

# Interpretation and Reporting of Sequence Variants in Cancer

## A Practice-Based Guide

July 19, 2018

PierianDx

PierianDx  
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St. Louis, MO, 63108  
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PATIENT INFORMATION	
Name:	DOE, PAULIN
Date of Birth:	05/11/1941
Gender:	Female
Disease:	Non-small cell
Specimen Type:	Biopsy sample
Indication:	NSCLC
Specimen Quality:	

VARIANT RESULT SUMMARY	
Variants Detected	Therapies or Prognostic Indication (in patient malignancy)
ALK-EML4 fusion	✓ Responsive to Crizotinib, Brigatinib, Ceritinib
EGFR vIII deletion	Yes

VARIANT INTERPRETATION DETAILS	
VARIANTS DETECTED	INTERPRETATIONS
EML4-ALK fusion	Interpretation: EML4 is inv. Lymphoma Kinase/Janus kinase superfamily. ALK is found to anaplastic large cell lymphoma (2011). Abnormal fusion of p. transcribed, which is one of EML4-ALK fusion to identify and the intracellular catalytic (PIM2: 28279422). Transcription such as PI3K/AKT, JAK/STAT most widely reported in non-28279422). In NSCLC, ALK benefit from targeted therapy (2018).
EGFR vIII deletion	Interpretation: EGFR encodes MAPK and PI3K-mTOR pathway while preventing apoptosis covering exon 2-7 (NM_005196.4). EGFR vIII deletion is a truncated receptor leading to ligand-independent EGFRvIII occurs in multiple patients and in a smaller population.

The Journal of Molecular Diagnostics, Vol. 19, No. 1, January 2017



### SPECIAL ARTICLE

#### Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

#### A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists

Marilyn M. Li,<sup>1\*</sup> Michael Datto,<sup>2\*</sup> Eric J. Duncavage,<sup>3\*</sup> Shashikant Kulkarni,<sup>4\*</sup> Neal I. Lindeman,<sup>5\*</sup> Somak Roy,<sup>6,7\*</sup> Apostolia M. Tsimberidou,<sup>8,9\*</sup> Cindy L. Vnencak-Jones,<sup>10,11</sup> Dayna J. Wolf,<sup>12\*</sup> Anas Younes,<sup>13,14\*</sup> and Marina N. Nikiforova<sup>15,16\*</sup>

From the Interpretation of Sequence Variants in Solid Tissues Working Group of the Clinical Practice Committee,\* Association for Molecular Pathology, Bethesda, Maryland; the Department of Pathology and Laboratory Medicine, Division of Genetic Diagnostics, the Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; the Duke University School of Medicine,<sup>7</sup> Durham, North Carolina; the Department of Pathology and Immunology,<sup>11</sup> Washington University School of Medicine, St. Louis, Missouri; Baylor Genetics,<sup>8</sup> Houston, Texas; the Brigham and Women's Hospital,<sup>9</sup> Harvard Medical School, Boston, Massachusetts; the University of Pittsburgh Medical Center,<sup>10</sup> Pittsburgh, Pennsylvania; the Department of Investigational Cancer Therapeutics,<sup>12</sup> University of Texas MD Anderson Cancer Center, Houston, Texas; the Department of Pathology, Microbiology and Immunology,<sup>13</sup> Vanderbilt University Medical Center, Nashville, Tennessee; the Department of Pathology and Laboratory Medicine,<sup>14</sup> Medical University of South Carolina, Charleston, South Carolina; and the Memorial Sloan-Kettering Cancer Center,<sup>15</sup> New York, New York.

Accepted for publication October 13, 2016.  
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Widespread clinical laboratory implementation of next-generation sequencing–based cancer testing has highlighted the importance and potential benefits of standardizing the interpretation and reporting of molecular results among laboratories. A multidisciplinary working group tasked to assess the current status of next-generation sequencing–based cancer testing and establish standardized consensus definitions, annotation, interpretation, and reporting conventions for somatic sequence variants was convened by the Association for Molecular Pathology with liaison representation from the American College of Medical Genetics and Genomics, American Society of Clinical Oncology, and College of American Pathologists. On the basis of the results of professional surveys, literature review, and the Working Group's subject matter expert consensus, a four-tiered system to categorize somatic sequence variations based on their clinical significance is proposed: tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants of unknown clinical significance; and tier IV, variants deemed benign or likely benign. Cancer genomics is a rapidly evolving field; therefore, the clinical significance of any variant in therapy, diagnosis, or prognosis should be

Shalini Verma, MD, FCAP

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Andy Bredemeyer, PhD

Vice President Product

PierianDx

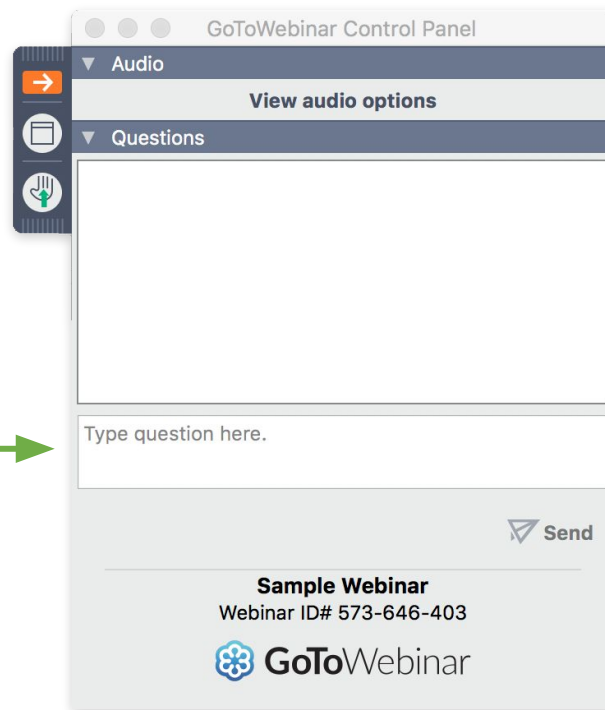




We Want to Hear from You!

# How to Submit Questions

Type questions here



The screenshot shows the GoToWebinar Control Panel interface. On the left is a vertical sidebar with three icons: an orange arrow, a document icon, and a hand icon. The main panel has a title bar 'GoToWebinar Control Panel' and two expandable sections: 'Audio' and 'Questions'. The 'Questions' section is expanded, showing a large empty text area for questions. Below this is a smaller text input field with the placeholder text 'Type question here.' and a 'Send' button with a checkmark icon. At the bottom, it displays 'Sample Webinar' and 'Webinar ID# 573-646-403' along with the GoToWebinar logo.



# Leaders in Clinical Genomics

Today



40+ leading lab clients  
in sharing network

Staff of 60+ medical  
and scientific experts

2014

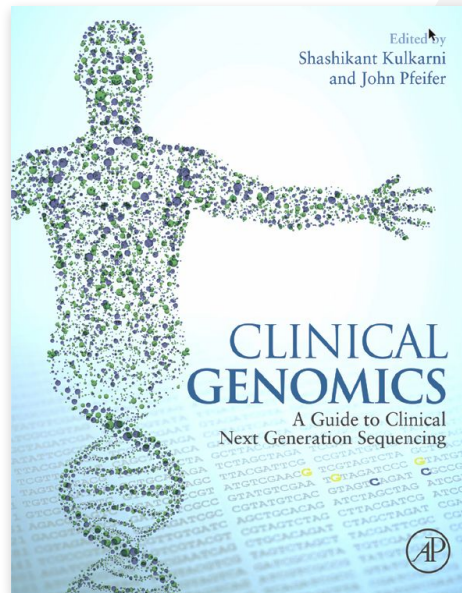
PierianDx established  
after ~50 labs visit  
WashU to learn how  
clinical NGS is  
operationalized.

2011

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

2003

WashU plays critical  
role in Human Genome  
Project.



## CLINICAL GENOMICS

Edited by  
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PierianDx founders among first to validate and  
clinically report on somatic cancer NGS panels.



# Agenda



1

Recommendation for Evidence Based Categorization

2

Application and Reporting in Molecular Pathology Practice

3

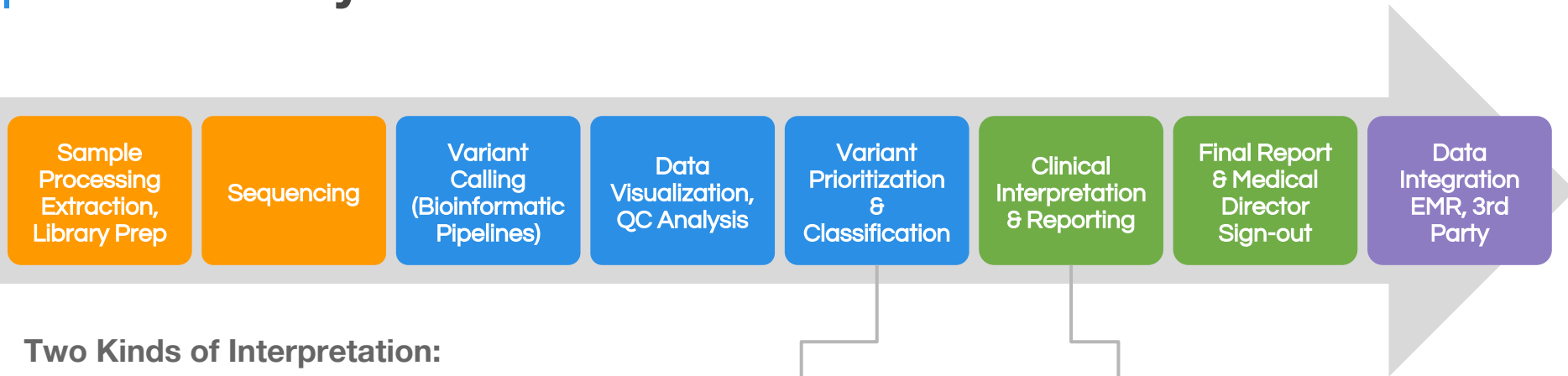
Additional Considerations

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, ie. 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.

