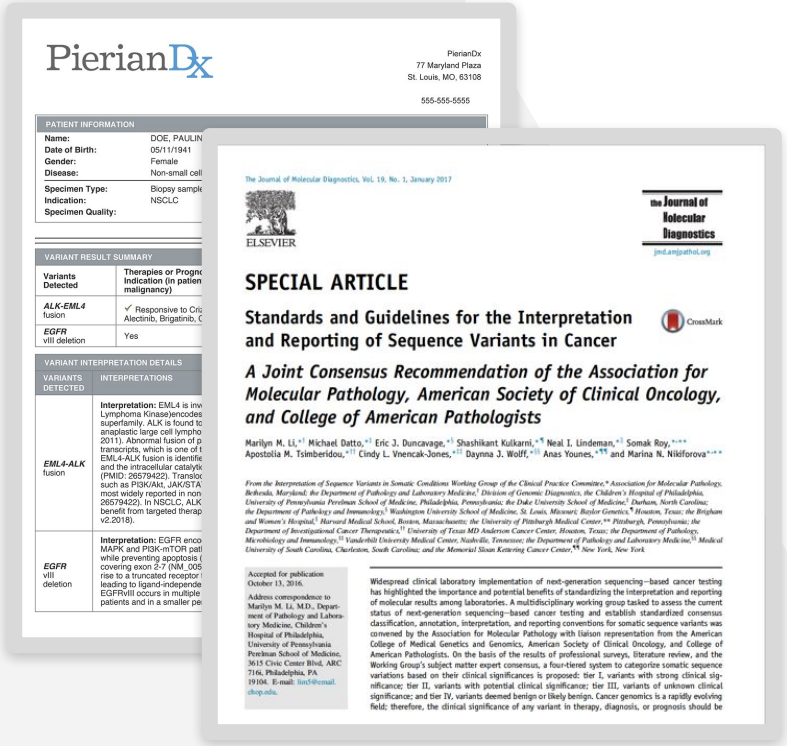
PierianDx

Interpretation and Reporting of Sequence Variants in Cancer

A Practice-Based Guide

July 19, 2018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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 patients with malignancy)
ALK-EML4 fusion	✓ Responsive to Crizotinib, Alectinib, Brigatinib, C
EGFR vIII deletion	Yes

VARIANT INTERPRETATION DETAILS

VARIANTS DETECTED	INTERPRETATIONS
EML4-ALK fusion	**Interpretation:** EML4 is inv Lymphoma Kinase)encodes superfamily. ALK is found to anaplastic large cell lympho 2011). Abnormal fusion of p transcripts, which is one of t EML4-ALK fusion is identifie and the intracellular catalyti (PMID: 26579422). Transloc such as PI3K/Akt, JAK/STA most widely reported in non- 26579422). In NSCLC, ALK benefit from targeted therap v2.2018).
EGFR vIII deletion	**Interpretation:** EGFR enco MAPK and PI3K-mTOR pat while preventing apoptosis (covering exon 2-7 (NM_005 rise to a truncated receptor leading to ligand-independe EGFRvIII occurs in multiple patients and in a smaller pe

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ELSEVIER

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jmd.amjpathol.org

SPECIAL ARTICLE

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

CrossMark

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

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From the Interpretation of Sequence Variants in Somatic Conditions Working Group of the Clinical Practice Committee, Association for Molecular Pathology, Bethesda, Maryland; the Department of Pathology and Laboratory Medicine,† Division of Genomic Diagnostics, the Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; the Duke University School of Medicine,‡ Durham, North Carolina; the Department of Pathology and Immunology,§ Washington University School of Medicine, St. Louis, Missouri; Baylor Genetics,¶ Houston, Texas; the Brigham and Women's Hospital,‖ Harvard Medical School, Boston, Massachusetts; the University of Pittsburgh Medical Center,** Pittsburgh, Pennsylvania; the Department of Investigational Cancer Therapeutics,†† University of Texas MD Anderson Cancer Center, Houston, Texas; the Department of Pathology, Microbiology and Immunology,‡‡ Vanderbilt University Medical Center, Nashville, Tennessee; the Department of Pathology and Laboratory Medicine,§§ Medical University of South Carolina, Charleston, South Carolina; and the Memorial Sloan Kettering Cancer Center,¶¶ New York, New York*

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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 classification, 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 significances 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

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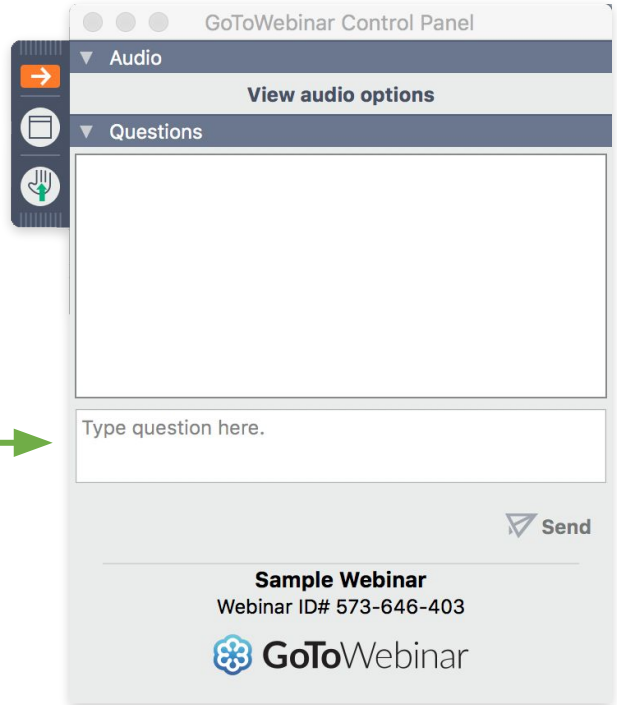
We Want to Hear from You!

How to Submit Questions

Type questions here



Pioneers in Precision Medicine

Leaders in Clinical Genomics

PierianDx

Today

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

2014

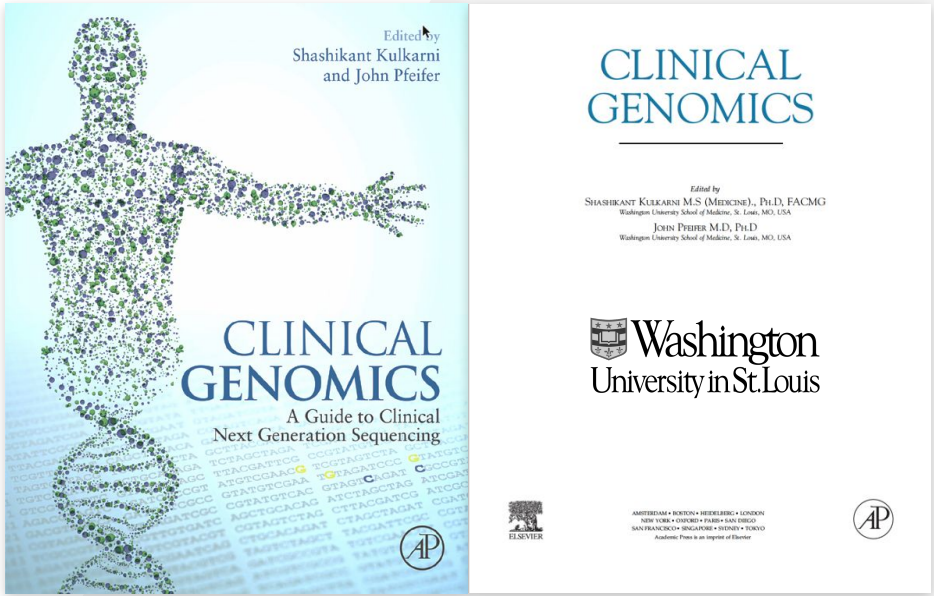
- PierianDx established after ~50 labs visit WashU to learn how clinical NGS is operationalized.

2011

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

2003

- WashU plays critical role in Human Genome Project.



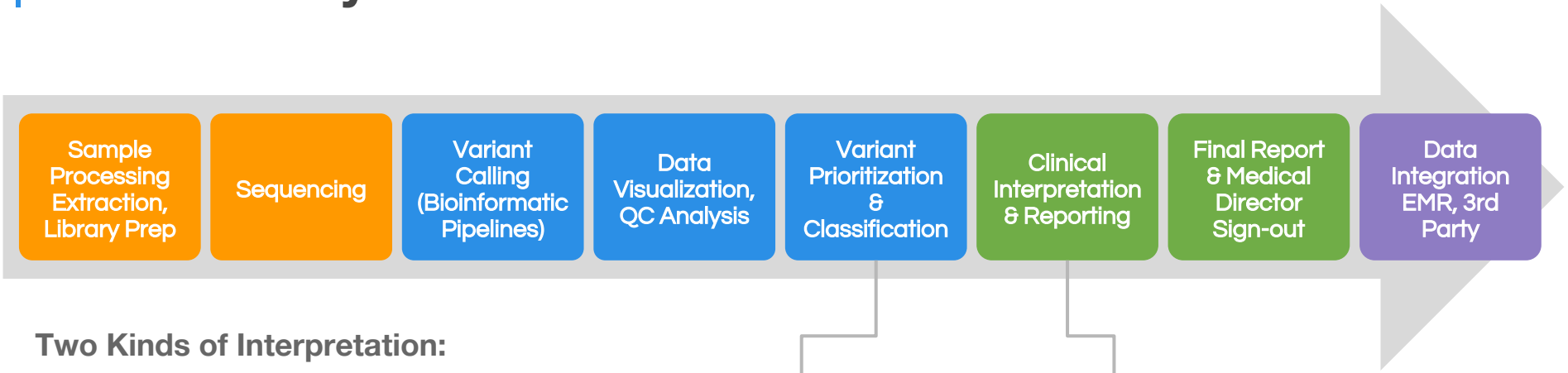
PierianDx founders among first to validate and clinically report on somatic cancer NGS panels.

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, 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.

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"

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

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

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 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

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

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

Evidenced Based Categorization

Application and Reporting

Application of Evidenced Based Categorization (somatic)

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.

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

Additional Guidance

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.

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

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.

