Interpretation and Reporting of Sequence Variants in Cancer

A Practice-Based Guide

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Pioneers in Precision Medicine

Leaders in Clinical Genomics

Today

- 40+ leading lab clients in sharing network
- Staff of 60+ medical and scientific experts

2014

- PierianDx established after ~50 labs visit WashU to learn how clinical NGS is operationalized.

2011

- WashU builds CLIA lab; develops Clinical Genomicist Workspace (CGW) for NGS testing.

2003

- WashU plays critical role in Human Genome Project.

PierianDx founders among first to validate and clinically report on somatic cancer NGS panels.
Webinar Objectives
Agenda

1. Recommendation for Evidence Based Categorization
2. Application and Reporting in Molecular Pathology Practice
3. Additional Considerations
4. PierianDx Comprehensive Support Non-Small Cell Lung Cancer Example
Variant Analysis Workflow

Two Kinds of Interpretation:

**Analytic Interpretation:** examining raw data and forming a conclusion about the quality or quantity of the analyte, i.e. producing a reportable result.

**Clinical Interpretation:** describing what the result means for the patient, either in general or based on specific knowledge of that patient’s situation.

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Laboratory Reports in Molecular Pathology—Gulley et al, Arch Pathol Lab Med—Vol 131, June 2007
Evidenced Based Categorization

Recommendations
Clinical Interpretation of Somatic Variants

Evidence Based Categorization

“Interpretation of somatic variants should be focused on their impact on clinical care”


Steps in clinical interpretation and classification of a sequence variant in cancer:

1. Collect evidence for variant’s clinical significance
2. Determine clinical impact of the variant (Diagnostic, Prognostic, Therapeutic, Preventive)
3. Evaluate strength of the evidence for clinical impact
# Evidence Based Categorization

## Evidence (Type and Source)

**Variant**
1. Mutation type: [Activating, LOF (missense, nonsense, indel, splicing), CNVs, fusions]
2. Present/Absent in somatic databases
3. Frequency (VAF), Potential Germline [50%, 100%]
4. Present/Absent in germline databases
5. Minor allele frequency (MAF) [>1%, < 1%]
6. Present/Absent in population databases
7. Functional Characterization- functional study, population study, other, prediction algorithms (reference only)
8. Pathway involvement

## Clinical Impact

### Diagnosis

### Prognosis

### Therapeutic

### Preventive

## Strength of Clinical and/or Experimental Evidence (based on sources)

**Level A:** FDA approved therapies, Professional Guidelines

**Level B:** Well Powered studies with consensus from experts in the field

**Level C:** Multiple small studies with some consensus, Clinical trials

**Level D:** Preclinical studies, small studies or a few case reports without consensus
## Clinical Interpretation of Somatic Variants

### Evidence Based Categorization

<table>
<thead>
<tr>
<th>Tier</th>
<th>Classification (Therapeutic, Prognostic, Diagnostic)</th>
<th>Evidence Level</th>
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<tbody>
<tr>
<td>I</td>
<td>Variants of Strong Clinical Significance</td>
<td>A B</td>
</tr>
<tr>
<td>II</td>
<td>Variants of Potential Clinical Significance</td>
<td>C D</td>
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<tr>
<td>III</td>
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<td></td>
</tr>
<tr>
<td>IV</td>
<td>Benign or Likely Benign Variants</td>
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</tbody>
</table>

**Variant Information**  
(with references)

1. **Evidence (all types)**  
   *(in patient’s disease and other diseases, as applicable)*

2. **Clinical Impact (Significance)**  
   *(in patient’s disease and other diseases)*

3. **Strength of Clinical and/or Experimental Evidence**  
   *(based on sources)*

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Evidenced Based Categorization

Application and Reporting
4 Steps for Drafting Interpretations

1. Consider
   Correct pathological diagnosis: (site, diagnosis, subtype, stage, grade) of patient
   Intended use: for the test results (especially when diagnosis not known, eg. occult primary)