**Analytic  
Interpretation**

**Clinical  
Interpretation**





## Evidenced Based Categorization **Recommendations**



# Evidence Based Categorization

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

J Mol Diagn. 2017 Jan;19(1):4-23.

## 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) [ $\geq 1\%$ ,  $< 1\%$ ]
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

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



# Evidence Based Categorization

## 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)*

J Mol Diagn. 2017 Jan;19(1):4-23.

Tier	Classification <i>(Therapeutic, Prognostic, Diagnostic)</i>	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	

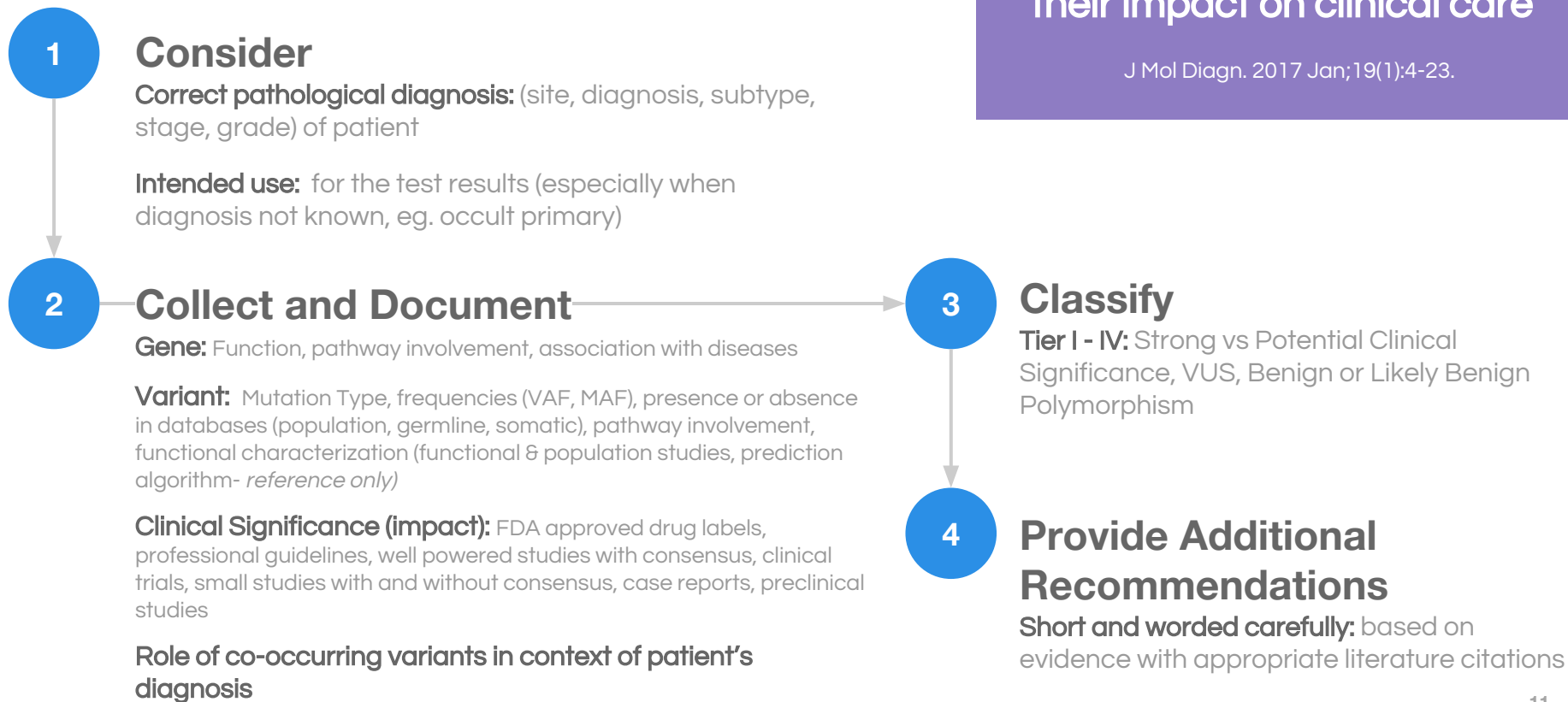


Evidenced Based Categorization

# **Application and Reporting**



# 4 Steps for Drafting Interpretations



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

J Mol Diagn. 2017 Jan;19(1):4-23.



# Reporting Clinical Significance

I

II

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.

III

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.

## ALWAYS REMEMBER

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.

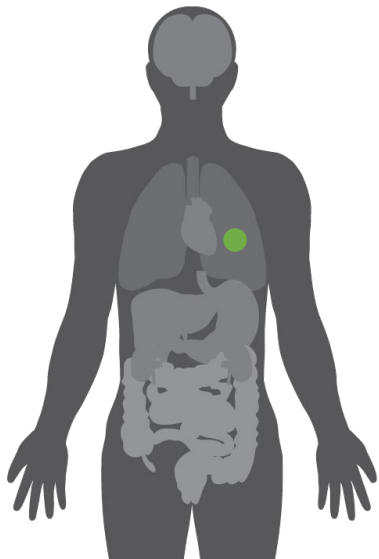
- **Often, these factors are unknown** to the molecular professional reporting results.
- Failure to take these other factors into consideration when recommending a specific therapy can lead to confusion, conflict between patient and oncology team, and anxiety.

**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\_116  
411887del12

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

## Evidence Summary

### 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 (eg.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



## Example - Tier I



**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

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

## 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





Clinical Interpretation of Somatic Variants

# **Additional Considerations**



# Polymorphisms

Do NOT miss variants with  
 $MAF \geq 1\%$  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  $\geq 1\%$  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

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.**



# 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.



# 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

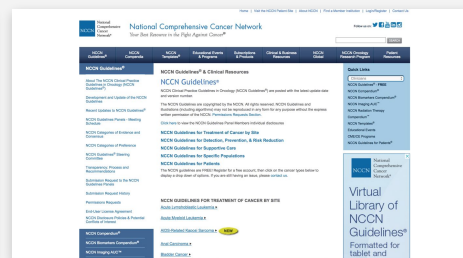
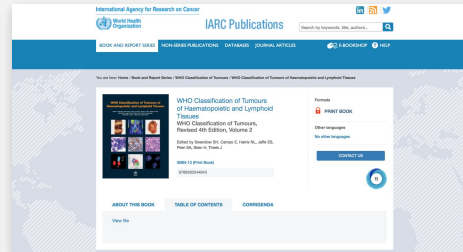
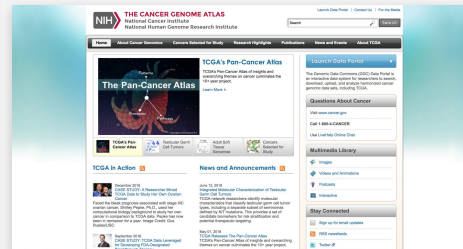
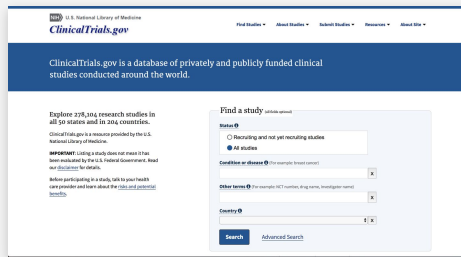
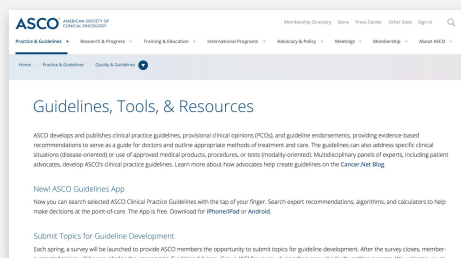
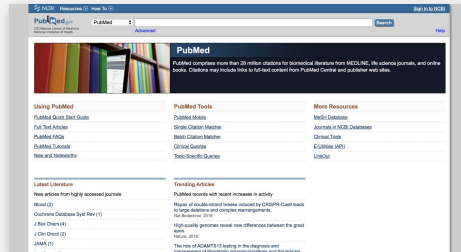
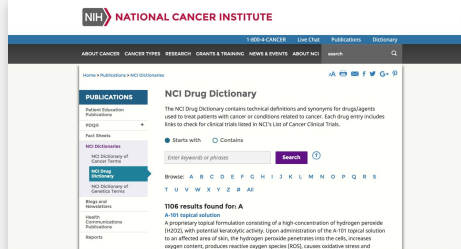
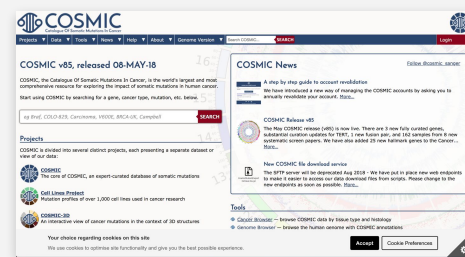
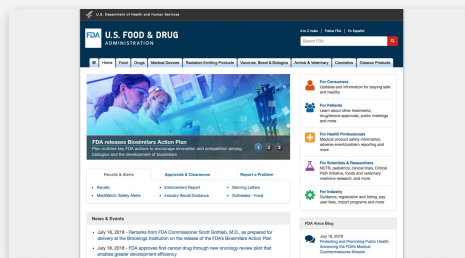
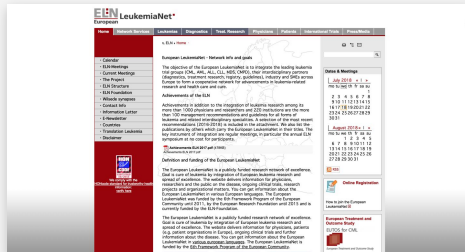
■ NCI Drug Dictionary

