standards and guidelines for reporting germline variants. These guidelines can be found at www.acmg.net.

- ❏ Whenever a pathogenic germline variant is suspected during tumor-only testing, recommend that confirmation be performed with the normal tissue sample**.** Also, recommend/provide appropriate genetic counseling as necessary.

- ❏ Enact policies for testing germline samples for a variant found in a malignancy, to confirm germline or somatic origin**.** These policies should state the use of a clinically validated germline test after appropriate patient consent is received, or per the request of a clinician.

- ❏ If secondary findings are revealed in germline testing, disclose the positive germline results for all 53 genes, per ACMG guidelines. This disclosure is recommended even when the germline variant is only being evaluated as part of a tumor-normal study.

- ❏ Always consider the likelihood of germline pathogenic variants in a tumor-only somatic mutation study.

- ❏ Remember that germline variants may also serve as an inclusion criteria in clinical trials.

What about Mutation Function and *In Silico* Prediction Algorithms?

The joint consensus guidelines state that you should not use the results from prediction algorithms as the sole evidence for variant classification and clinical decision-making. Instead, use data with consensus from published functional studies, population studies, and potential drug response studies, to determine the functionality of a mutation.

Additionally, you should keep in mind that in general, missense and splice site prediction tools have a moderate specificity of 60% to 80%. And they have a tendency of over-predicting the deleterious impact of the alterations. Thus, one should exercise caution when interpreting *in silico* scores. Use these data for reference only.

Step 5: Draft the Interpretation

After you classify the variant, you can begin to draft the interpretation. The most important point to make about the interpretation is that all variants must be carefully reviewed by trained and certified molecular diagnostic professionals in the context of each complete case.

Also important:

- ❏ Consider exact disease subtype, stage of the disease, and/or treatment background of the patient's disease while performing evidence-based variant categorization and drafting clinical interpretation for the variants in the molecular pathology report.
- ❏ The molecular pathology reports should be short, simple, and to the point.
- ❏ Report Tier I, Tier II, and Tier III variants in descending order of clinical importance. Do not include Tier IV, or benign or likely benign alterations, in the report.
- ❏ Report all detected genetic alterations in standard nomenclature. Also, use colloquial nomenclature so that the genetic data are clearly communicated to the treating physician.
- ❏ Report pertinent negatives in a disease-specific manner for Tier 1 drug/cancer combinations. For example, in patients with lung cancer, a definitive lack of an *EGFR* mutation must be stated. In a patient with melanoma, a definitive lack of *BRAF* mutation must be stated.
- ❏ If the germline variants are not reported in some of the genes in an NGS test in a laboratory, then specifically state this fact in the interpretation.
- ❏ If the NGS test does not allow definitive differentiation between the germline and somatic variant, then specifically state this fact in the interpretation.

- ❏ If there's any uncertainty about the results due to issues such as sequence quality, sample adequacy, tumor content, or lack of biomedical knowledge, then you must clearly state these concerns in the report.
- ❏ If there is a need for orthogonal testing to better evaluate the consequences of some of the variants in the light of guideline recommendations, then such a need can be specifically identified in the report. For example, somatic mutations in MMR are one of the causes of microsatellite instability (MSI) or MMR protein deficiency (dMMR) in several cancers and identification of dMMR or MSI-high status impacts treatment decisions. Need for IHC/PCR based orthogonal testing to further confirm NGS findings or correlation of findings obtained from NGS and orthogonal testing can be explicitly mentioned in such reports

References

1. Li, M., Datto, M., Duncavage, E., Kulkarni, S., Lindeman, N., Roy, S., Tsimberidou, A., Vnencak-Jones, C., Wolff, D., Younes, A. and Nikiforova, M. (2017). Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer. The Journal of Molecular Diagnostics, 19(1), pp.4-23.

2. Verma, S. and Bredemeyer, A. (2018). A Practice-based Guide to Interpretation and Reporting of Sequence Variants in Cancer. [online] Info.pieriandx.com. Available at: https://info.pieriandx.com/a-practice-based-guide-to-interpretation-and-reporting-of-sequence-variants-in-cancer-0 [Accessed 2 Aug. 2018].

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For a companion webinar to this reference guide, visit
pieriandx.com/interpretation-reporting-sequencing-variants-in-cancer