2. Collect and Document
   Gene: Function, pathway involvement, association with diseases
   Variant: Mutation Type, frequencies (VAF, MAF), presence or absence in databases (population, germline, somatic), pathway involvement, functional characterization (functional & population studies, prediction algorithm - reference only)
   Clinical Significance (impact): FDA approved drug labels, professional guidelines, well powered studies with consensus, clinical trials, small studies with and without consensus, case reports, preclinical studies
   Role of co-occurring variants in context of patient’s diagnosis

3. Classify
   Tier I - IV: Strong vs Potential Clinical Significance, VUS, Benign or Likely Benign Polymorphism

4. Provide Additional Recommendations
   Short and worded carefully: based on evidence with appropriate literature citations

“Interpretation of somatic variants should be focused on their impact on clinical care”
Additional Guidance

Reporting Clinical Significance

I. It’s essential the alteration is interpreted in clinicopathologic context

Recommendations for specific clinical trials should NOT be made; general statements about availability of relevant trials or citing results of published trials are acceptable.

II. Provide the most critical information in reports - concise, clear, and prominent.

Comments may include functional, prognostic, or predictive significance of the variant for particular tumor type, impact on biochemical pathway(s), and prevalence in relevant cancers.

III. Treatment or other management decisions are based on many pieces of medical information

Suitability for treatment is based on many factors other than diagnosis (as written on requisition form) and genotype discovered through testing.

- Often, these factors are unknown to the molecular professional reporting results.
- Failure to take these other factors into consideration when recommending a specific therapy can lead to confusion, conflict between patient and oncology team, and anxiety.

Treatment suggestions should be

- Evidence based
- Relevant to the patient’s diagnosis
- Clear and state that the report contains generalized treatment suggestions incorporating the data points available to the laboratory (i.e., diagnosis and genotype), but that additional factors need to be incorporated into crafting a treatment plan for each individual.
Example - Tier III (VUS)

**Age:** 57  
**Gender:** Male  
**Indication:** Non-Small Cell Lung Cancer  

NGS testing revealed **MET** variant: chr7:g.116411876_116411888del12

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**Gene level:**
- **MET** is a potential therapeutic target in a number of cancers, including NSCLC;
- Per NCCN guidelines, **MET** exon 14 skipping mutations are of clinical relevance (therapeutic significance) in NSCLC

**Variant Level:**
- 12 bp deletion within the **MET** intron 13, located 15bp upstream of 5’ end of **MET** exon 14  
- Never reported in literature  
- Not characterized as splice site variant in literature; Not characterized for its functional consequence on **MET** exon 14 skipping  
- **VAF:** 20%, **MAF** <1% (in all population databases)  
- **MET** intron 13 deletions overlapping the region of this variant have been reported in lung adenocarcinomas and other tumors in few case studies, and in some of these cases **MET** inhibitors (e.g., crizotinib) have been tried for treatment

**Clinical Impact:** No clinical significance info available for this variant

**Classification:** Tier III - Variant of Uncertain Significance

**Recommendation:** Since **MET** intron 13 deletions overlapping the region of patient’s variant have been reported in lung adenocarcinomas and other tumors in a few case studies, and because in some of these cases **MET** inhibitors (e.g., crizotinib) have been tried for treatment, clinical correlation is recommended

"Interpretation of somatic variants should be focused on their impact on clinical care"  
Example - Tier I

**Clinical Interpretation of Somatic Variants**

**Age:** 53 Years  
**Gender:** Female  
**Indication:** Hairy Cell Leukemia  

NGS testing revealed **BRAF** variant:  
*BRAF* p.V600E  
chr7:g.140453136A>T  
NM_004333.4:c.1799T>A

**Evidence Summary**

**Gene level:**  
- *BRAF* is an oncogene, involved in RAS/MAPK pathway

**Variant Level:**  
- Missense mutation  
- VAF = 60%, MAF<1% (all population databases)  
- most frequent *BRAF* mutation identified in human cancers and occurs in virtually all HCL patients  
- V600E occurs in the kinase domain of *BRAF*; causes activation of RAF/MEK/ERK signaling pathway

**Clinical Impact:**  
- Diagnostic and Therapeutic significance (NCCN, HCL v2.2018)  
- *BRAF* V600E has been reported in majority of patients with classic HCL; is useful for the distinction of classic HCL from HCL-variant and other splenic B-cell lymphomas.  
- Vemurafenib (*BRAF* V600E kinase inhibitor) has demonstrated activity in relapsed or refractory HCL