■ LeukemiaNET

■ WHO Class of Tumors

■ Etc.

www.pierianrx.com





## 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

## Need for creating a Clinical Genomics “Workstation”

MOLECULAR ONCOLOGY 8 (2014) 859 - 873

### 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, eg. 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



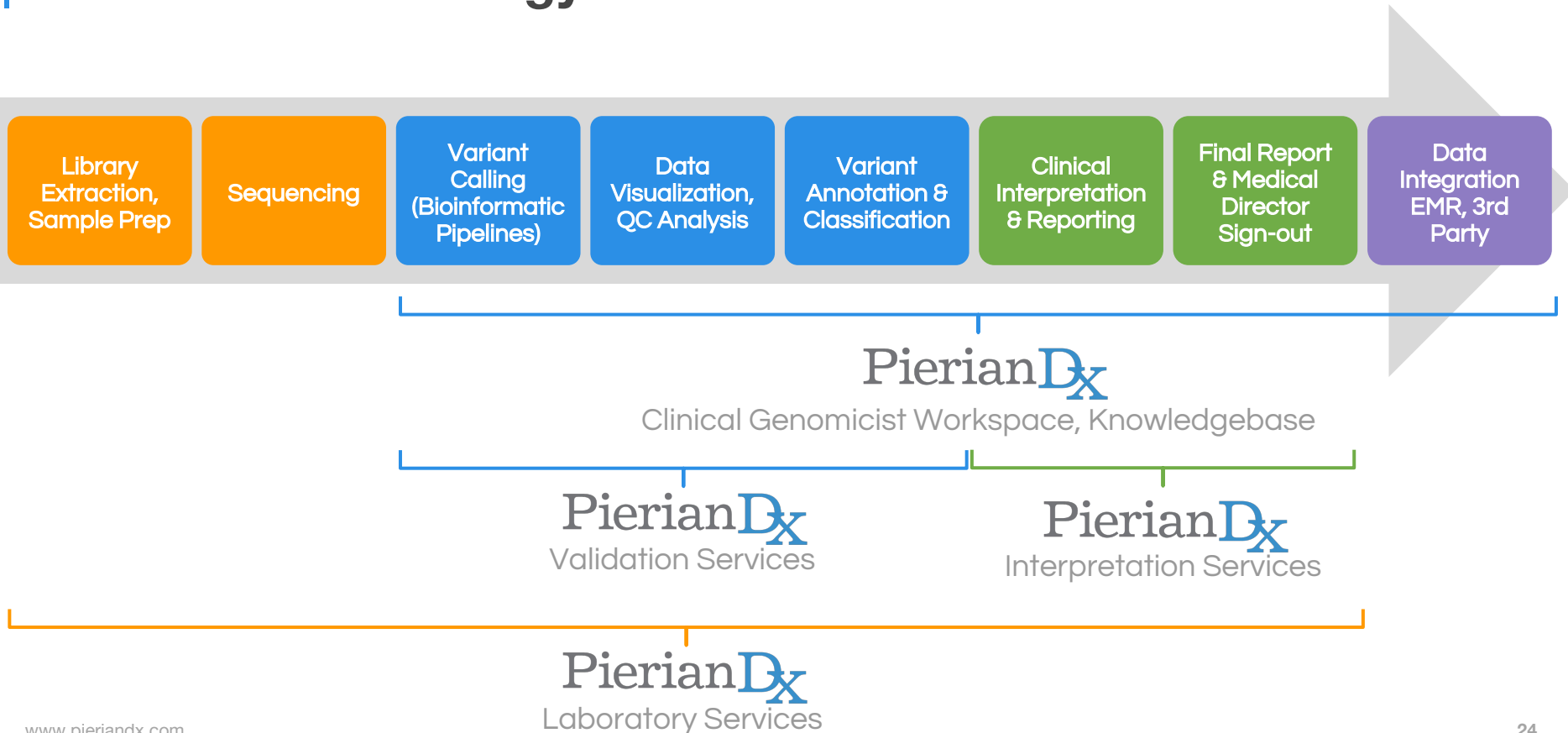


PierianDx: Comprehensive Support

# **NSCLC Example**



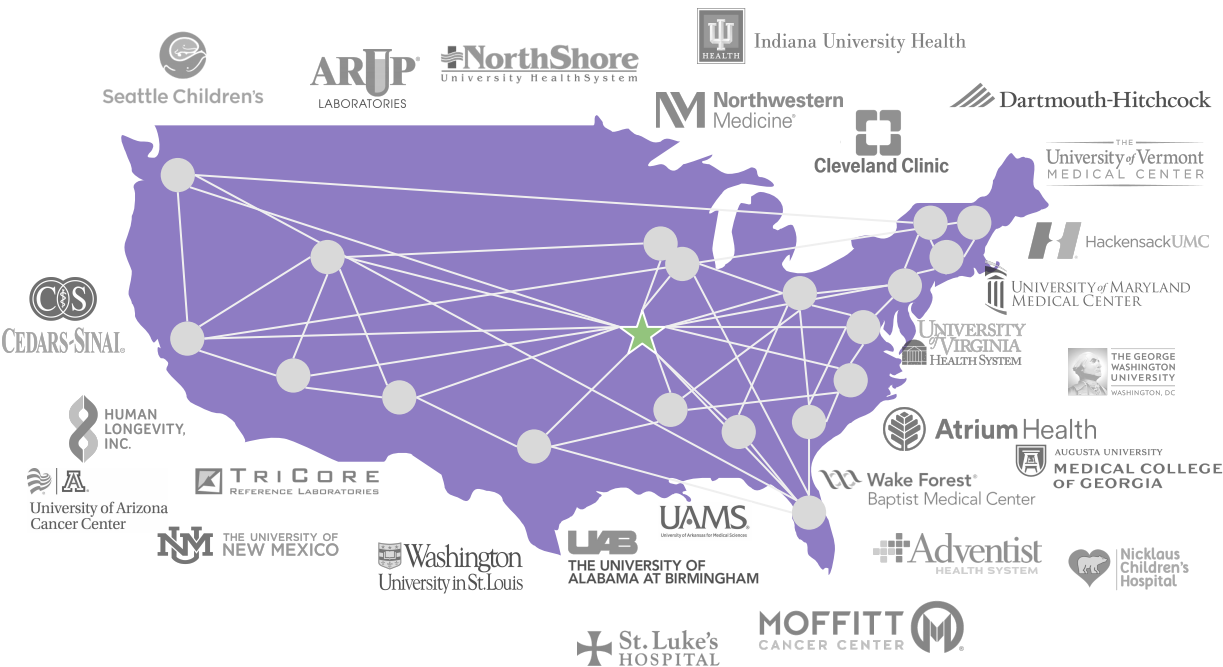
# PierianDx Technology and Services





# Creating the Largest, Peer Shared Interpretation System

## More Rapid, Accurate Interpretation

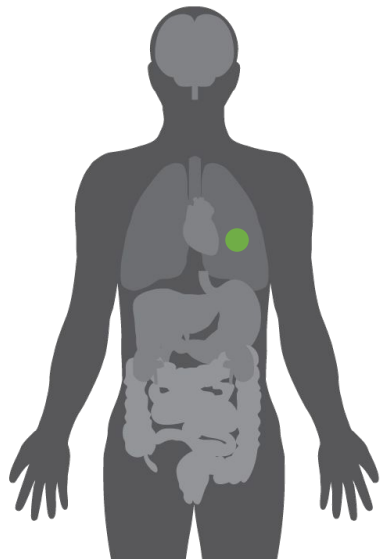


### Most clinically robust, up-to-date knowledgebase

- 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



# 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

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

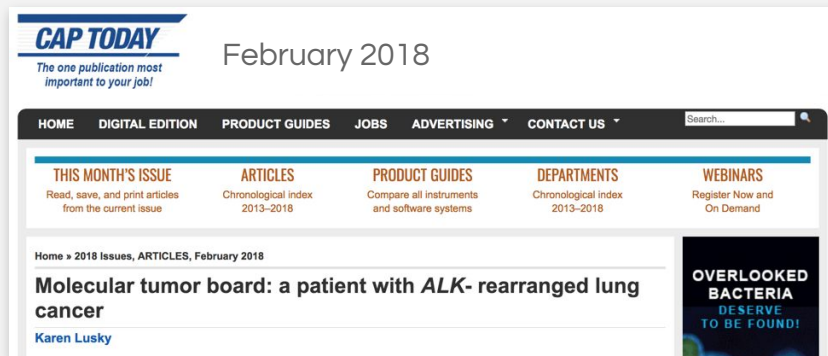
## Initial assessment

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





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

## Evidence

(Type and Source)

### Variant

1. Mutation type: [Activating, LOF (missense, nonsense, indel, splicing), CNVs, fusions]
2. Present/Absent in somatic databases
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### 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



# 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...”

J Mol Diagn. 2017 Jan;19(1):4-23.