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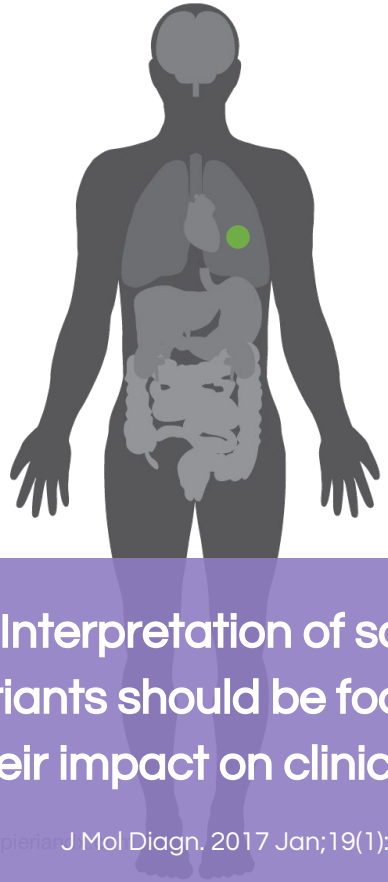
Clinical Interpretation of Somatic Variants

Example - Tier III (VUS)



Age: 57

Gender: Male

Indication: Non-Small Cell Lung Cancer

NGS testing revealed *MET* variant: chr7:g.116411876_116411887del12

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

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

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

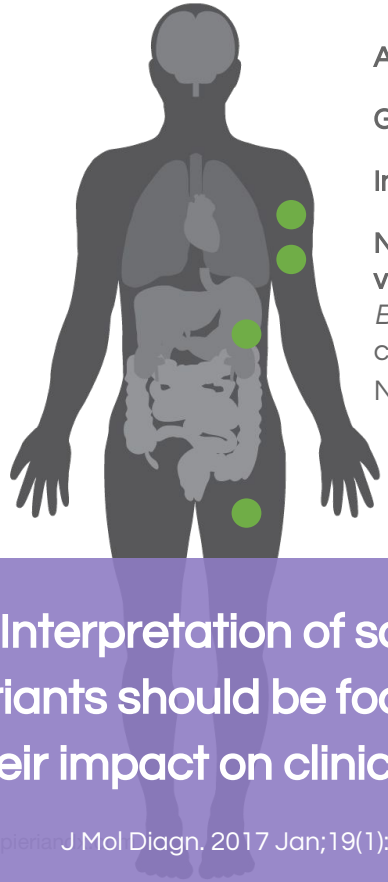
Clinical Impact: No clinical significance info available for this variant

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

Clinical Interpretation of Somatic Variants

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"

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

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

Additional Considerations

Polymorphisms

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)

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

Additional Considerations

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.

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

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.**

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.

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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.

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

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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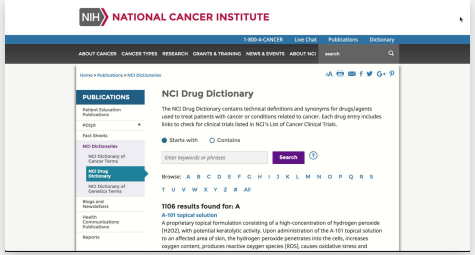
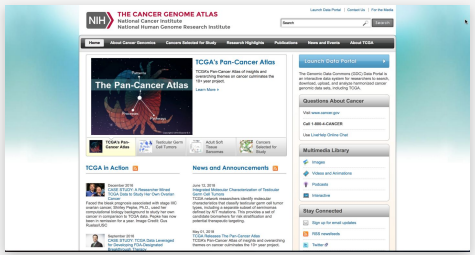
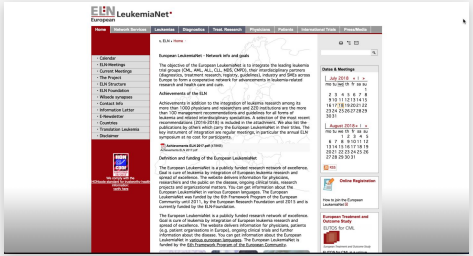
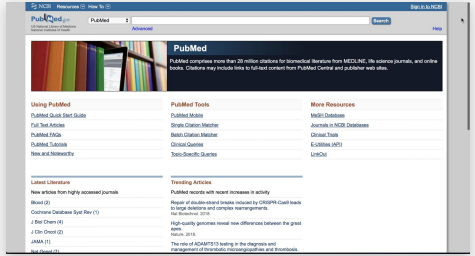
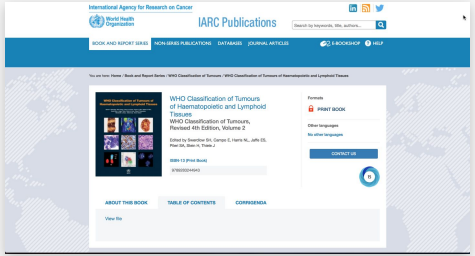
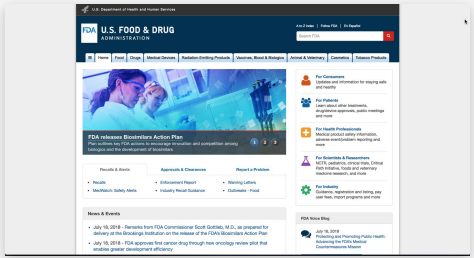


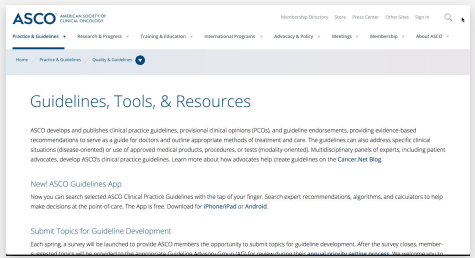


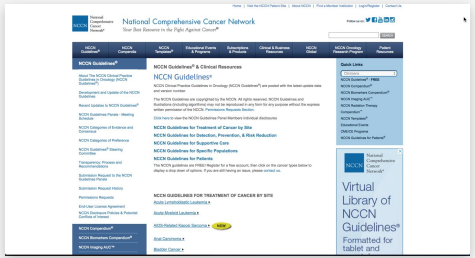


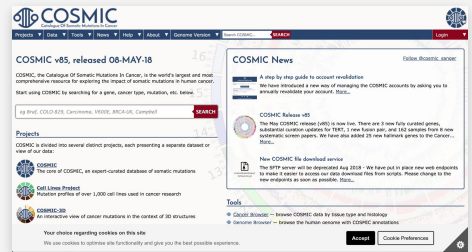


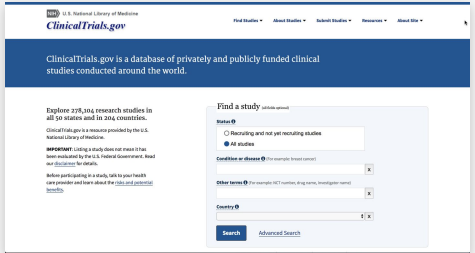


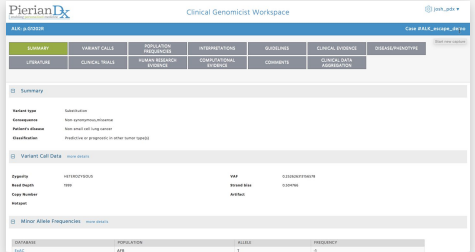












Additional Considerations

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.

MOLECULAR ONCOLOGY 8 (2014) 859 - 873

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:

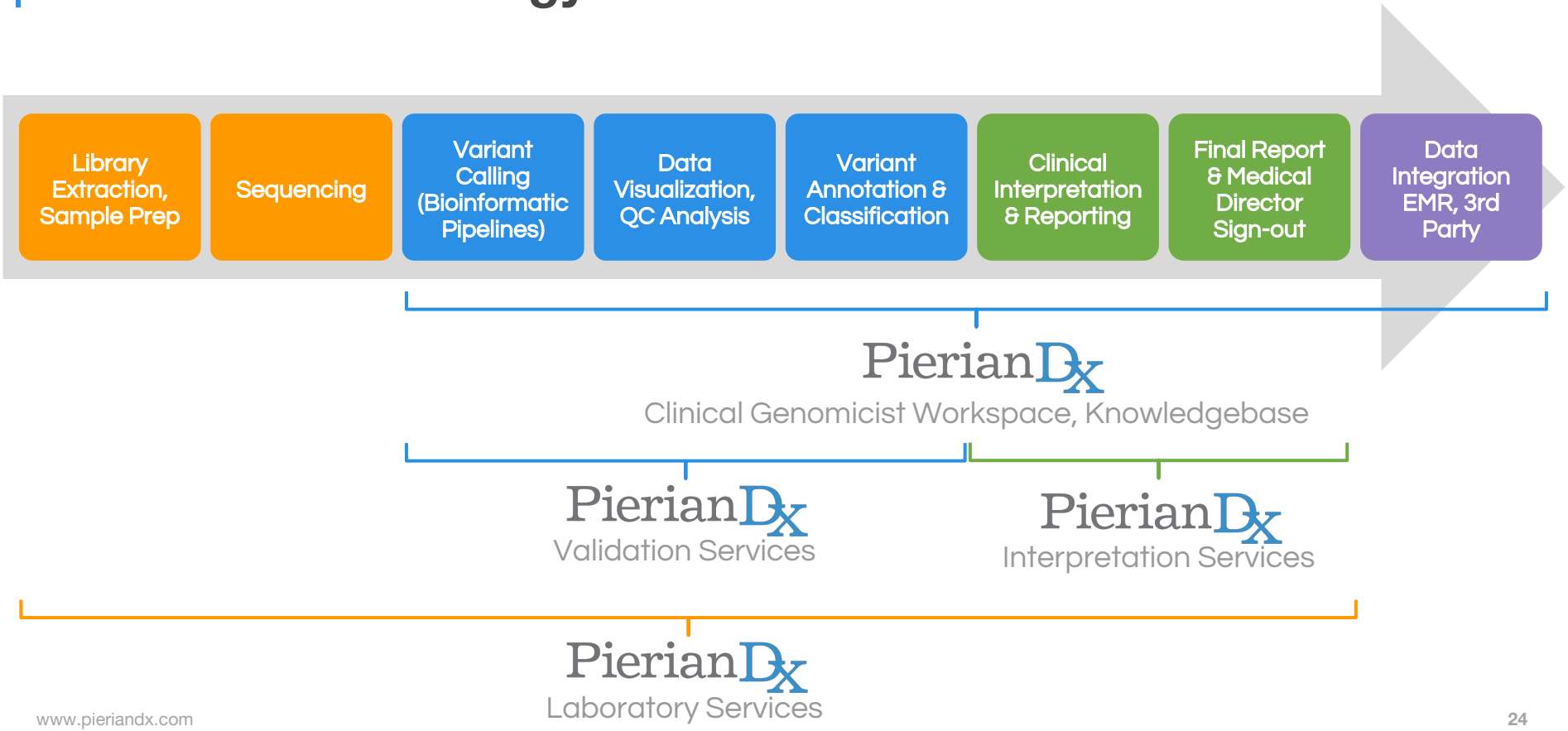
- Previously classified and interpreted variants
- Curated annotations on the most frequent and relevant variants in multiple tumor types, based on the publicly available resources (population and variant databases)
- Is designed to accommodate new clinical and preclinical data
- Is routinely updated 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