**Classification:** Tier I - Variant of Strong Clinical Significance  
**Recommendation:** No additional recommendations

“Interpretation of somatic variants should be focused on their impact on clinical care”

Clinical Interpretation of Somatic Variants

Additional Considerations
Do NOT miss variants with MAF ≥1% that may be of clinical significance

Example: Pathogenic variants in genes associated with cancer predisposition syndrome, activating mutations with predictive or prognostic impact

Minor Allele Frequency (MAF):
1. There is no standardized cutoff for MAF to be used for eliminating polymorphic or benign variants.
2. In the absence of paired normal tissue, the AMP/ASCO/CAP work group recommends using 1% (0.01) as a primary cutoff
3. Aggregate global MAF is most commonly used; may consider using ethnicity-specific MAFs based on the ethnic background of the patients (when known)

Use of Population Databases to Exclude Polymorphisms:
1. Use these databases with caution (individuals participating in these sequencing studies were assumed to be healthy / free of subclinical diseases at the time of participation in the study)
2. Several well-known classic cancer-associated and targetable somatic alterations are included as germline variants in population database

Polymorphism:
1. No existing published evidence of cancer association
2. Are benign or likely benign variants
3. Observed at significant allele frequency in the general or specific subpopulation databases
4. It is NOT recommended to include tier IV alterations in the report
5. Thus, review disease/mutation databases (eg. ClinVar) and literature for assertion of pathogenicity and clinical significance, for variants with ≥1% MAF

Example:
*KDR Q472H* (rs1870377) is a known polymorphism (population frequencies: ExAC = 22%; NHLBI = 19.4%) ; an Activating Mutation, Pathogenic germline variant in melanoma, melanoma patients with *KDR Q472H* (rs1870377) might benefit from anti-angiogenesis treatment (PMID: 26631613)
Germline Pathogenic Variants

Additional Considerations

Tumor sequencing with or without matched normal tissues may reveal variants that are of germline origin.

AMP/ASCO/CAP Joint Consensus recommends:

1. **Report germline variants with known evidence of clinical impact**

2. **Report germline pathogenic variants in genes associated with hereditary cancer syndrome that has an established guideline for clinical surveillance along with recommendation for cancer genetic counseling.**

Additional Guidance

1. **Distinguish somatic variants from inherited germline variants.**

2. **Labs must have policies addressing detection, disclosure/non-disclosure, interpretation/reporting of germline variants.**

3. **Follow ACMG/AMP standards and guidelines for interpretation of germline variants.**

4. **When a pathogenic germline variant is suspected during tumor-only testing:**
   a. **Confirmation of the variant with a normal tissue sample, along with appropriate genetic counseling, should be recommended.**
   b. **Labs should have a policy about testing germline sample for a variant found in a malignancy to confirm germline or somatic origin - use clinically validated germline test after appropriate patient consent is received or per request of a clinician.**

5. **For secondary findings revealed in germline testing, the ACMG recommends disclosure of positive germline results for 53 genes. Disclosure is recommended even when the germline variant is only being evaluated as part of a tumor/normal study.**

6. **It is prudent to also consider the likelihood of germline pathogenic variants in a tumor-only somatic mutation study.**

7. **Germline variants may also serve as clinical trial inclusion criteria.**

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Additional Considerations

Mutation Function

Results of prediction algorithms should never be used as the sole evidence for variant classification or clinical decision making.

* Utilize data with consensus from published functional studies, population studies and potential drug response studies to determine functionality of a mutation.

In Silico Prediction Algorithms

1. In general, missense and splice site prediction tools have a moderate specificity (approximately 60% to 80%) with a tendency of over-predicting deleterious impact.

2. **Exercise caution** when interpreting in silico scores.

3. Information to be used for reference only.
Additional Considerations

Reporting

 Detected variants should be carefully reviewed by appropriately trained and certified molecular diagnostic professionals in the context of each complete case.

* Histologic findings, and evidence-based variant categorization must be performed before reporting.