Called variants

Variant allele fraction (VAF)

Calculated variant consequence

Clinical Genomicist Work: x

Secure https://demo.pierianDX.com/cgw/report/edit?orderId=8873

New filter Save Add filter group

Report: Review: Artifacts: CUSTOMIZE COLUMNS

	STATUS	LEVEL	GENE	VARIANT	CONSEQUENCE	KNOWLEDGEBASE SOURCES	STRAND BIAS	VAF	ZYGOSITY
		1-Pr	EML4 ALK	t(2;2)(p23;p21)(chr2:g.294463...	Fusion inframe	Interpretation			
		1-Pr	ALK	p.G1202R	Non-synonymous,missense	Clinical eviden...	0.504766	0.2526263131565...	HETEROZYGOUS
		2-P	TP53	p.G115V, p.G22V, p.G134V, p.G...	Non-synonymous	Interpretation,...	0.522016	0.5012683916793...	HETEROZYGOUS
		3-R	APC	c.4479G>A, c.4425G>A, c.430...	Synonymous	Clinical eviden...	0.512992	0.3775870772337...	HETEROZYGOUS
		3-R	TP53	p.P72R, p.P33R	Non-synonymous	Clinical eviden...	0.5	1	HOMOZYGOUS
			CDKN2A	chr9:g.21967751_21975132(0_0)	Non-synonymous				UNKNOWN
		4-V	KDR	c.2679T>G	Synonymous		0.510066	0.0354969574036...	HETEROZYGOUS
		4-V	KDR	c.798+54G>A	No established biological imp...		0.5	1	HOMOZYGOUS
		4-V	CSF1R	c.*35delinsT	No established biological imp...		0.5	1	HOMOZYGOUS
		4-V	SMAD4	c.1335A>G	Synonymous		0.525449	0.0527373179306...	HETEROZYGOUS

Flag variant as an artifact

Displaying 11 of 17 results



# Variant Annotation and Classification

## Variant Table

"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."

J Mol Diagn. 2017 Jan;19(1):4-23.

- Identify false-positives
- Establish frequencies of mutations

Clinical Genomicist Work: x Andy

Secure https://demo.pierianDX.com/cgw/report/edit?orderId=8873

New filter Save Add filter group

Report: Review: Artifacts: CUSTOMIZE COLUMNS

	STATUS	LEVEL	GENE	VARIANT	CONSEQUENCE	KNOWLEDGEBASE SOURCES	STRAND BIAS	SAF	ZYGOSITY
		1-Pr	EML4 ALK	t(2;2)(p23;p21)(chr2:g.294463...	Fusion inframe	Interpretation			
		1-Pr	ALK	p.G1202R	Non-synonymous,missense	Clinical eviden...	0.504766	0.2526263131565...	HETEROZYGOUS
		2-P	TP53	p.G115V, p.G22V, p.G134V, p.G...	Non-synonymous	Interpretation,...	0.522016	0.5012683916793...	HETEROZYGOUS
		3-R	APC	c.4479G>A, c.4425G>A, c.430...	Synonymous	Clinical eviden...	0.512992	0.3775870772337...	HETEROZYGOUS
		3-R	TP53	p.P72R, p.P33R	Non-synonymous	Clinical eviden...	0.5	1	HOMOZYGOUS
			CDKN2A	chr9:g.21967751_21975132(0_0)	Non-synonymous				UNKNOWN
		4-V	KDR	c.2679T>G	Synonymous		0.510066	0.0354969574036...	HETEROZYGOUS
		4-V	KDR	c.798+54G>A	No established biological imp...		0.5	1	HOMOZYGOUS
		4-V	CFR1R	c.*35delinsT	No established biological imp...		0.5	1	HOMOZYGOUS
		4-V	SLAD4	c.1335A	ymous		0.525449	0.0527373179306...	HETEROZYGOUS

Flag variant as an artifact

Artifact

Displaying 11 of 17 results



# 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...”

J Mol Diagn. 2017 Jan;19(1):4-23.

Variant filtering for guided, standardized variant review

Automated variant classification

The screenshot displays the Clinical Genomicist Workbench interface. The top navigation bar shows the URL <https://demo.pierianx.com/cgw/report/view?orderId=8873>. The main content area is divided into two sections. The top section, titled "Variants for review (4)", contains a filter panel with the following settings: Level: 1, 2, 3, 4; Consequence: Non-synonymous, Non-s...; VAF: 0.05 -; Strand bias: 0.02 - 0.98; More: Select. Below this, there is an "OR" section with Variant type/subtype: Fusion and More: Select. The bottom section displays a table of variant results. The table has columns for STATUS, LEVEL, GENE, VARIANT, DNA CHANGE, VARIANT TYPE/SUBTYPE, and CONSEQUENCE. The first row is highlighted with a purple box, showing a variant at LEVEL 1 for the EML4 gene. The second row shows a variant at LEVEL 1 for the ALK gene. The third row shows a variant at LEVEL 2 for the TP53 gene. The fourth row shows a variant at LEVEL 3 for the TP53 gene. The table is titled "CUSTOMIZE COLUMNS" and shows "Displaying 1-4 of 4 results".

STATUS	LEVEL	GENE	VARIANT	DNA CHANGE	VARIANT TYPE/SUBTYPE	CONSEQUENCE
	1	EML4	t(2;2)(p23;p21)(chr2:g.294463...	t(2;2)(p23;p21)(chr2...	Fusion	Fusion inframe
	1	ALK	p.G1202R	chr2:g.29443613C>T	Substitution	Non-synonymous,missense
	2	TP53	p.G115V, p.G22V, p.G134V, p.G...	chr17:g.7578469C>A	Substitution	Non-synonymous
	3	TP53	p.P72R, p.P33R	chr17:g.7579472G>C	Substitution	Non-synonymous



# Variant Annotation and Classification

## Clinical Practice Guidelines

### EML4/ALK Fusion

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

J Mol Diagn. 2017 Jan;19(1):4-23.

### For recognizable recurrent variants, get:

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

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/summary/10595\\_8546\\_0\\_1\\_2](https://demo.pierianDX.com/cgw/variantDetails/summary/10595_8546_0_1_2)

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EML4, ALK: t(2;2)(p23;p21)(chr2:g.29446394::ochr2:g.42522654) Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

Disease:  Source:  Management Impact:  More:

CUSTOMIZE COLUMNS

ACTION	SOURCE	DISEASE	GENERIC DRUG NAME	MANAGEMENT IMPACT	DRUG IMPACT	URL
	FDA	Metastasis from malignant tumor of lung, Non-small cell carcinoma, Non-small cell lung cancer	Alectinib	Therapeutic	RESPONSIVE	<a href="https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208434s004lbl.pdf">https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208434s004lbl.pdf</a>
	FDA	Metastasis from malignant tumor of lung, Non-small cell carcinoma, Non-small cell lung cancer	Alectinib	Therapeutic	RESPONSIVE	<a href="https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208434s004lbl.pdf">https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/208434s004lbl.pdf</a>
	NCCN	Non-small cell carcinoma, Non-small cell lung cancer, Secondary malignant neoplasm of brain	Ceritinib; Alectinib; Brigatinib	Therapeutic	RESPONSIVE	<a href="https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf">https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf</a>
	NCCN	Non-small cell carcinoma, Non-small cell lung cancer, Secondary malignant neoplasm of brain	Alectinib	Therapeutic	RESPONSIVE	<a href="https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf">https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf</a>
	NCCN	Metastasis from malignant tumor of lung, Non-small cell carcinoma, Non-small cell lung cancer, Secondary malignant neoplasm of central nervous system	Alectinib	Therapeutic	RESPONSIVE	<a href="https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf">https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf</a>
		Non-small cell	Alectinib	Therapeutic	RESPONSIVE	<a href="https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf">https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf</a>



# Variant Annotation and Classification

## Clinical Interpretations

### EML4/ALK Fusion

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

J Mol Diagn. 2017 Jan;19(1):4-23.