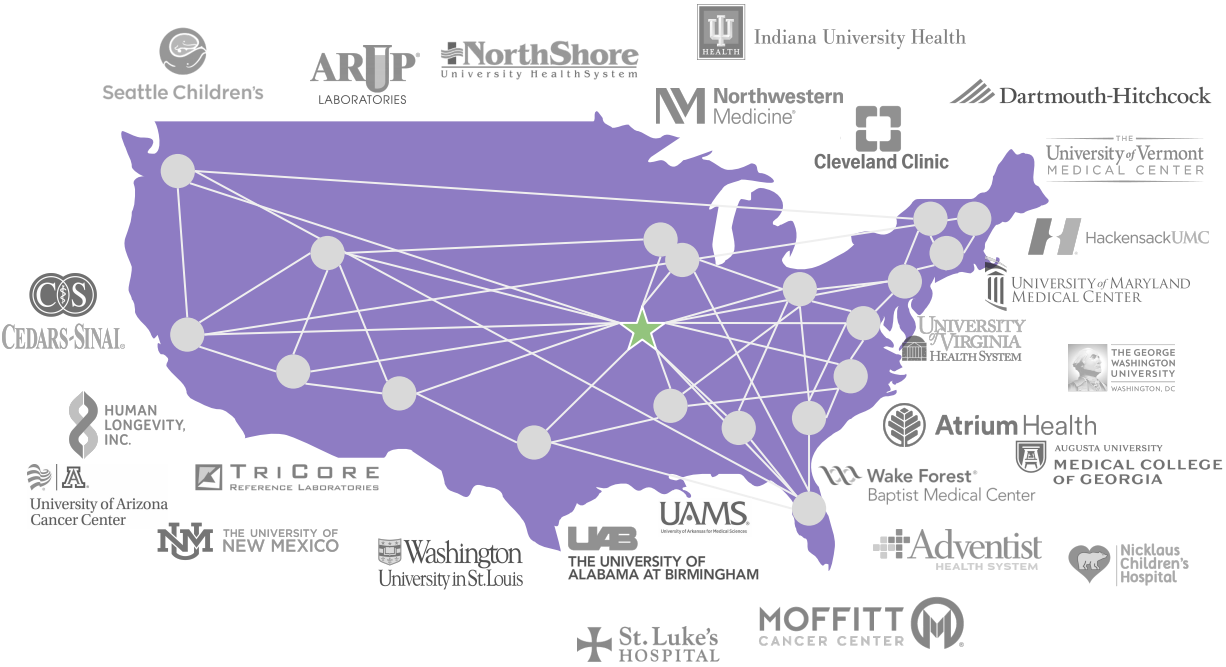
Complete Clinical Genomics Support

PierianDx Technology and Services



Creating the Largest, Peer Shared Interpretation System

More Rapid, Accurate Interpretation

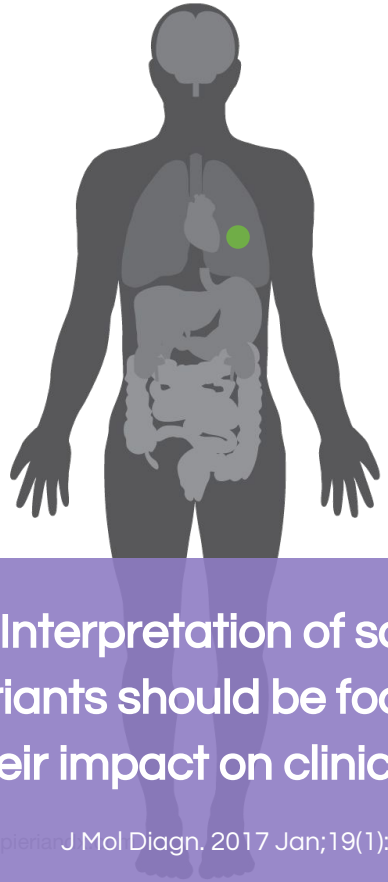


Most clinically robust, up-to-date knowledgebase

- 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

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

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

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

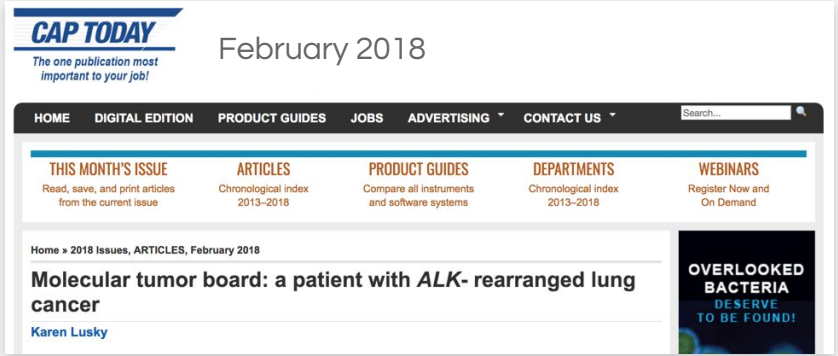
Initial assessment

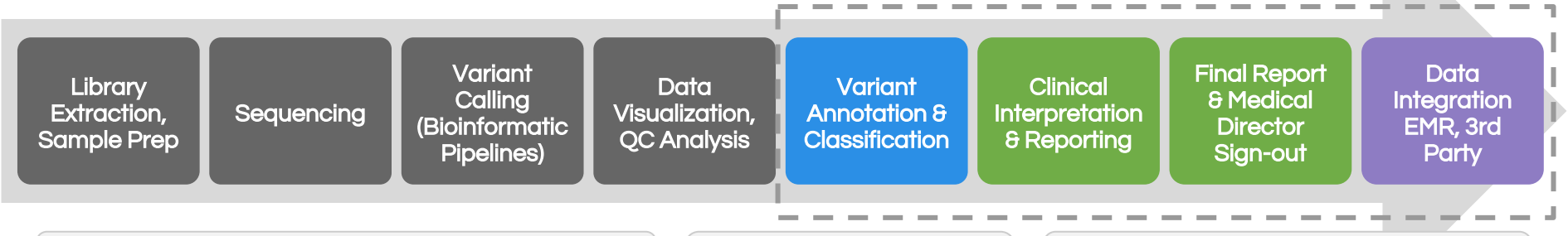
Selected variants identified by NGS:

- 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





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

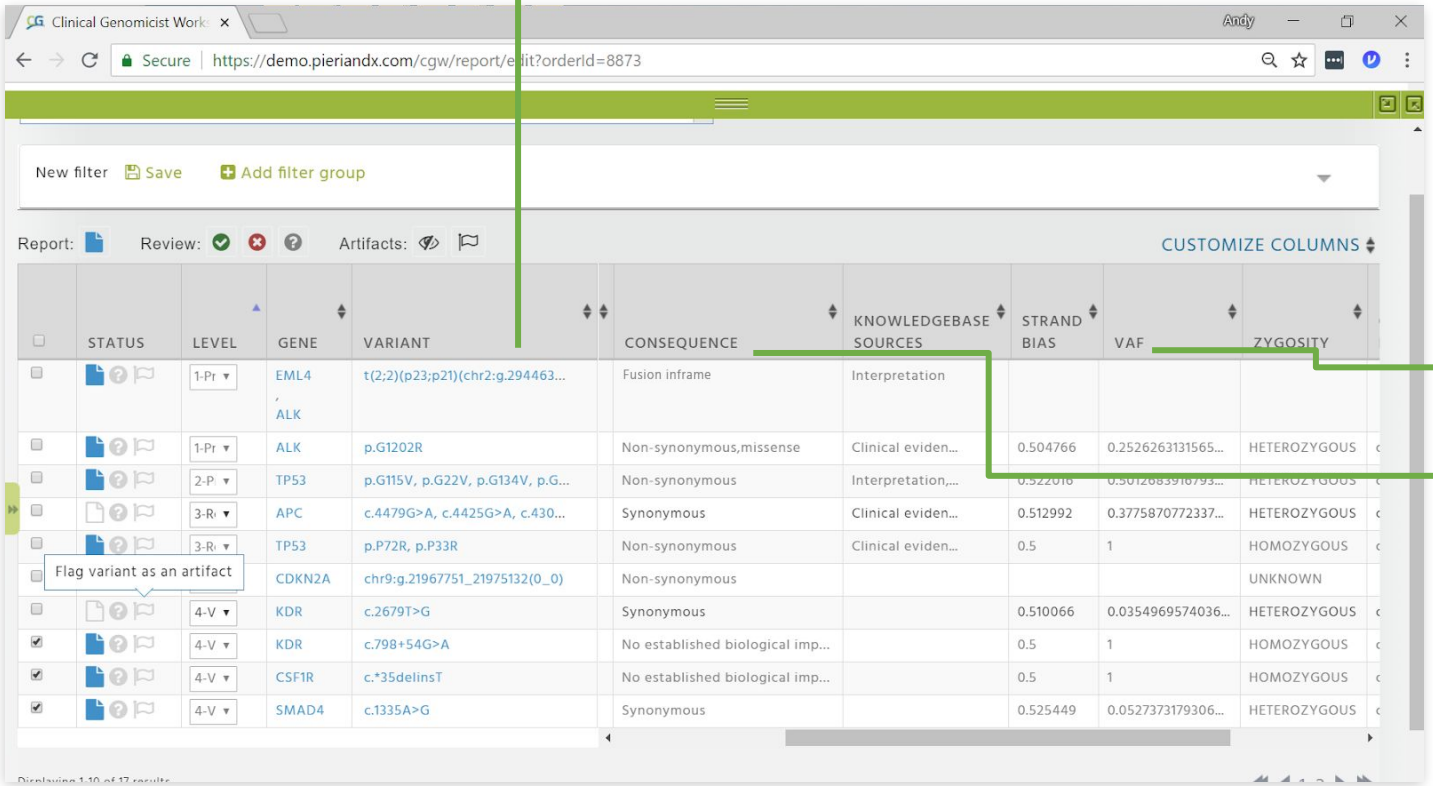
Level B: Well Powered studies with consensus from experts in the field

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

Level D: Preclinical studies, small studies or a few case reports without consensus