Additional Guidance

1. Reports should be short, simple and to the point.
2. Tiers I to III must be reported in descending order of clinical importance.
3. It is NOT recommended to include Tier IV or benign/likely benign variants/alterations in the report.
4. All detected genetic alterations should be annotated and reported as designated by the HUGO Gene Nomenclature Committee.
   a. Colloquial nomenclature should also be included in addition to the standard nomenclature.
5. Pertinent negatives should be reported, in a disease-specific manner. Pertinent negatives should be included for Tier I drug/cancer combinations (eg, the definitive lack of an EGFR mutation in a patient with lung cancer or the definitive lack of a BRAF mutation in a patient with melanoma).
6. If germline variants are not reported in some of the genes in an NGS panel, the initial report should specifically state that fact.
7. If the NGS test does not allow definitive differentiation between germline and somatic variants, report should clearly state that.
8. Uncertainty, if present, must be communicated in reports; this includes issues of sequence quality, sample adequacy, tumor content, and biomedical knowledge.
Reviewing Evidence

Data Sources

- COSMIC
- FDA
- ASCO
- TCGA
- NCCN
- PubMed
- ClinicalTrials.gov
- NCI Drug Dictionary
- LeukemiaNET
- WHO Class of Tumors
- Etc.

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Additional Considerations

Conclusion

“With hundreds to thousands of tumor variants observed in the coding region of an individual’s genome...it will not be possible for molecular pathologists and oncologists to identify and appropriately annotate the clinical significance of each variant by manually assigning an individual interpretation to the variants found in every patient.”

J Clin Oncol 31:1825-1833

1. There is a need for prioritization strategies for the identification and reporting of clinically significant genetic variants.

2. After narrowing down the list of candidate variants, the biggest challenge is to interpret the remaining genomic alterations within a biological context.

3. Manually annotating each single variant in terms of clinical significance in every possible tumor type is a daunting challenge.

4. Clinical interpretation of most variants identified in NGS-based cancer diagnostic tests usually involves manual searching of the published literature, a burdensome process for those collating and making sense of the information.
Conclusion

Additional Considerations

Need for creating a Clinical Genomics “Workstation”

Genomic information management software that:

- Has computational tools to support rigorous analysis and clinical interpretation of comprehensive genomic data
  - Assists in determining real and reportable variants
  - Helps in prioritizing non-synonymous mutations over synonymous and intronic variants
  - Recognizes cancer mutations that have been previously annotated and reported
  - Is dynamic in nature

Integrated with a medical knowledgebase that:

- Contains curated information stored for future use and comprises of:
  - Previously classified and interpreted variants
  - Curated annotations on the most frequent and relevant variants in multiple tumor types, based on the publicly available resources (population and variant databases)
  - Is designed to accommodate new clinical and preclinical data
  - Is routinely updated with new scientific and clinical knowledge as it becomes available, e.g., novel variants of biological importance, variants with novel therapeutic relevance.
  - Such information is gathered with a regular and systematic review of drug regulatory and approval status, consensus guidelines, peer-reviewed publications and clinical trial databases.
  - Is integrated with the genomic information management software for automated report generation
PierianDx: Comprehensive Support

NSCLC Example
Complete Clinical Genomics Support

PierianDx Technology and Services

Library Extraction, Sample Prep | Sequencing | Variant Calling (Bioinformatic Pipelines) | Data Visualization, QC Analysis | Variant Annotation & Classification | Clinical Interpretation & Reporting | Final Report & Medical Director Sign-out | Data Integration EMR, 3rd Party

Clinical Genomicist Workspace, Knowledgebase

PierianDx Validation Services

PierianDx Interpretation Services

PierianDx Laboratory Services

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Creating the Largest, Peer Shared Interpretation System

More Rapid, Accurate Interpretation

Most clinically robust, up-to-date knowledgebase

- 1,100+ somatic genes curated
- ~6mb of sequence coverage
- Largest sharing network
- Interpretations 100% clinical
- Compare classifications and interpretations across sites
- Practice guidelines, FDA therapeutics, clinical trials - updated weekly
- 18 million published articles
Case Example
Non-Small Cell Lung Cancer

Age: 72
Gender: Male
Indication: NSCLC; previous identification of EML4/ALK rearrangement; disease progression after treatment with second-generation ALK inhibitor

Initial assessment
Selected variants identified by NGS:
- EML4/ALK fusion
- ALK p.G1202R
- TP53 p.G154V
- TP53 p.P72R

ALK G1202R is a secondary mutation described to confer resistance to first- and second-generation ALK inhibitors. This patient has progressed on ALK inhibitors.