Leverage pathologist and geneticist variant assessment expertise through the shared interpretation knowledgebase

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/summary/10595\\_8546\\_0\\_1\\_2](https://demo.pierianDX.com/cgw/variantDetails/summary/10595_8546_0_1_2)

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EML4, ALK: t(2;2)(p23;p21)(chr2:g.29446394::ochr2:g.42522654) Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

Nomenclature:  Gene:  Interpretation:  Date updated:  Disease:  More:

CUSTOMIZE COLUMNS

ACTION	DATE UPDATED	PUBLISHER	GENE	DISEASE	NOMENCLATURE	CLASSIFICATION	INTERPRETATION
	04/06/2018 14:52	demo	EML4	Non-small cell lung cancer	t(2;2)(p23;p21)(chr2:g.29446394::ochr2:g.42522654)	Predictive or prognostic in tumor type	An <i>EML4-ALK</i> gene rearrangement, t(2;2)(p23;p21)(chr2:g.29446394::ochr2:g.42522654), was identified by next generation sequencing. Rearrangements involving the <i>ALK</i> gene are an uncommon occurrence observed in 3-7% of NSCLC (Pillai RN, Ramalingam SS; <i>Curr Oncol Rep</i> 14; 105-10; 2012 Apr). <i>EML4-ALK</i> gene translocations have been significantly associated with adenocarcinoma and advanced stage and are generally mutually exclusive of mutations in <i>EGFR</i> and <i>KRAS</i> (Zhao F, et al.; <i>PLoS One</i> 10; e0117333; 2015). <i>ALK</i> rearrangements have been established in the literature as predictive for therapeutic responses in the setting of non-small cell lung cancer to tyrosine kinase inhibitors including Crizotinib (Gainor J; <i>Ann Oncol</i> 26; ii14; 2015 Mar).
	04/06/2018 15:03	demo	EML4	Non-small cell lung cancer	t(2;2)(p23;p21)(chr2:g.29446394::ochr2:g.42522654)	Predictive or prognostic in tumor type	EML4 is involved in microtubule formation (RefSeq, Jan 2015). ALK (Anaplastic Lymphoma Kinase) encodes a receptor tyrosine kinase, which belongs to the insulin receptor superfamily. ALK is found to be rearranged, mutated, or amplified in a series of tumors including anaplastic large cell lymphomas, neuroblastoma, and non-small cell lung cancer (NSCLC) (RefSeq, Jan 2011). Abnormal fusion of parts of EML4 gene with portions of ALK gene generates EML4-ALK fusion transcripts, which is one of the primary mutations associated with NSCLC (RefSeq, Jan 2015). An



# Variant Annotation and Classification

## Clinical Interpretations

ALK p.G1202R

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

Auto-classify

Point to relevant literature and trials

Kick start your report editing

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/summary/10595\\_8546\\_0\\_0\\_0](https://demo.pierianDX.com/cgw/variantDetails/summary/10595_8546_0_0_0)

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Case #ALKescape

ALK: p.G1202R

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

Nomenclature:  Gene:  Interpretation:  Date updated:  Disease:  More:

CUSTOMIZE COLUMNS

ACTION	DATE UPDATED	PUBLISHER	GENE	DISEASE	NOMENCLATURE	CLASSIFICATION	INTERPRETATION
	06/03/2018 23:24	PierianDX	ALK	Non-small cell lung cancer	p.G1202R	Predictive or prognostic in tumor type	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, 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 onstrated to confer resistance to crizotinib in-vitro in cells harboring (PMID: 22277784). 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: 22277784, 28122866). In a case study in a patient with NSCLC with EML4-ALK fusion, ALK G1202R alteration conferred high-level resistance to crizotinib (PMID: 22277784) as well as to next-generation ALK inhibitors (ceritinib, alectinib, and brigatinib (PMID: 28122866). Moreover, the secondary resistant mutation ALK G1202 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 (NCT03052608, phase 3; NCT02584634, phase 2).
	04/06/2018 17:35	demo	ALK	Non-small cell lung cancer	NP_004295.2:p.G1202R	Predictive or prognostic in tumor type	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



# Variant Annotation and Classification

## Clinical Trials

ALK p.G1202R

“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.”

J Mol Diagn. 2017 Jan;19(1):4-23.

Assess trial matches for consideration of Tier 2C classification evidence

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | https://demo.pierianDX.com/cgw/variantDetails/summary/10595\_8546\_0\_0\_0

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ALK: p.G1202R Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

Phase:  Disease:  Gene/Variant:  More:

CUSTOMIZE COLUMNS

ACTION	CLINICAL TRIAL NAME	DISEASE	PHASE	INCLUSION CRITERIA	EXCLUSION CRITERIA	TREATMENT	TRIAL SITE NAME	TRIAL SITE ADDRESS	GENERIC DRUG NAME
	ENSIGN: Phase II Window of Opportunity Trial of Stereotactic Body Radiation Therapy and In Situ Gene Therapy Followed by Nivolumab in Metastatic Squamous or Non-Squamous Non-Small Cell Lung Carcinoma and Metastatic Uveal Melanoma	Metastasis from malignant tumor of lung, Non-small cell carcinoma, Nonsquamous non-small cell neoplasm of lung, Squamous cell carcinoma of lung	II	Patients that have stage IV, metastatic squamous or non-squamous NSCLC that has progressed after a platinum-based chemotherapy	Patients that have received prior treatment with gene therapy are ineligible. Patients with EGFR or ALK genomic tumor	Gene Therapy ADV/HSV-tk; Valacyclovir; Stereotactic Body Radiation	Houston Methodist Hospital	Houston, Texas, 77030, United States	Nivolumab
	A Phase IIa Trial of sEphB4-HSA in Combination With Anti PD-1 Antibody (Pembrolizumab, MK3475) in Patients With Non-small Cell Lung and Head/Neck Cancer	Metastasis from malignant tumor of lung, Non-small cell carcinoma, Non-small cell lung cancer	II	Patients with locally advanced or metastatic non-small cell lung cancer that has progressed after at least 1 line of platinum based chemotherapy; Patients may have received up to 2 prior lines of	active central nervous system (CNS) metastases and/or carcinomatous meningitis, Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti cytotoxic T-	Recombinant EphB4-HSA Fusion Protein	USC / Norris Comprehensive Cancer Center	Los Angeles, California, 90033, United States	Pembrolizumab



# Variant Annotation and Classification

## Human Research Evidence

ALK p.G1202R

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

J Mol Diagn. 2017 Jan;19(1):4-23.

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/summary/10595\\_8546\\_0\\_0\\_12](https://demo.pierianDX.com/cgw/variantDetails/summary/10595_8546_0_0_12)

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TP53: p.G115V, p.G22V, p.G134V, p.G154V Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

COSMIC URLs:

COSMIC Mutation ID: 342244 - p.G115V, p.G22V, p.G134V, p.G154V

COSMIC Mutation ID: 342245 - p.G115V, p.G22V, p.G134V, p.G154V

COSMIC Mutation ID: 342243 - p.G115V, p.G22V, p.G134V, p.G154V

COSMIC Mutation ID: 342246 - p.G115V, p.G22V, p.G134V, p.G154V

COSMIC Mutation ID: 1649372 - p.G115V, p.G22V, p.G134V, p.G154V

COSMIC Mutation ID: 4271946 - p.G115V, p.G22V, p.G134V, p.G154V

COSMIC Mutation ID: 6815 - p.G115V, p.G22V, p.G134V, p.G154V

Filter by All cancers

Available Tracks

filter tracks

☒ COSMIC

☒ TCGA

☒ Variant

Genes 1

Genome Track View Help

0 10,000,000 20,000,000 30,000,000 40,000,000 50,000,000 60,000,000 70,000,000 80,000,000

chr17 chr17:7577469..7579725 (2.26 Kb) Go

77,500 7,578,000 7,578,500 7,579,000 7,579,500

Reference Sequence

Zoom In to see sequence

<https://demo.pierianDX.com/cgw/>



# Variant Annotation and Classification

## Computational Evidence

ALK p.G1202R

"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.**"

J Mol Diagn. 2017 Jan;19(1):4-23.

Use with caution

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/summary/10595\\_8546\\_0\\_0\\_0](https://demo.pierianDX.com/cgw/variantDetails/summary/10595_8546_0_0_0)

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ALK: p.G1202R Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

Algorithm:  Score:  More:

CUSTOMIZE COLUMNS

ALGORITHM	SCORE	INTERPRETATION
MutationTaster	1	Disease
Polyphen2Hvar	0.998	Probably Damaging
Polyphen2Hdiv	1	Probably Damaging
LRT	0.000236	Deleterious
SIFT	0	Deleterious
FATHMM	-2.59	Damaging
MutationAssessor	3.36	Medium

Displaying 1-7 of 7 results



# Variant Annotation and Classification

## Literature Search

ALK p.G1202R

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

Clinical Genomicist Workspace || Patient Data and Knowledgebase Browser - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/dataAnnotationKB/10595\\_8546\\_0\\_0\\_0?aaSyntax=G1202R&gene=ALK&cytoBand=&literatureTab=true](https://demo.pierianDX.com/cgw/variantDetails/dataAnnotationKB/10595_8546_0_0_0?aaSyntax=G1202R&gene=ALK&cytoBand=&literatureTab=true)

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Search:  Start typing here

From Date:  To Date:  Clinical Relevance:  Study Type:  Variant Type:  Consequence:  Inheritance Pattern:

Amino Acid Position: From:  To:

Genome Browser | Publications

Filter by: Disease | Drug | Phenotype | Cytoband

- Small Cell Lung Carcinoma - 5
- Pulmonary Sclerosing Hemangioma - 5
- Pulmonary Blastoma - 5
- Pancoast Syndrome - 5
- Multiple Pulmonary Nodules - 5
- Lung Neoplasms - 5
- Carcinoma, Non-Small-Cell Lung - 5
- Carcinoma, Bronchogenic - 5
- Bronchial Neoplasms - 5
- Vipoma - 1