Variant Annotation and Classification

Variant Table

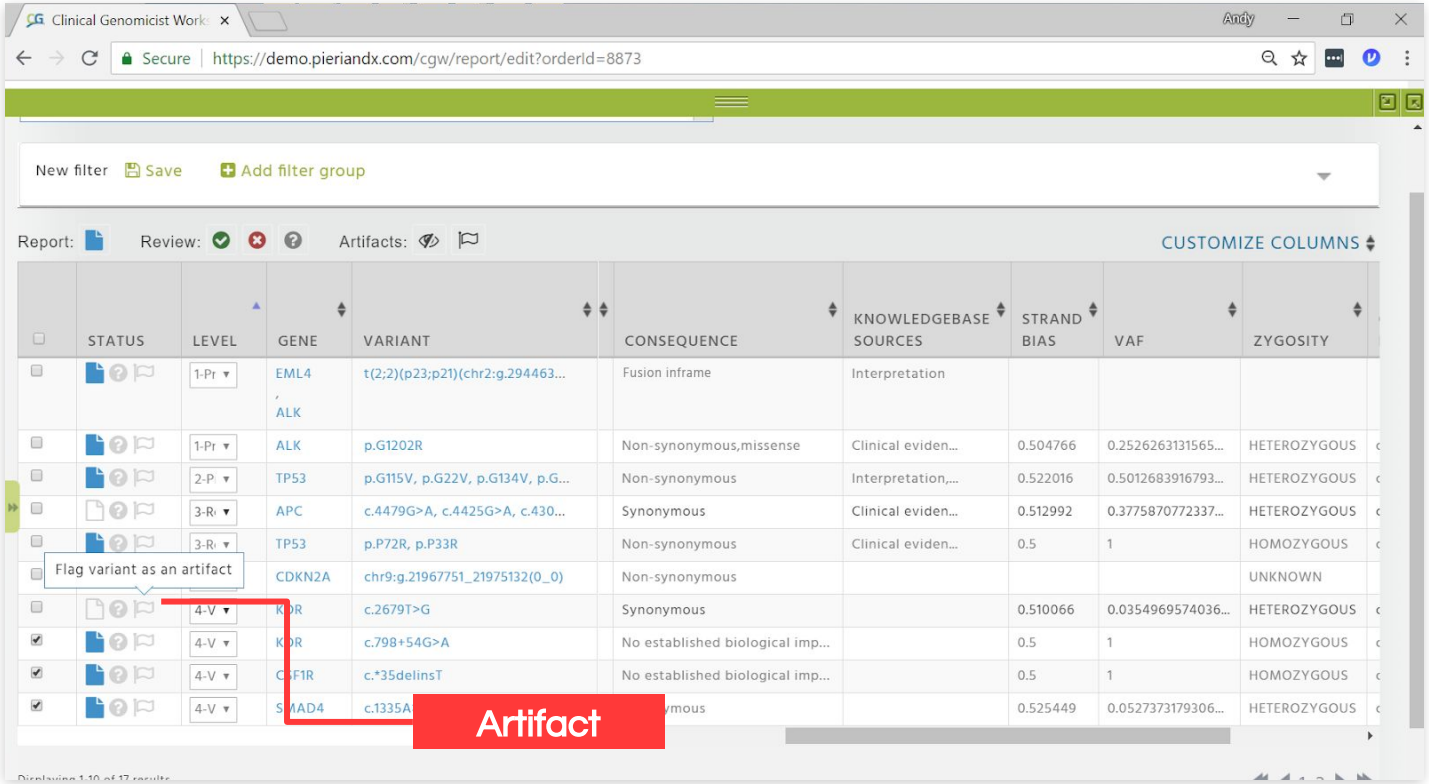


"Certain metrics for called variants are **critical for variant interpretation, such as supporting reads (depth of coverage) and variant allele frequency (VAF)**, and should be included in variant evaluation..."

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

Variant Annotation and Classification

Variant Table



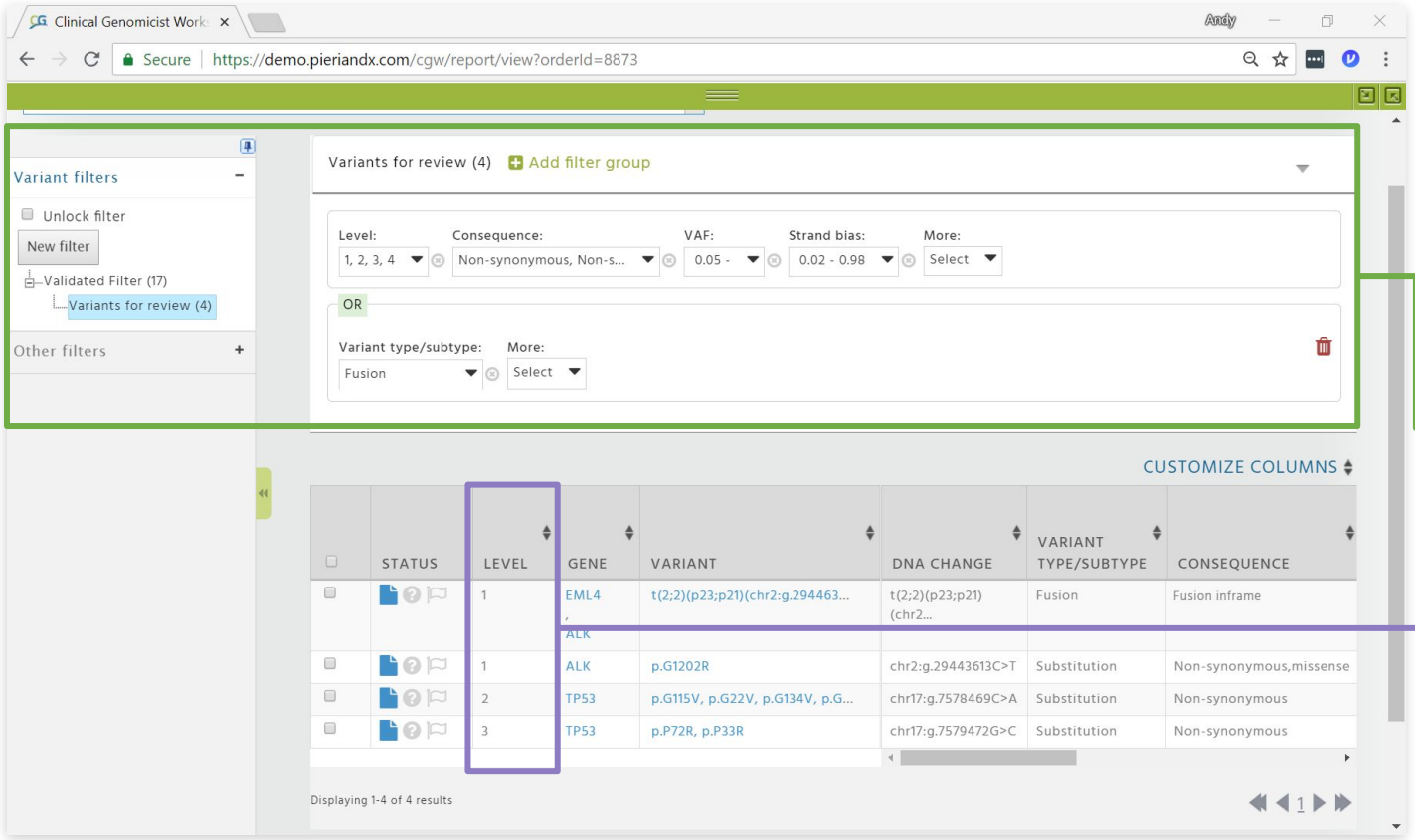
"It is important to emphasize that **clinical laboratories should establish a well-annotated in-house database** for both tracking variants identified within the laboratory and to provide consistent annotations."

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

- Identify false-positives
- Establish frequencies of mutations

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

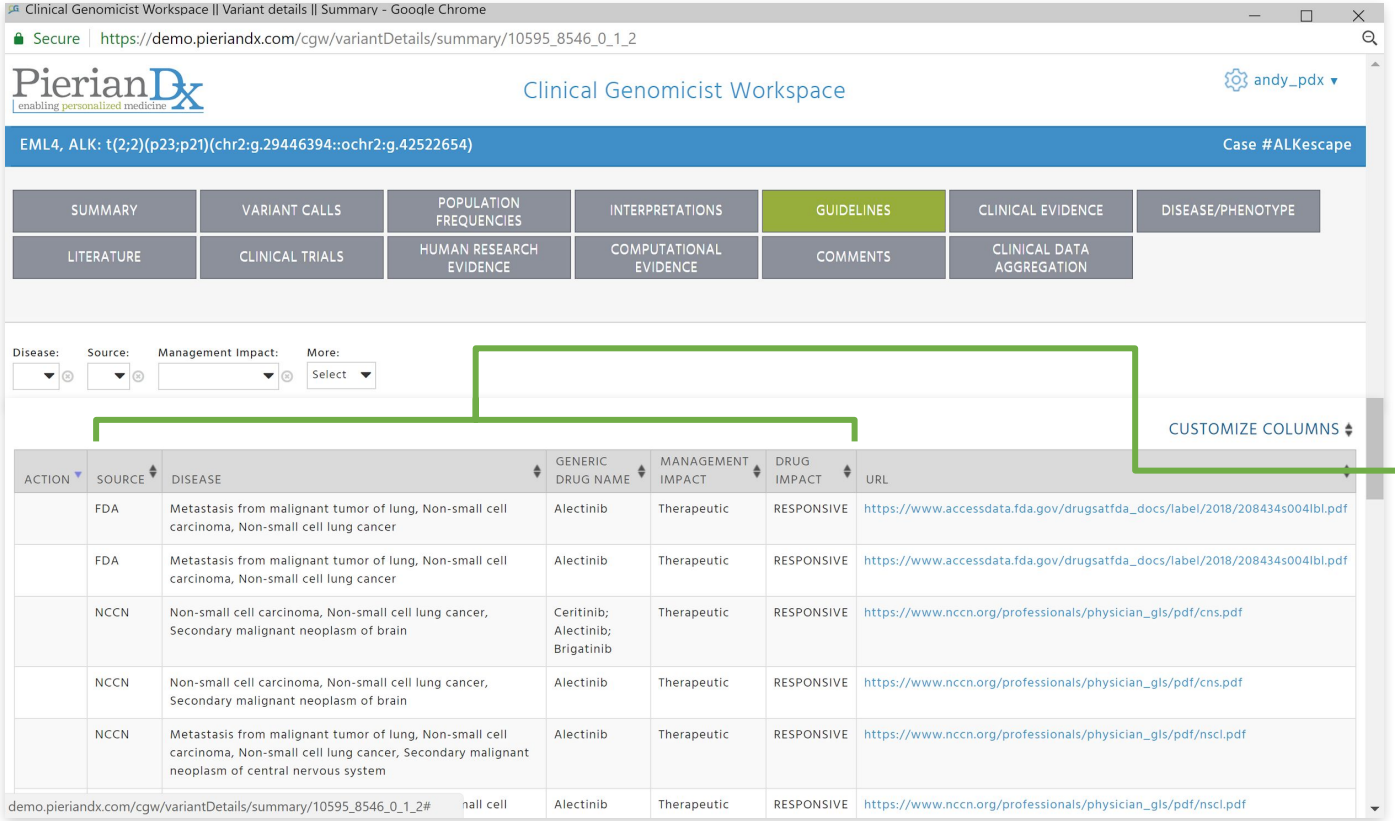
Variant Annotation and Classification

Clinical Practice Guidelines

"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.