TP53 mutations in lung cancer are reported to be negative prognostic factors

“Interpretation of somatic variants should be focused on their impact on clinical care”

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February 2018

Molecular tumor board: a patient with ALK-rearranged lung cancer
Karen Lusky
Evidence (Type and Source)

Variant
1. Mutation type: [Activating, LOF (missense, nonsense, indel, splicing), CNVs, fusions]
2. Present/Absent in somatic databases
3. Frequency (VAF), Potential Germline [50%, 100%]
4. Present/Absent in germline databases
5. Minor allele frequency (MAF) [≥1%, < 1%]
6. Present/Absent in population databases
7. Functional Characterization - functional study, population study, other, prediction algorithms (reference only)
8. Pathway involvement

Clinical Impact Information sources
1. FDA-approved therapies
2. Professional Guidelines
3. Well powered studies with consensus
4. Investigational therapies (including clin. trials)
5. Small studies with and without consensus
6. Case reports
7. Preclinical studies

Clinical Impact

Diagnosis

Prognosis

Therapeutic

Preventative

Strength of Clinical and/or Experimental Evidence (based on sources)

Level A: FDA approved therapies, Professional Guidelines

Level B: Well Powered studies with consensus from experts in the field

Level C: Multiple small studies with some consensus, Clinical trials

Level D: Preclinical studies, small studies or a few case reports without consensus
“Certain metrics for called variants are critical for variant interpretation, such as supporting reads (depth of coverage) and variant allele frequency (VAF), and should be included in variant evaluation…”

“It is important to emphasize that clinical laboratories should establish a well-annotated in-house database for both tracking variants identified within the laboratory and to provide consistent annotations.”


- Identify false-positives
- Establish frequencies of mutations
Variant Annotation and Classification

Variant Table

“Certain metrics for called variants are critical for variant interpretation, such as supporting reads (depth of coverage) and variant allele frequency (VAF), and should be included in variant evaluation…”

Variant Annotation and Classification

Clinical Practice Guidelines

For recognizable recurrent variants, get:

- Direct, curated knowledge (NCCN, ASCO, FDA)
- Treatment, prognostic, and diagnostic practice guidelines and drug labels

“Peer-reviewed literature, clinical practice guidelines, and large-scale cancer mutation databases remain primary resources for evidence...”

We strongly encourage somatic variant data sharing...to facilitate accurate interpretation of somatic variants.”


Leverage pathologist and geneticist variant assessment expertise through the shared interpretation knowledgebase.
Variable Annotation and Classification

Clinical Interpretations

For less well characterized recurrent variants **without** practice guidelines, interpretations can help:

1. **Auto-classify**
2. **Point to relevant literature and trials**
3. **Kick start your report editing**

**ALK p.G1202R**

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Gene</th>
<th>Interpretation</th>
<th>Date updated</th>
<th>Disease</th>
<th>More</th>
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**ALK (Anaplastic Lymphoma Kinase)** encodes a receptor tyrosine kinase, which belongs to the insulin receptor superfamily and activates the mitogen-activated protein kinase (MAPK) pathway (RefSeq, Jan 2011, Uniprot.org). ALK missense substitution, p.G1202R, was identified. Codon G1202 lies in the protein kinase domain of ALK (Uniprot.org). ALK G1202R is postulated to be on the solvent-exposed region of the ALK kinase domain adjoining the crizotinib-binding site and intrasted to confer resistance to crizotinib in vitro in cells harboring (PMID: 22377794). ALK G1202R has been reported in lung adenocarcinoma including non-small cell carcinoma (NSCLC) (COSMIC, Feb 2018). This variant has been reported as a secondary mutation in ALK+ NSCLC patients (PMID: 22377786, 28122866). In a case study in a patient with NSCLC with EML4-ALK fusion, ALK G1202R alteration conferred high-level resistance to crizotinib (PMID: 22777654) which suggests that ALK inhibitors (ceritinib, alecibib, and brigatinib (PMID: 28122866)). Moreover, the secondary resistant mutation ALK G1202R is susceptible to the third-generation inhibitor lorlatinib (PMID: 28122866). Currently, ALK inhibitor, Lorlatinib is tested in clinical trials for the ALK-positive NSCLC patients (NCT03652408, phase 3; NCT02564834, phase 2).
“Recommendations for specific clinical trials should not be made, although general statements about availability of relevant trials or citing results of published trials are acceptable.”