18 publications found

JOURNAL	DATE	TITLE	ANNOTATIONS	REFERENCE TEXT
ONCO TARGETS THER	2017 Apr 25	The activity, safety, and evolving role of brigatinib in patients with ALK-rearranged non-small cell lung cancers.	<b>Gene:</b> "ALK", "FES", "MERTK", "NM", "T" <b>Drug:</b> "Crizotinib", "Brigatinib", "... <b>Syntaxes:</b> "G1202R", "L1196M", "I1171S/T, V1180L, L1196M, L1152R/P, E1210K, and G1269A. In patients with ... ...Early data point to ALK <b>G1202R</b> and ALK E1210K as ... ...to ALK G1202R and ALK <b>E1210K</b> as potential mechanisms ...	
LUNG CANCER	2017 Mar 14	Dual occurrence of ALK G1202R solvent front mutation and small cell lung cancer transformation as resistance mechanisms to second generation ALK inhibitors without prior exposure to crizotinib. <b>Phenotype:</b> "Pericardial effusion" <b>Syntaxes:</b> "G1202R", "C706Y"	<b>Gene:</b> "ALK", "FES", "MERTK", "NM", "T" <b>Drug:</b> "Crizotinib", "Tumor Infiltration" <b>Phenotype:</b> "Pericardial effusion" <b>Syntaxes:</b> "G1202R", "C706Y"	

<https://demo.pierianDX.com/cgw/>



# Variant Annotation and Classification

## Clinical Interpretations

TP53 p.G154V

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

J Mol Diagn. 2017 Jan;19(1):4-23.

PierianDx network-sourced interpretations point to valuable evidence

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/summary/10595\\_8546\\_0\\_0\\_12](https://demo.pierianDX.com/cgw/variantDetails/summary/10595_8546_0_0_12)

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Clinical Genomicist Workspace

andy\_pdx

TP53: p.G115V, p.G22V, p.G134V, p.G154V Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

Nomenclature:  Gene:  Interpretation:  Date updated:  Disease:  More:

CUSTOMIZE COLUMNS

ACTION	DATE UPDATED	PUBLISHER	GENE	DISEASE	NOMENCLATURE	CLASSIFICATION	INTERPRETATION
	09/16/2015 18:16	Washu	TP53		NP_000537:p.G154V	Predictive or prognostic in tumor type	A non-synonymous p.G154V variant was detected in exon 1 of <i>TP53</i> that has previously been described in various tumor types, most frequently in lung cancer (COSMIC, IARC database). It has also been described as a germline mutation in two cases of brain cancer (IARC <i>TP53</i> database). Thus, if clinically indicated, germline testing of a peripheral blood sample may be considered. This variant which affects the NDBL/beta-sheet structural motif is predicted to have a deleterious effect on the translated p53 protein rendering it nonfunctional (IARC <i>TP53</i> database). Indeed, a recent study demonstrated that <i>TP53</i> mutations, to include this variant, were associated with significantly reduced survival in non-small cell lung cancer (PMID: 22980975). Thus, this variant may be clinically actionable in this patient.
	06/03/2018 23:24	PierianDx	TP53	Non-small cell lung cancer	p.G154V	Predictive or prognostic in tumor type	TP53 is a tumor suppressor and regulates expression of target genes, by inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism (PMID: 20182602). A TP53 missense substitution, G154V, was identified. Codon G154 lies within the DNA binding domain of the TP53 protein (exon 6) (UniProt.org). It is predicted to impair the normal function of the protein (classified as non-functional and deleterious on the IARC TP53 database, Jan 2018). TP53 G154V has been reported in multiple tumor types including non-small cell carcinoma (NSCLC) (COSMIC, Feb 2018). A meta-analysis in lung cancer patients suggested that TP53 mutations are associated with smoking-induced lung cancer (PMID: 24126199). TP53 mutations have been reported to be a negative predictor of the outcome of lung adenocarcinoma patients, and NSCLC patients with TP53 alterations may be relatively more resistant to chemotherapy and radiation (PMID: 28240049; 26689115; 26647728).



# 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.”

J Mol Diagn. 2017 Jan;19(1):4-23.

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/summary/10595\\_8546\\_0\\_0\\_12](https://demo.pierianDX.com/cgw/variantDetails/summary/10595_8546_0_0_12)

PierianDx enabling personalized medicine Clinical Genomicist Workspace andy\_pdx

TP53: p.G115V, p.G22V, p.G134V, p.G154V Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

Phase:  Disease:  More:

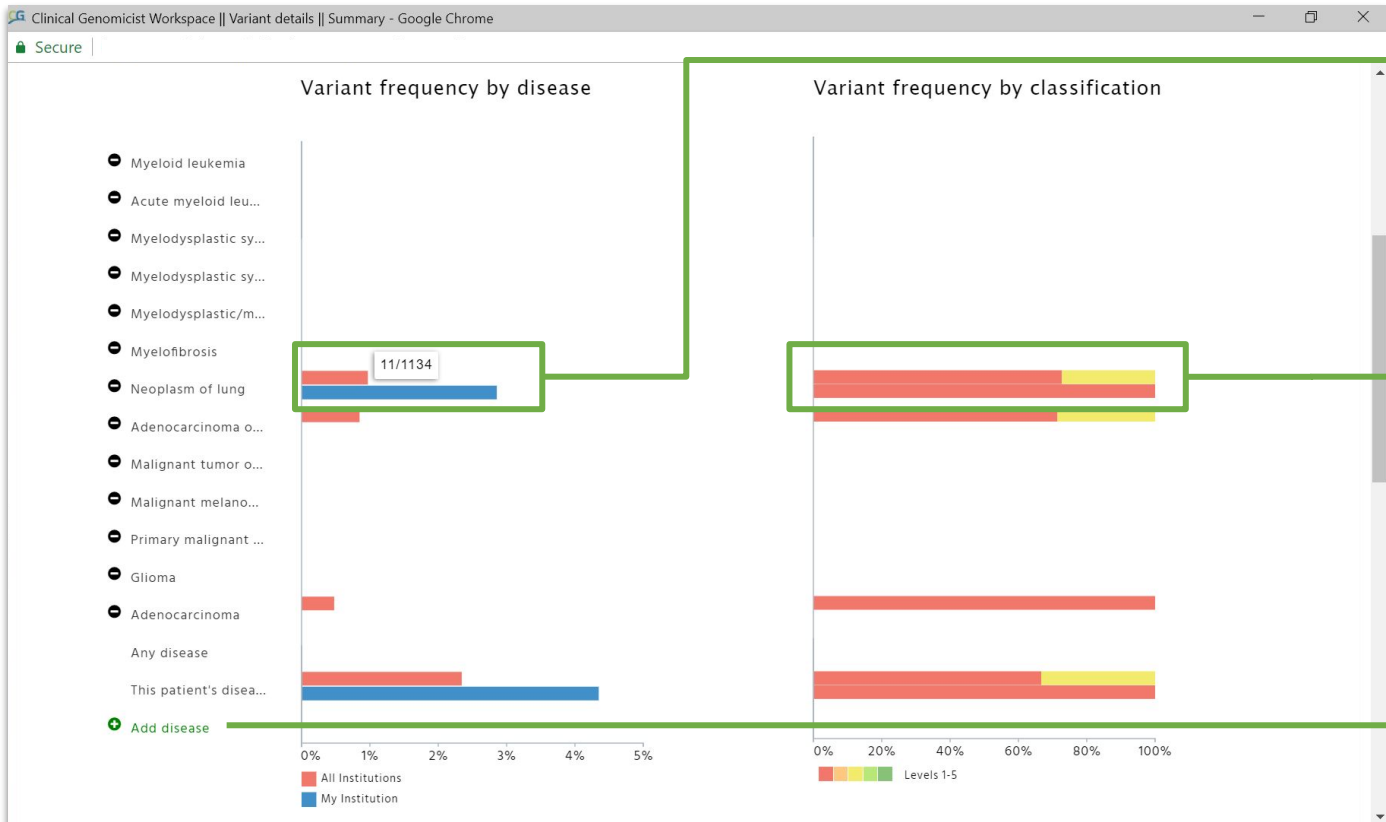
CUSTOMIZE COLUMNS

ACTION	CLINICAL TRIAL NAME	DISEASE	PHASE	INCLUSION CRITERIA	EXCLUSION CRITERIA	TRIAL SITE NAME	GENERIC DRUG NAME
	A Phase II Study of the PARP Inhibitor Olaparib (AZD2281) Alone and in Combination With AZD1775, AZD5363, or AZD6738 in Advanced Solid Tumors	Malignant Neoplasm (Morphology), Malignant neoplastic disease, Neoplasm, Neoplastic disease, Secondary malignant neoplastic disease	II	Patients with histologically documented metastatic cancer (solid tumors, not including hematologic malignancies) are included. Patients with progressive cancer at the time of study entry are included. Prior treatment	Patients with known germline BRCA mutations in breast or ovarian cancers will be excluded from the study, however testing is not required for inclusion in the study. Patients with hematologic malignancies (includes patients with	Yale Cancer Center Dana-Farber Cancer Institute Vanderbilt-Ingram Cancer Center	Olaparib; AZD1775