EML4/ALK Fusion

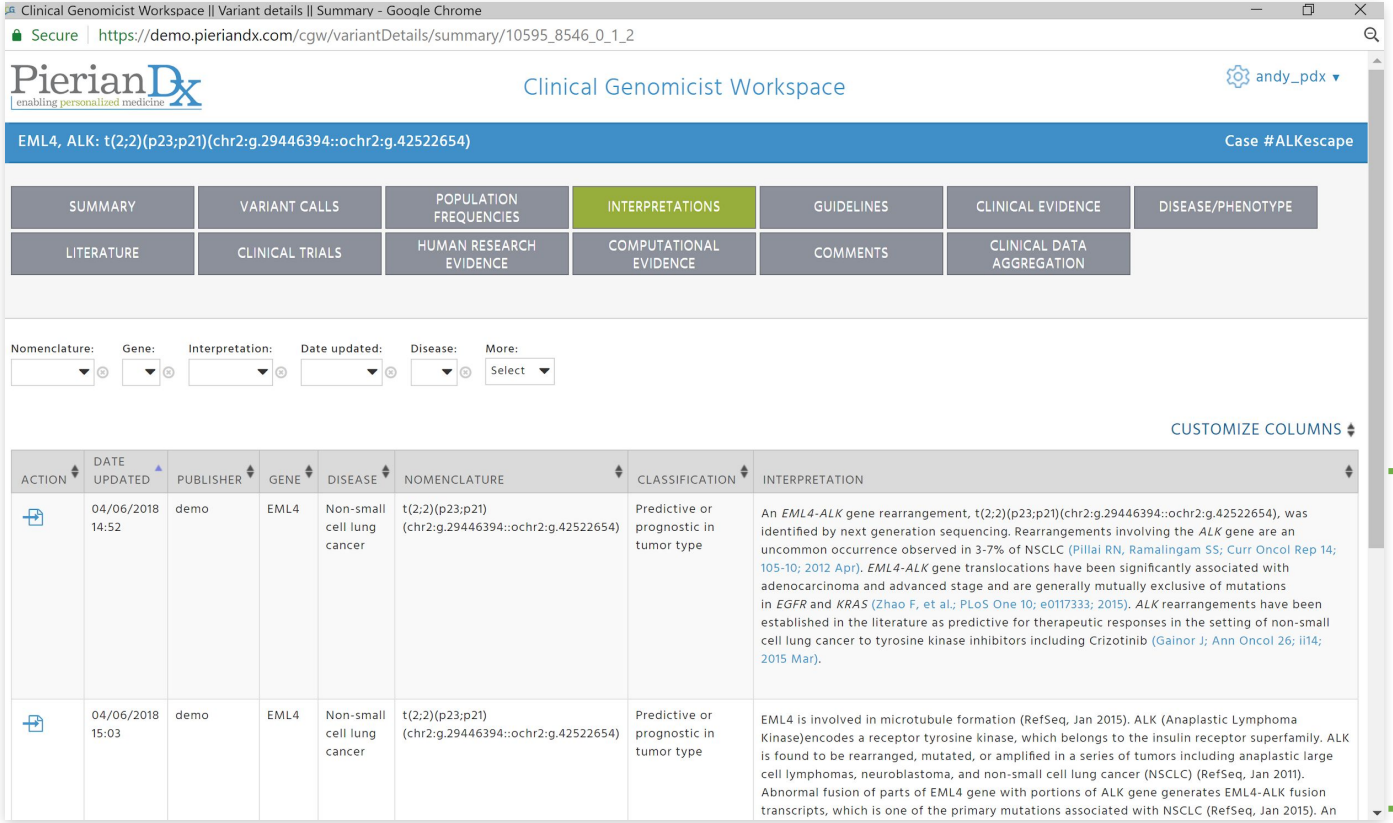


For recognizable recurrent variants, get:

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

Clinical Interpretations

EML4/ALK Fusion



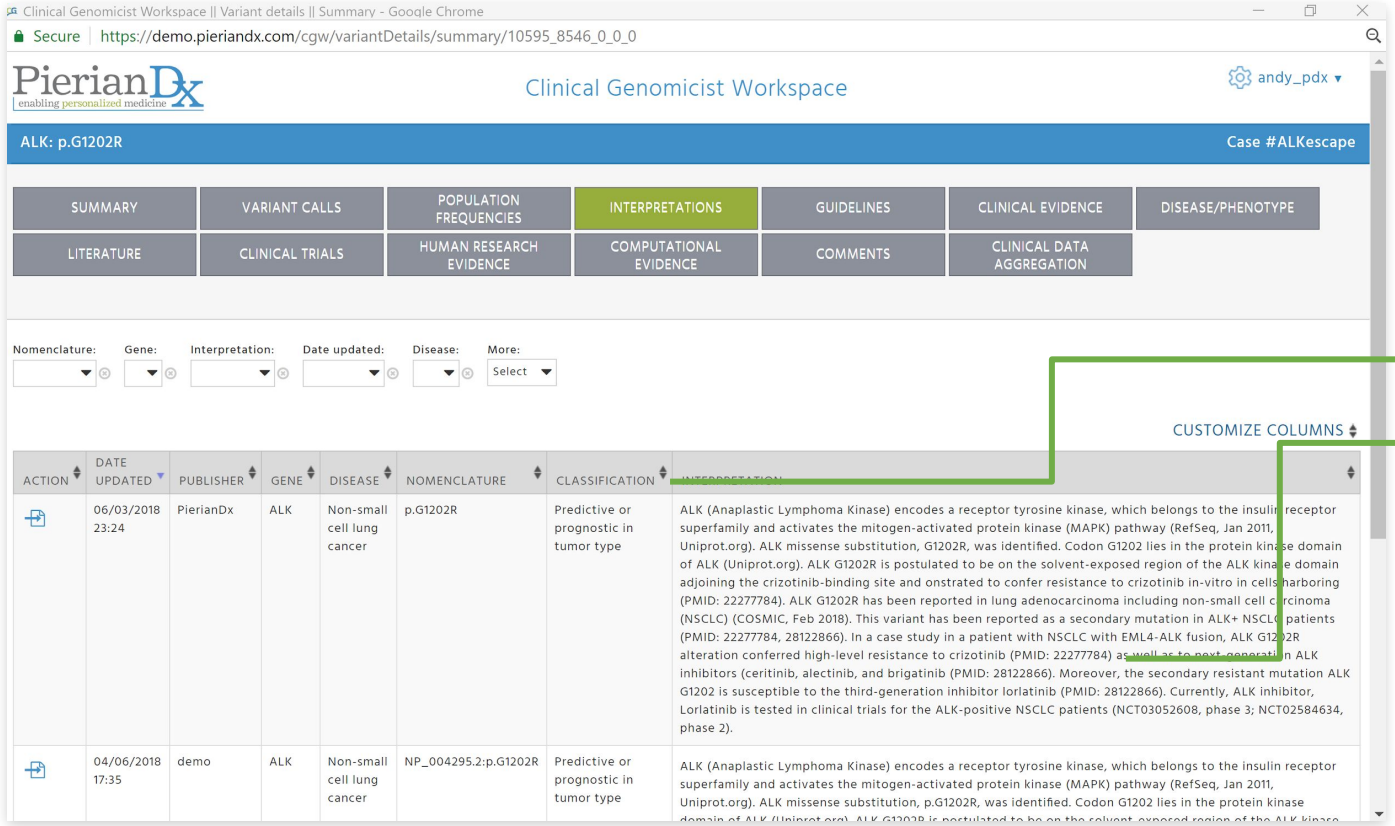
"**We strongly encourage somatic variant data sharing** ...to facilitate accurate interpretation of somatic variants."

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

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

Clinical Interpretations

ALK p.G1202R

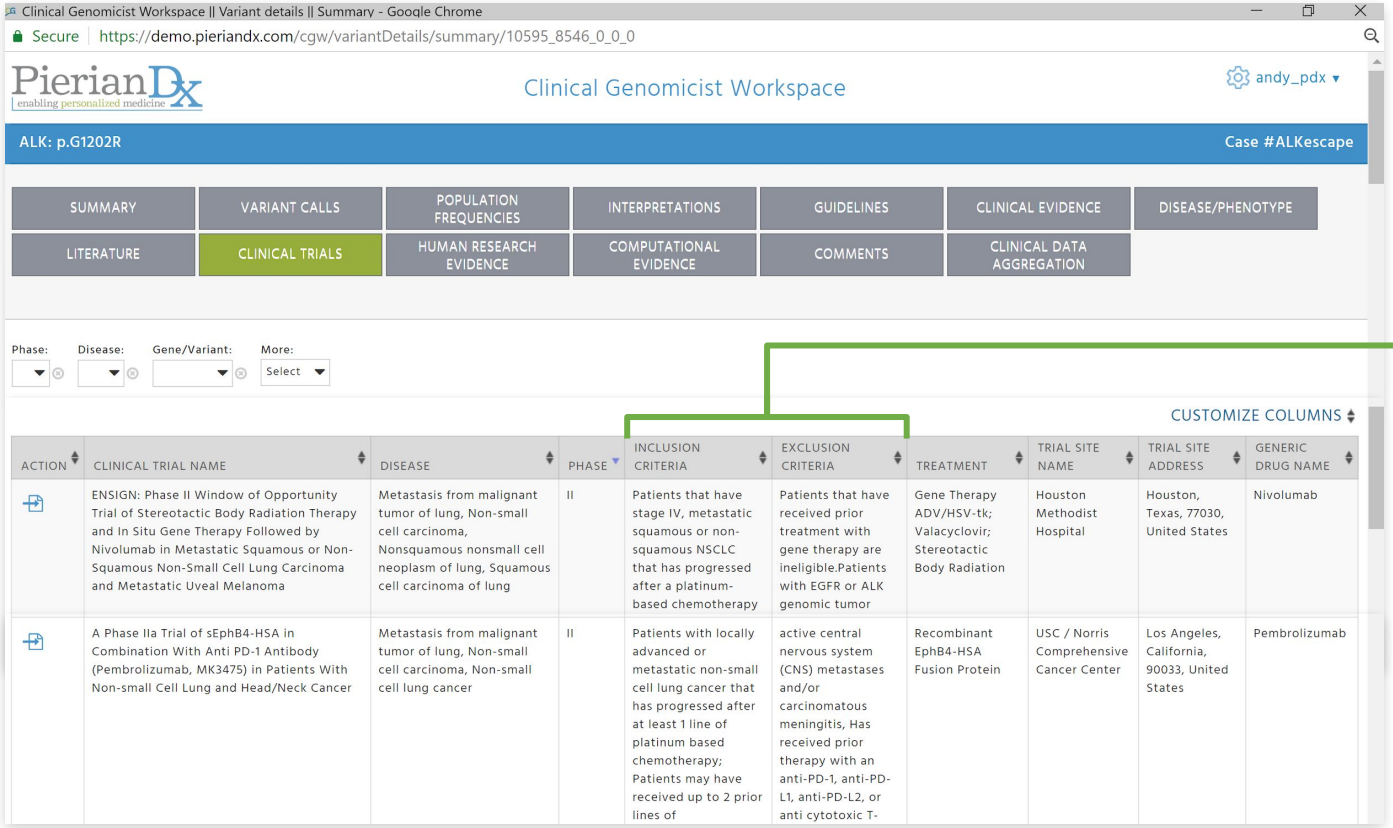


For less well characterized recurrent variants **without practice guidelines**, interpretations help:

- Auto-classify
- Point to relevant literature and trials
- Kick start your report editing

Clinical Trials

ALK p.G1202R



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 nonsmall 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

"**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."