Assess trial matches for consideration of Tier 2C classification evidence.
Variant Annotation and Classification

Human Research Evidence

“[Cancer specific] databases provide information about the incidence and prevalence of sequence variants across the spectrum of different cancers and subtypes”

“The interpretation of these predictions in the context of cancer gene function is usually not straightforward, especially for activating mutations. ... Clinical laboratories should exercise caution when interpreting in silico scores.”


Use with caution
Variant Annotation and Classification

Literature Search

Kickstart literature search directly from the Variant Details Page

Quickly filter by relevant categories of info

Highlighted reference text

Link directly to full text article
Variant Annotation and Classification

Clinical Interpretations

TP53 p.G154V

“We strongly encourage somatic variant data sharing ...to facilitate accurate interpretation of somatic variants.”


PierianDx network-sourced interpretations point to valuable evidence
Variant Annotation and Classification

Clinical Trials

TP53 p.G154V

“Recommendations for specific clinical trials should not be made, although general statements about availability of relevant trials or citing results of published trials are acceptable.”

Clinical Data Aggregation

Variant Annotation and Classification

Explore frequency of detection in patient cases across the network and within your laboratory.

Learn how others in the network have classified this variant for this cancer.

Search frequency in other tumor types.

**Variant: TP53 p.G154V**

- **Variant frequency by disease**
  - Myeloid leukemia
  - Acute myeloid leu...
  - Myelodysplastic sy...
  - Myelodysplastic sy...
  - Myelodysplastic/m...
  - Myelodysplasia
  - Neoplasm of lung
  - Adenocarcinoma o...
  - Malignant tumor o...
  - Malignant melanoma...
  - Primary malignant ...
  - Glioma
  - Adenocarcinoma
  - Any disease
  - This patient's disea...
  - Add disease

- **Variant frequency by classification**
  - Levels 1-5

- **Frequency counts**
  - 11/1154
Variant Annotation and Classification

Clinical Evidence

TP53 p.P72R

Conflicting ClinVar submissions, but consensus is clear.
“These databases are frequently used to filter out variants that are deemed polymorphic/benign based on an arbitrary cutoff of minor allele frequency (MAF).”


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<th>FREQUENCY</th>
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MAF > 1% in all population databases
Age: 72
Gender: Male

**Indication:** NSCLC; previous identification of EML4/ALK rearrangement; disease progression after treatment with second-generation ALK inhibitor

**Variants identified by NGS:**
- EML4/ALK fusion
- ALK p.G1202R
- TP53 p.G154V
- TP53 p.P72R

**Clinical Impact:**
Therapeutic (in light of combination of ALK rearrangement and p.G1202R mutation) and prognostic (TP53 mutation)

**Classifications:**
- EML4/ALK fusion: Tier I, Level A evidence
- ALK p.G1202R: Tier II, Level C evidence
- TP53 p.G154V: Tier II, Level C evidence
- TP53 p.P72R: Tier IV

**Clinical Interpretation:**

"Interpretation of somatic variants should be focused on their impact on clinical care"
Clinical Interpretation and Reporting

Final Report

“...reports should be short, simple, and to the point.”

All clinically critical information should be at the beginning of the report and formatted in a prominent manner to increase the likelihood that it is seen and understood...

“...reports should be short, simple, and to the point.”
"...the report should also contain several other elements that may be relevant for more thorough analysis of the results or for comparison with other results obtained from this patient over time."
Ideally, the report should be in a format that enables integration with an electronic health record.

An aesthetically beautiful report that must be scanned (eg, a printed or PDF file) into a patient’s chart is, in the long-term, less valuable for that patient than a report that can be integrated into the structured environment of an electronic health record.”

Thank You

Q&A

Type questions here

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