# Clinical Data Aggregation

TP53 p.G154V



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 Annotation and Classification

## Clinical Evidence

TP53 p.P72R

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure | [https://demo.pierianDX.com/cgw/variantDetails/summary/10595\\_8546\\_0\\_0\\_13](https://demo.pierianDX.com/cgw/variantDetails/summary/10595_8546_0_0_13)

TP53: p.P72R, p.P33R Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

Nomenclature:  Gene:  More:

CUSTOMIZE COLUMNS

SOURCE	GENE	CLASSIFICATION	CLINICAL SIGNIFICANCE	DISEASE LABEL	LAB	SOURCE URL
Clinvar	TP53	3	Uncertain significance	Li-Fraumeni syndrome 1	Pathway Genomics	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000144668/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000144668/</a>
Clinvar	TP53	5	Benign	Hereditary cancer-predisposing syndrome	Color Genomics, Inc.	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000132165/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000132165/</a>
Clinvar	TP53	5	Benign	Hereditary cancer-predisposing syndrome	Ambry Genetics	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000132165/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000132165/</a>
Clinvar	TP53	4	Likely benign	Li-Fraumeni syndrome	Invitae	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000233585/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000233585/</a>
Clinvar	TP53	5	Benign	Li-Fraumeni syndrome	Invitae	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000300782/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000300782/</a>
Clinvar	TP53	5	Benign	Li-Fraumeni syndrome	Illumina Clinical Services Laboratory, Illumina	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000300782/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000300782/</a>
Clinvar	TP53	5	Benign	not specified	Mayo Clinic Genetic Testing Laboratories, Mayo Clinic	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000079202/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000079202/</a>
Clinvar	TP53	5	Benign	not specified	ARUP Laboratories, Molecular Genetics and Genomics	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000079202/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000079202/</a>
Clinvar	TP53	5	Benign	not specified	PreventionGenetics	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000079202/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000079202/</a>
Clinvar	TP53	5	Benign	not specified	EGL Genetic Diagnostics, Eurofins Clinical	<a href="http://www.ncbi.nlm.nih.gov/clinvar/RCV000079202/">http://www.ncbi.nlm.nih.gov/clinvar/RCV000079202/</a>

Conflicting ClinVar submissions, but consensus is clear



# Variant Annotation and Classification

## Population Frequencies

TP53 p.P72R

"These databases are frequently used to **filter out variants that are deemed polymorphic/benign** based on an arbitrary cutoff of minor allele frequency (MAF)."

J Mol Diagn. 2017 Jan;19(1):4-23.

MAF > 1% in all population databases

Clinical Genomicist Workspace || Variant details || Summary - Google Chrome

Secure https://demo.pierianDX.com/cgw/variantDetails/summary/10595\_8546\_0\_0\_13

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TP53: p.P72R, p.P33R Case #ALKescape

SUMMARY	VARIANT CALLS	POPULATION FREQUENCIES	INTERPRETATIONS	GUIDELINES	CLINICAL EVIDENCE	DISEASE/PHENOTYPE
LITERATURE	CLINICAL TRIALS	HUMAN RESEARCH EVIDENCE	COMPUTATIONAL EVIDENCE	COMMENTS	CLINICAL DATA AGGREGATION	

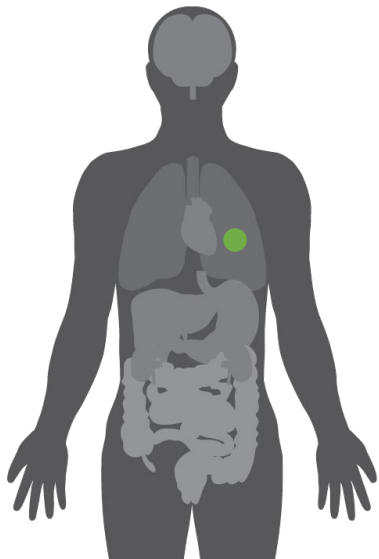
Database: Population: More: Select

CUSTOMIZE COLUMNS

DATABASE	POPULATION	ALLELE	FREQUENCY
ExAC	AFR	C	0.384
ExAC	AMR	C	0.711
ExAC	EAS	C	0.589
ExAC	EURF	C	0.730
ExAC	EURNF	C	0.734
ExAC	OTHER	C	0.665
ExAC	SAS	C	0.499
ExAC	TOTAL	C	0.660
NHLBI	EA	C	0.745
NHLBI	AA	C	0.405



# 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

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

## Evidence Summary

### 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:

Despite *ALK* rearrangement, progressed tumor not expected to respond to first- and second- generation ALK inhibitors due to p.G1202R secondary mutation. Consider trials offering third-generation inhibitors.



# Clinical Interpretation and Reporting

## Final Report

"The report is an essential part of any laboratory test and **should contain all the information required for the ordering physician and the patient** to know:

- Exactly was tested
- What results were obtained from the test
- Any additional preanalytic, analytic, or postanalytic factors that may influence the clinical interpretation of the results.

J Mol Diagn. 2017 Jan;19(1):4-23.

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77 Maryland Plaza  
St. Louis, MO 63108

PATIENT INFORMATION			
Name:	DOE, JOHAN	Accession Number:	ALKescape
Date of Birth:	10/7/1945	MR#:	45615663
Gender:	Male	Ordering Physician:	Geraldine Moore, MD
Disease:	Non-small cell lung cancer		
Specimen Type:	Core needle biopsy	Date Collected:	05/26/2018
Indication:	Liver metastasis	Date Ordered:	05/29/2018
Specimen Quality:	Adequate	Date Accessed:	05/30/2018

REVIEW STATUS: Final

CLINICAL INFORMATION AND TEST SUMMARY	OVERVIEW OF FINDINGS										
<b>Illumina TST 170 Panel</b> Targeted next-generation sequencing was performed on this specimen of NSCLC from a 72 year old male to evaluate genomic alterations. Tumor DNA and RNA were assessed across a total of 170 targeted genes using the Illumina TruSight Tumor 170 assay kit and variant detection software.	<b>7</b> Variants of therapeutic significance <b>0</b> Variants of diagnostic or prognostic significance										
	Variant clinical significance tiers per AMP/ASCO/CAP guidelines <table border="1"> <thead> <tr> <th>1A</th> <th>1B</th> <th>2C</th> <th>2D</th> <th>3</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>0</td> <td>2</td> <td>0</td> <td>3</td> </tr> </tbody> </table>	1A	1B	2C	2D	3	1	0	2	0	3
1A	1B	2C	2D	3							
1	0	2	0	3							

RESULT SUMMARY		
Variant Detected	Strong clinical significance	Potential clinical significance
<b>EML4/ALK fusion</b>	<div> <div>✓</div> Responsive to Crizotinib, Ceritinib, Alectinib, Brigatinib </div> <div> <div>✗</div> Non-Responsive to Erlotinib, Gefitinib, Afatinib </div>	
<b>ALK</b> p.G1202R VAF: 26%		<div> <div>    </div> Lorlatinib under investigation in Phase III trial NCT03053608 and Phase II trial NCT02584634 </div>
<b>TP53</b> p.G514V VAF: 51%		<div> <div>    </div> Olaparib + AZD1775 under investigation in Phase II trial NCT02576444 </div>

CLINICALLY RELEVANT RESULTS		
Variants of strong clinical significance (predictive, prognostic, diagnostic) based on:	<div> <div>FDA-approved therapy in tumor type, or professional guidelines</div> or <div>Well-powered studies with consensus of experts</div> </div>	
Variants of potential clinical significance (predictive, prognostic, diagnostic) based on:	<div> <div>FDA-approved therapy in other tumor type, investigational therapies, or multiple small studies</div> or <div>Preclinical studies or case reports</div> </div>	
<div> <div>EML4/ALK fusion</div> </div>	<p>Interpretation: AMP Somatic Variant Classification: <i>EML4-ALK</i> rearrangement is classified at Tier 1 based on Level A evidence.</p> <p><i>EML4</i> is involved in microtubule formation (RefSeq, Jan 2015). <i>ALK</i> (Anaplastic Lymphoma Kinase) encodes a receptor tyrosine kinase belonging to the insulin receptor superfamily. <i>ALK</i> is found to be rearranged, mutated, or amplified in a series of tumors including anaplastic large cell lymphomas, neuroblastoma, and NSCLC (RefSeq, Jan 2011). Abnormal fusion of parts of <i>EML4</i> gene with portions of <i>ALK</i> gene generates <i>EML4/ALK</i> fusion transcripts, one of the primary mutations associated with NSCLC (RefSeq, Jan 2015).</p> <p>An <i>EML4/ALK</i> fusion is identified from RNA isolated from this patient's tumor. The protein contains the N-terminal half of <i>EML4</i> and the intracellular catalytic domain of <i>ALK</i>. The known genomic event resulting in this fusion occurs through a paracentric inversion (PMID: 26579422). Translocation leads to activation of oncogenic signaling through multiple pathways such as PI3K/AKT, JAK/STAT, and MEK/ERK (PMID: 26872581). <i>EML4-ALK</i> gene rearrangements are most widely reported in NSCLC (COSMIC, January 2018; PMID: 26579422). In NSCLC, <i>ALK</i> fusions are biomarkers that predict benefit from targeted therapy such as the <i>ALK</i> kinase inhibitors crizotinib or ceritinib (NCCN, NSCLC, v2.2018).</p>	

"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..."