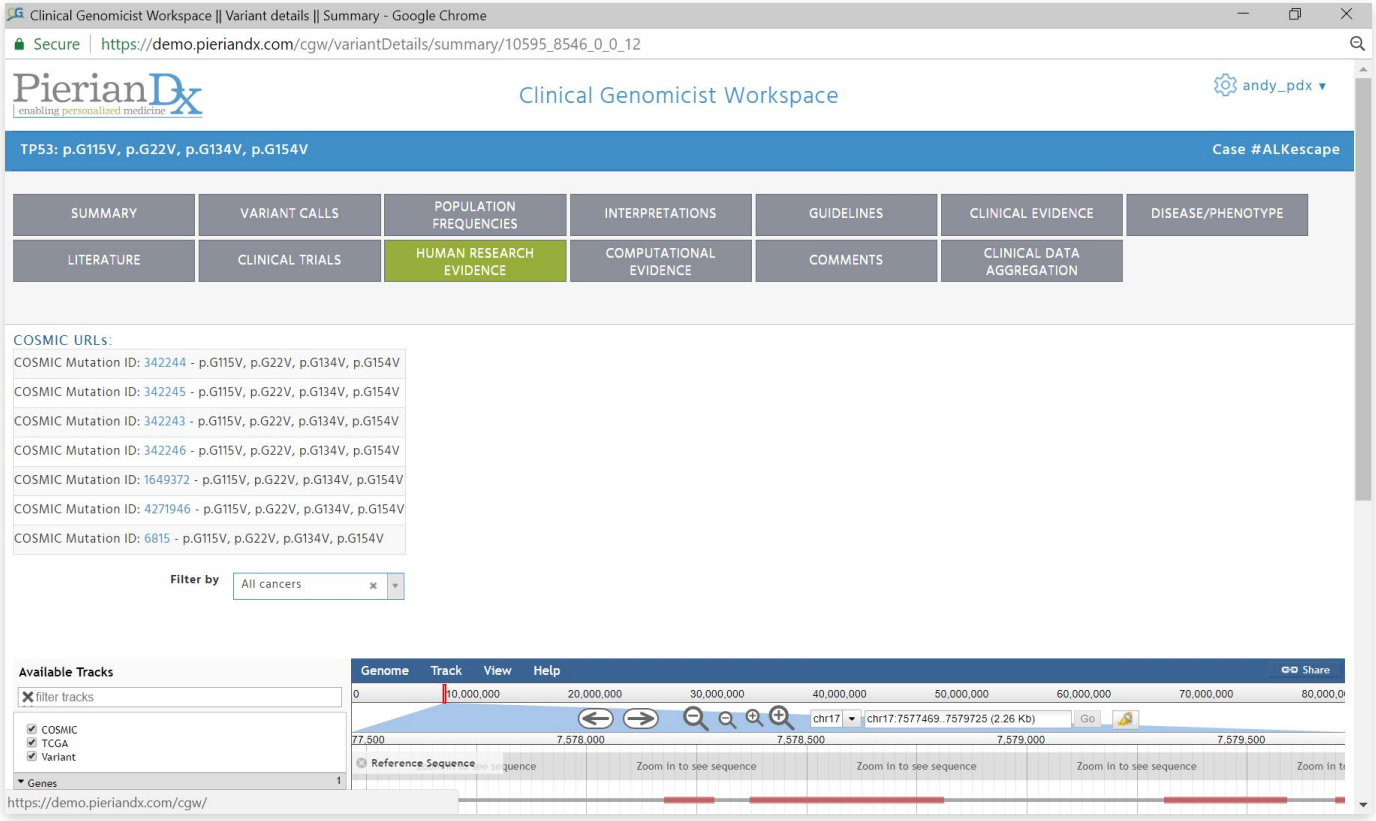
J Mol Diagn. 2017 Jan;19(1):4-23.

Assess trial matches for consideration of Tier 2C classification evidence

Variant Annotation and Classification

Human Research Evidence

ALK p.G1202R



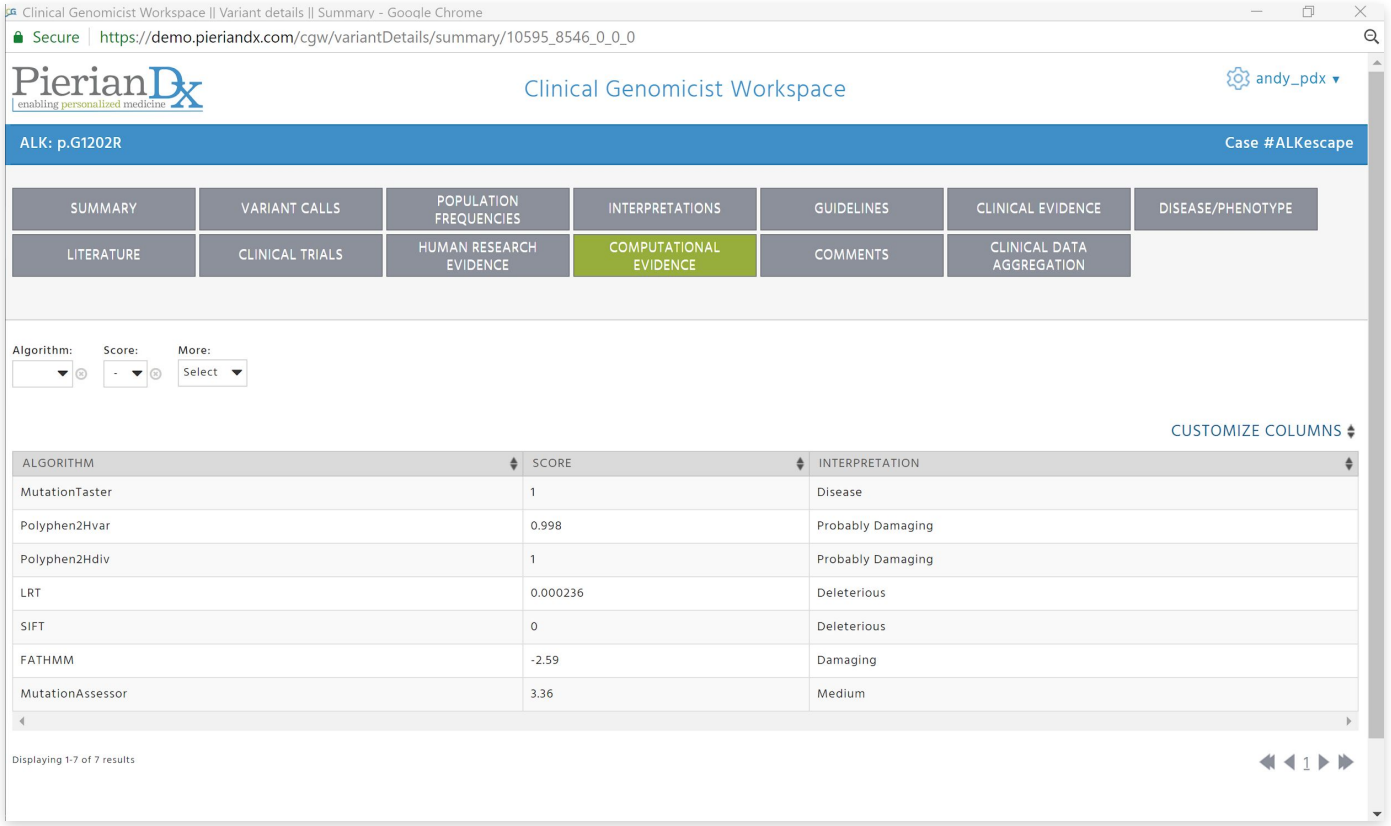
"[Cancer specific] databases provide information about the incidence and prevalence of sequence variants across the spectrum of different cancers and subtypes"

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

Variant Annotation and Classification

Computational Evidence

ALK p.G1202R



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

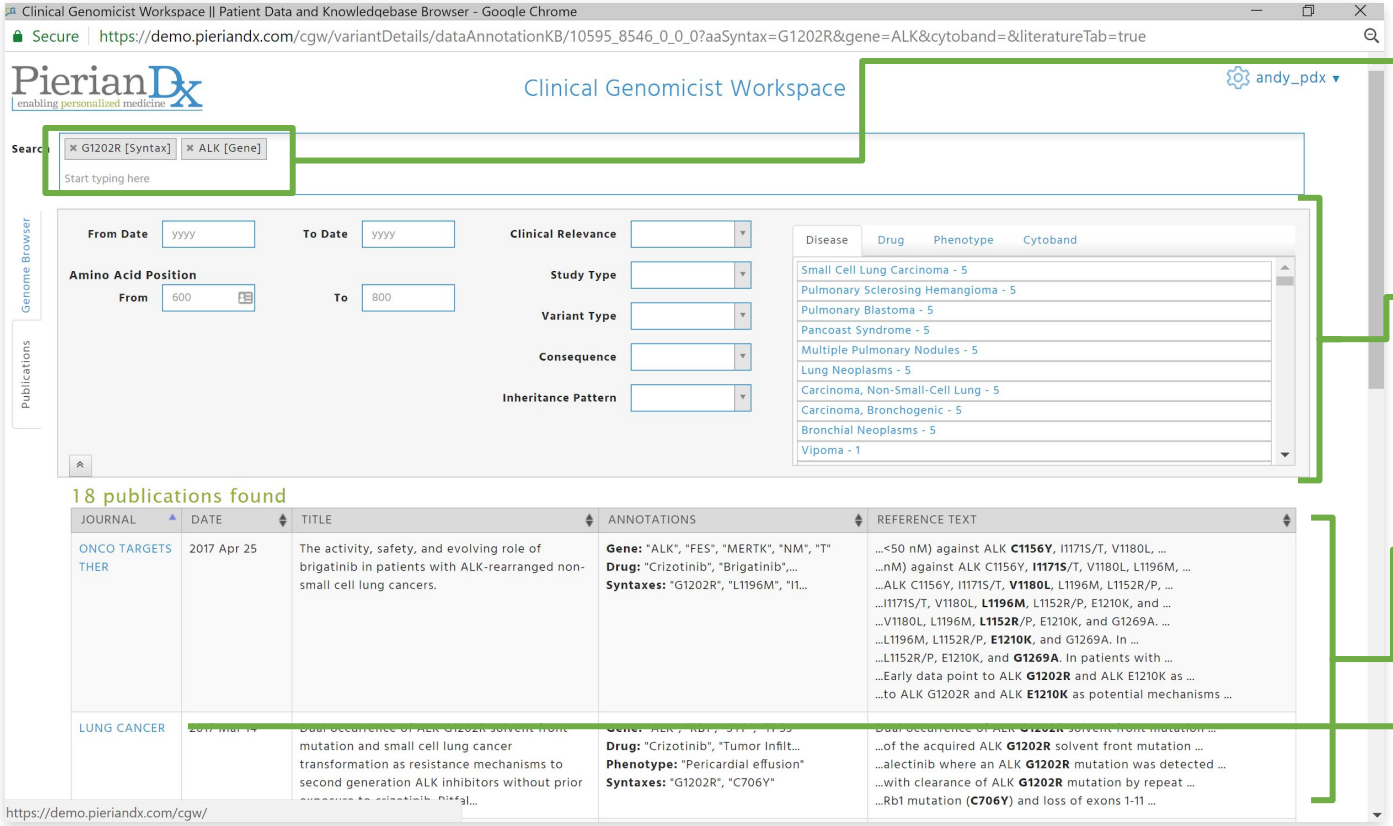
"The interpretation of these predictions in the the context of cancer gene function is usually not straightforward, especially for activating mutations. … **Clinical laboratories should exercise caution when interpreting *in silico* scores.**"

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

Use with caution

Literature Search

ALK p.G1202R



Kickstart literature search directly from the Variant Details Page

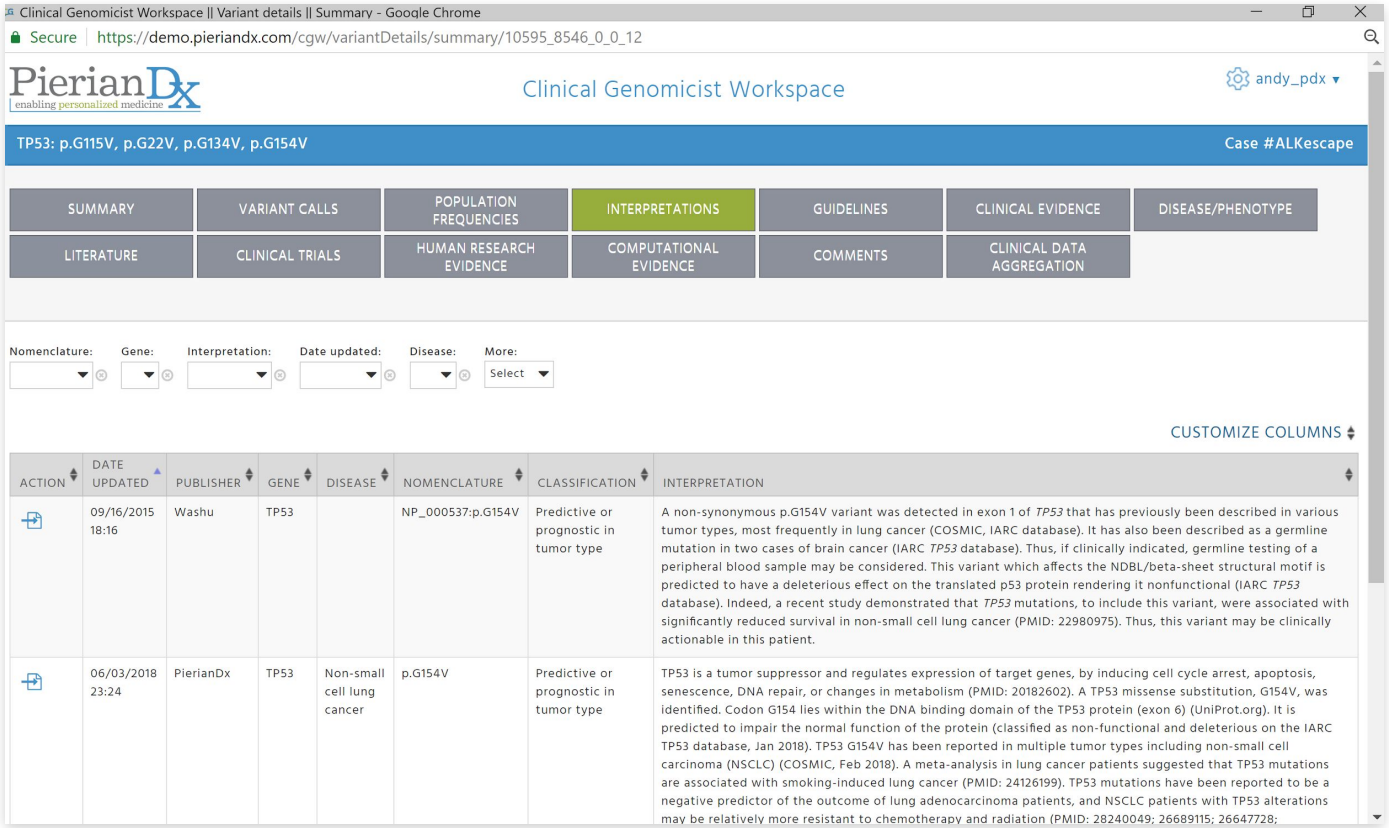
Quickly filter by relevant categories of info

Highlighted reference text

Link directly to full text article

Clinical Interpretations

TP53 p.G154V



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

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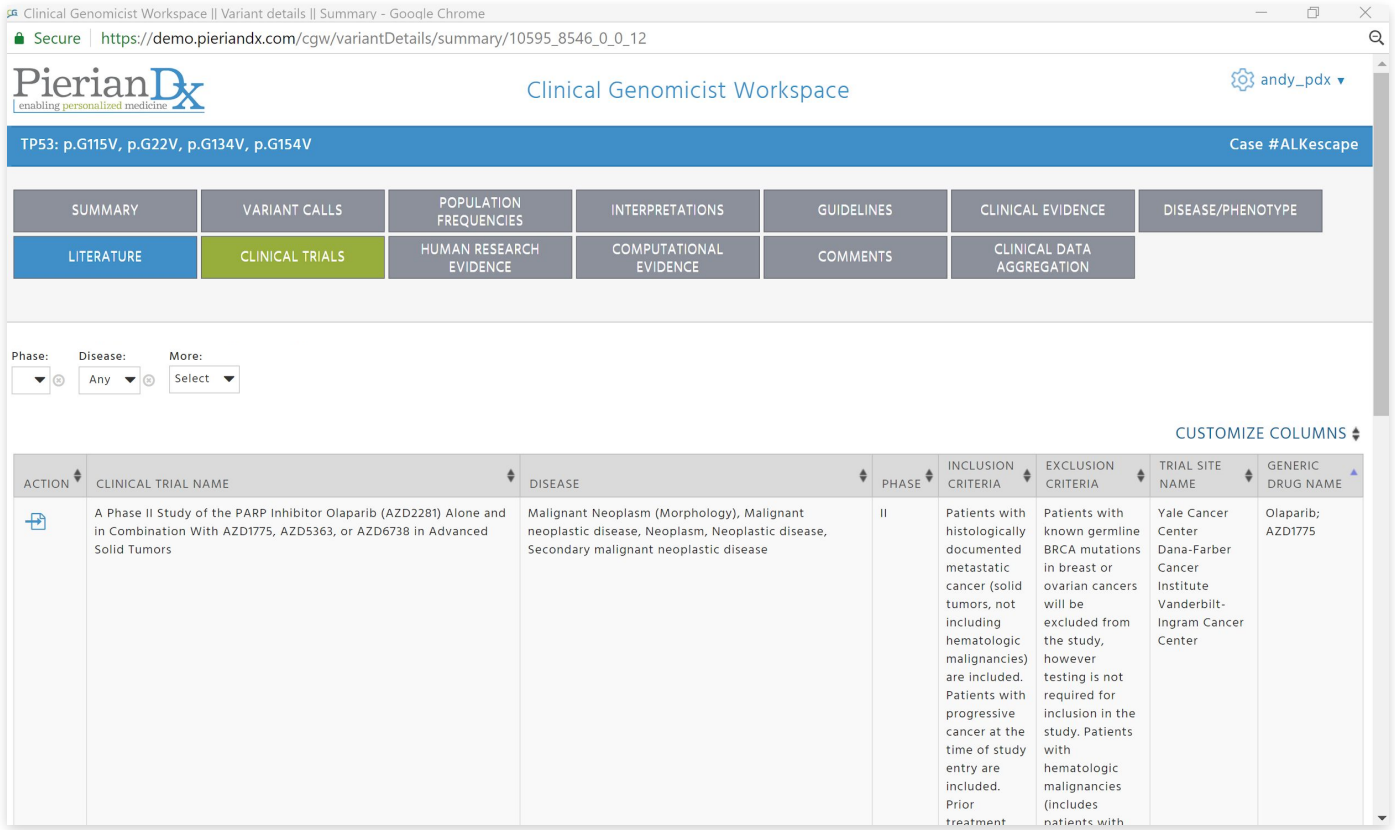
PierianDx network-sourced interpretations point to valuable evidence

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."

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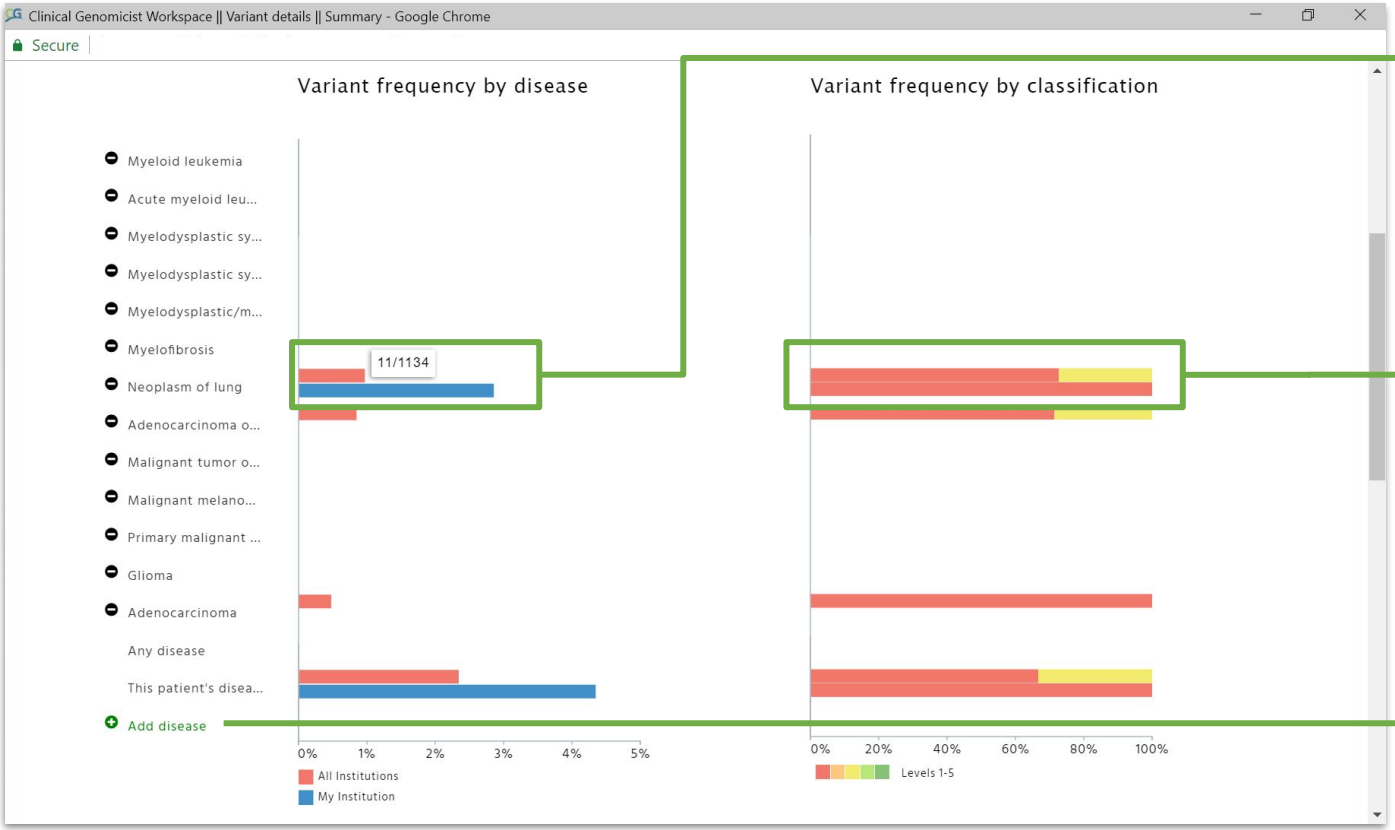
Variant Annotation and Classification