"It is desirable to include graphs, charts, and tables to increase the overall clarity of the report."

"...reports should be short, simple, and to the point."



# Clinical Interpretation and Reporting

## Other Elements

“...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...”

J Mol Diagn. 2017 Jan;19(1):4-23.

Genome build

DOE, JOHAN  
Accession #: ALKescape

CLINICALLY RELEVANT RESULTS, continued

Variants of strong clinical significance  
(predictive, prognostic, diagnostic) based on:

Variants of potential clinical significance  
(predictive, prognostic, diagnostic) based on:

FDA-approved therapy in tumor type,  
or professional guidelines

FDA-approved therapy in other tumor type,  
investigational therapies, or multiple small studies

or

Well-powered studies with  
consensus of experts

Preclinical studies  
or case reports

**ALK**  
p.G1202R

Interpretation: AMP Somatic Variant Classification: ALK p.G1202R is classified at Tier 2 based on level C evidence.

ALK (Anaplastic Lymphoma Kinase) encodes a receptor tyrosine kinase that belongs to the insulin receptor superfamily and activates the mitogen-activated protein kinase (MAPK) pathway (RefSeq, Jan 2011, Uniprot.org).

A missense substitution, ALK G1202R, was identified in the specimen. Codon G1202 lies in the protein kinase domain of ALK (Uniprot.org) and is postulated to be on the solvent-exposed region of the ALK kinase domain adjoining the crizotinib-binding site. This variant has been demonstrated to confer resistance to crizotinib in vitro in cells harboring an EML4/ALK fusion (PMID: 22277784). ALK G1202R has been reported in lung adenocarcinoma including NSCLC (COSMIC, Feb 2018). This variant has been reported as a secondary mutation in ALK+ NSCLC patients (PMID: 22277784, 28122866).

In patients with ALK-rearranged NSCLC, the ALK G1202R alteration has been reported to confer high-level resistance to crizotinib (PMID: 22277784) as well as to second-generation ALK inhibitors (ceritinib, alectinib, and brigatinib) (PMID: 28122866). Moreover, this mutation is susceptible to the third-generation inhibitor lorlatinib (PMID: 26144315, 27197608, 27432227). Lorlatinib is under investigation in clinical trials for ALK-positive NSCLC patients (NCT03052608, phase 3; NCT02584634, phase 2).

**TP53**  
p.G154V

Interpretation: AMP Somatic Variant Classification: TP53 p.G154V is classified at Tier 2 based on level C evidence.

TP53 is a tumor suppressor and regulates expression of target genes, by inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism (PMID: 20182602).

The TP53 missense substitution G154V was identified in this specimen. Codon G154 lies within the DNA binding domain of the TP53 protein (exon 6, Uniprot.org). It is predicted to impair the normal function of the protein (classified as non-functional and deleterious on the IARC TP53 database, Jan 2018). This variant has been reported in multiple tumor types including NSCLC (COSMIC, Apr 2018). A meta-analysis in lung cancer patients suggested that TP53 mutations are associated with smoking-induced lung cancer (PMID: 24126199). TP53 mutations have been reported to be a negative predictor of the outcome of lung adenocarcinoma patients, and NSCLC patients with TP53 alterations may be relatively more resistant to chemotherapy and radiation (PMID: 28240049, 26669115, 26647728, 21331359, 2011). In Asian patients with NSCLC, TP53 mutations were found to be enriched in patients harboring metastases (PMID: 29547728).

Olaparib in combination with AZD1775 (WEE1 inhibitor) is in phase II clinical trial to determine tumor overall response rate in patients with advanced solid tumors harboring TP53 mutations (NCT02576444). A p53-based vaccine (p53MVA) in combination with pembrolizumab is currently being investigated in TP53-mutation-harboring cancer patients including NSCLC (NCT02432963).

VARIANTS OF UNCERTAIN SIGNIFICANCE

**SLIT2**  
p.R1526T  
NM\_004787.1 c.4577G>C  
chr4 g.20620819G>C  
VAF: 49%

**INSR**  
p.D946E  
NM\_000208.2 c.2838C>G  
chr19 g.7132173G>C  
VAF: 44%

**INSR**  
p.V1012M  
NM\_000208.2 c.3034G>A  
chr19 g.7125518C>T  
VAF: 48%

TEST DETAILS

**Illumina TST170 Panel:** 170 genes were subjected to targeted next generation sequencing analysis. The versions/releases/builds/dates of the following databases were used to generate this report.

- Genomic Build: GRCh37.p13
- Genomic Annotation Sources: NCBI RefSeq v105
- dbNSFP: 3.0b2c
- ExAC: v1.0
- COSMIC: v83
- dbSNP: 149
- ClinVar: 20170905
- NHLBI ESP: v0.0.30

Coding exon coverage metrics: The following exons failed to achieve 250x coverage across 95% of coding exonic space:

Gene, Transcript ID (Exon)	Gene, Transcript ID (Exon)	Gene, Transcript ID (Exon)
<b>AKT3</b> NM_001206729.1 (14) NM_181690.2 (13)	<b>NOTCH2</b> NM_001200001.1 (2) NM_024408.3 (2)	<b>NTRK1</b> NM_001007792.1 (2)

Pierian

Page 2 of 4

Transcript reference sequence

Genomic coordinates

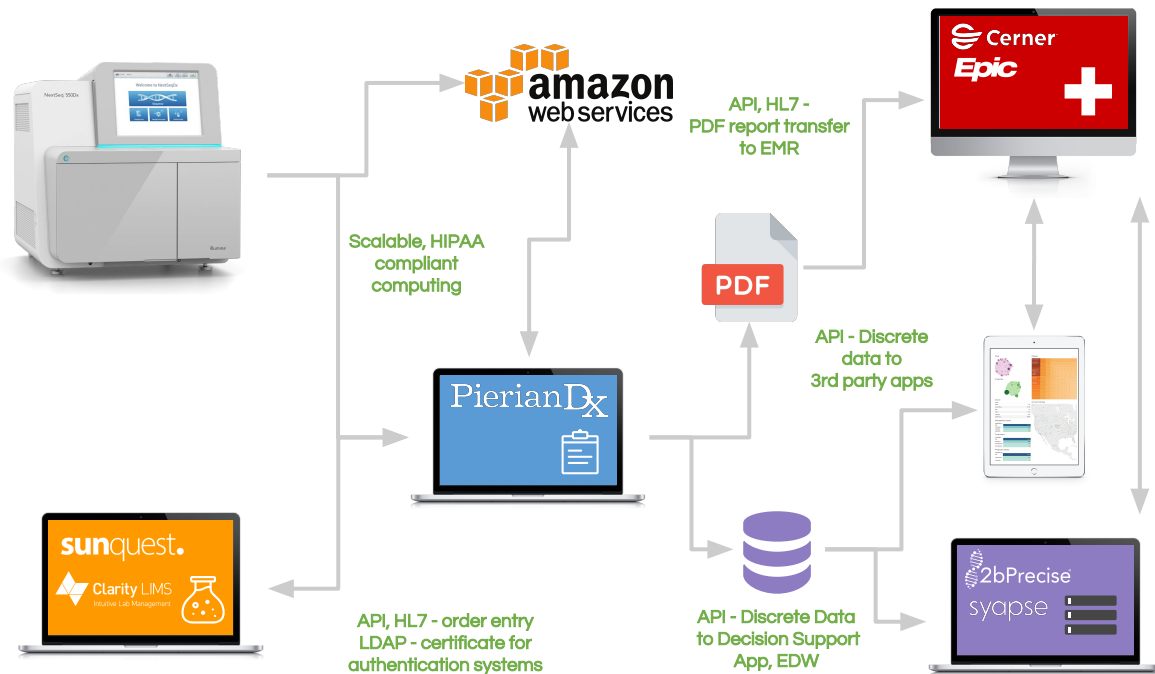
Variant allele fraction (VAF)

All genes and/or hotspots not meeting minimum required coverage should be declared



# Clinical Interpretation and Reporting

## HealthIT Integration



"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."**

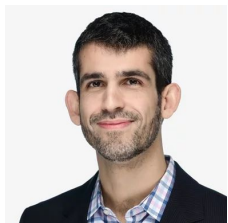
J Mol Diagn. 2017 Jan;19(1):4-23.



# Thank You Q&A



**Shalini Verma, MD, FCAP**  
Medical Director, Lab Director  
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**Andy Bredemeyer, PhD**  
Vice President Product  
andy.bredemeyer@pieriandx.com

Type questions here

A screenshot of the GoToWebinar Control Panel. The title bar says 'GoToWebinar Control Panel'. There are three tabs: 'Audio', 'Questions', and 'View audio options'. The 'Questions' tab is selected. Below the tabs is a large empty text area for questions. At the bottom of the panel, there is a 'Send' button with a checkmark icon. Below the 'Send' button, it says 'Sample Webinar' and 'Webinar ID# 573-646-403'. At the very bottom is the GoToWebinar logo.



Let's Work Together

# The Pierian Partnership



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150+ unique panels deployed

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60+ medical and scientific experts

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