Clinical Data Aggregation

TP53 p.G154V



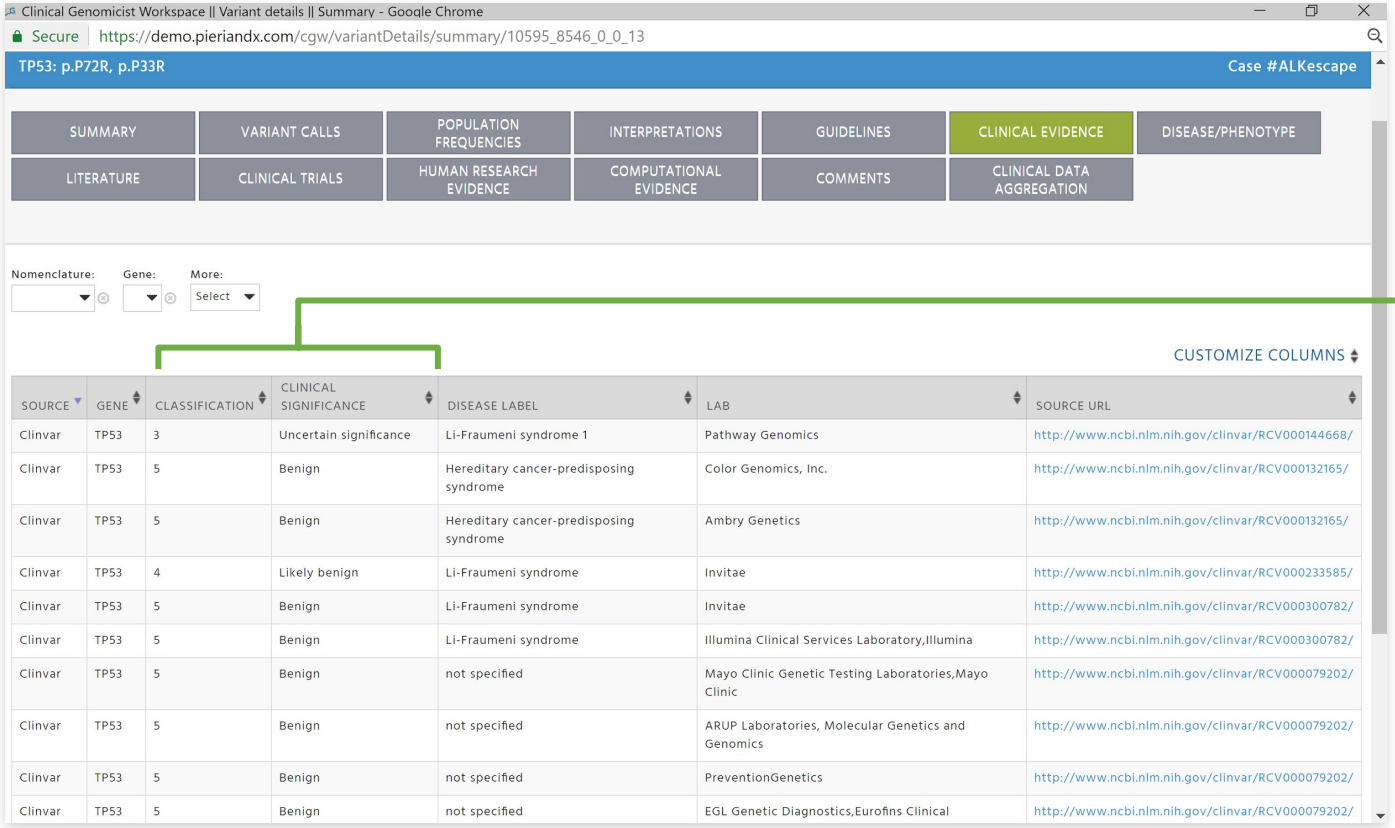
Explore frequency of detection in patient cases across the network and within your laboratory

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

Search frequency in other tumor types

Clinical Evidence

TP53 p.P72R

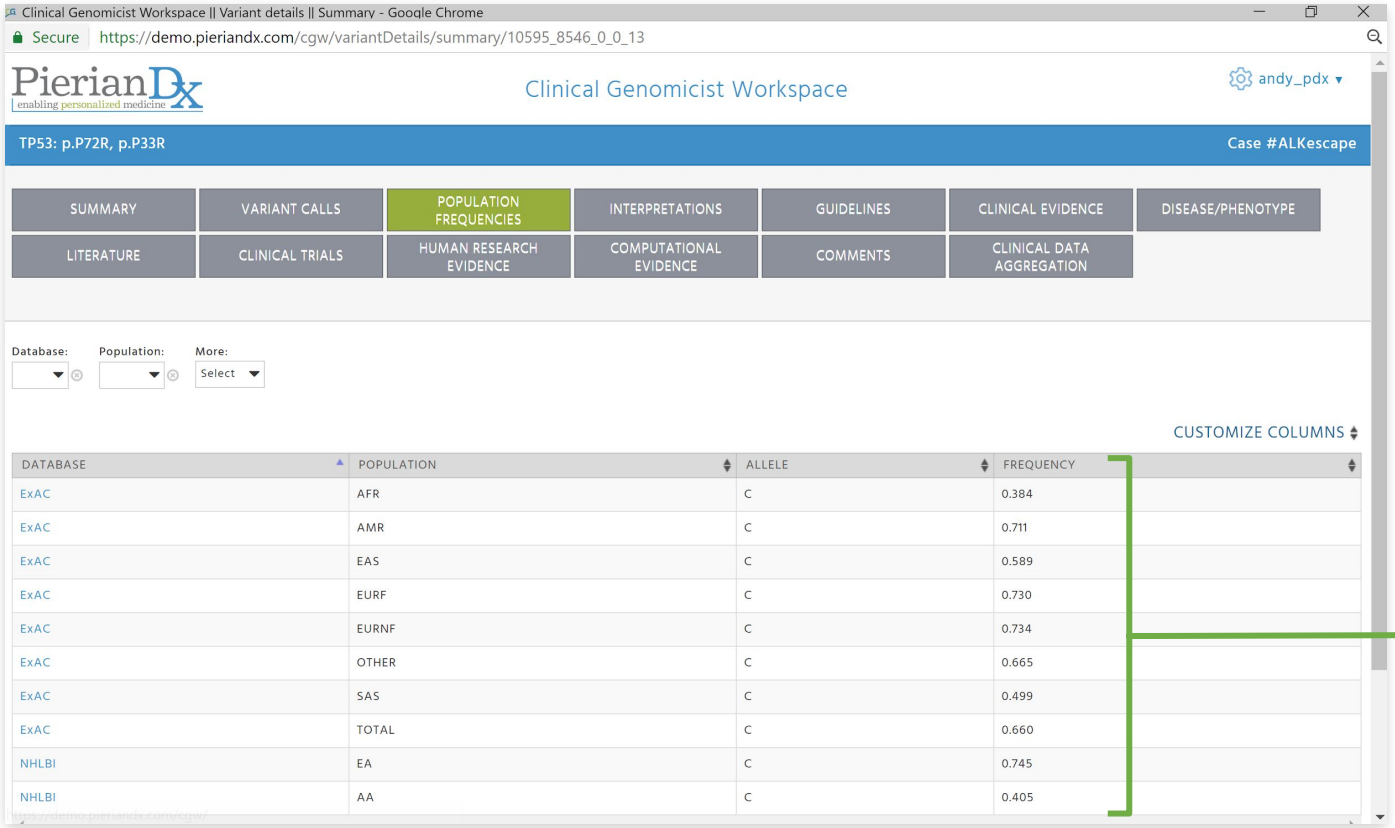


SOURCE	GENE	CLASSIFICATION	CLINICAL SIGNIFICANCE	DISEASE LABEL	LAB	SOURCE URL
Clinvar	TP53	3	Uncertain significance	Li-Fraumeni syndrome 1	Pathway Genomics	http://www.ncbi.nlm.nih.gov/clinvar/RCV000144668/
Clinvar	TP53	5	Benign	Hereditary cancer-predisposing syndrome	Color Genomics, Inc.	http://www.ncbi.nlm.nih.gov/clinvar/RCV000132165/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

Conflicting ClinVar submissions, but consensus is clear

Population Frequencies

TP53 p.P72R



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

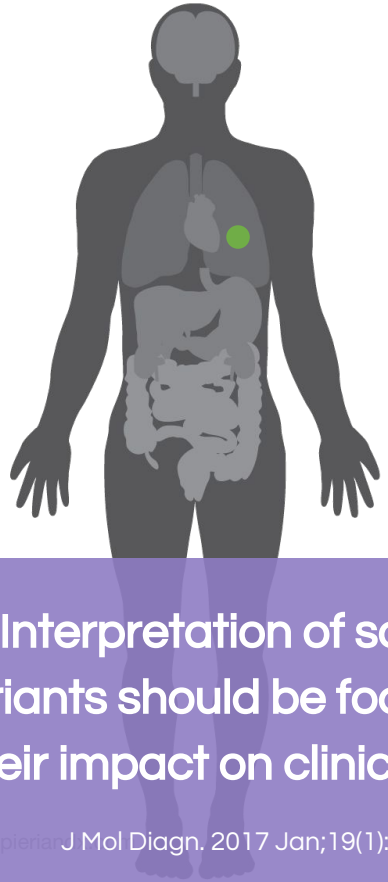
"These databases are frequently used to **filter out variants that are deemed polymorphic/ benign** based on an arbitrary cutoff of minor allele frequency (MAF)."

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

MAF > 1% in all population databases

Clinical Interpretation and Reporting

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"

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

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.

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.

PierianDx

PierianDx
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 Accessioned:	05/30/2018

REVIEW STATUS: Final

CLINICAL INFORMATION AND TEST SUMMARY

Illumina TST 170 Panel 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.

OVERVIEW OF FINDINGS

7 Variants of therapeutic significance

0 Variants of diagnostic or prognostic significance

Variant clinical significance tiers per AMP/ASCO/CAP guidelines

1A	1B	2C	2D	3
1	0	2	0	3

RESULT SUMMARY

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

CLINICALLY RELEVANT RESULTS

Variants of strong clinical significance (predictive, prognostic, diagnostic) based on: FDA-approved therapy in tumor type, or professional guidelines; or Well-powered studies with consensus of experts

Variants of potential clinical significance (predictive, prognostic, diagnostic) based on: FDA-approved therapy in other tumor type, investigational therapies, or multiple small studies; or Preclinical studies or case reports

EML4/ALK fusion

Interpretation: AMP Somatic Variant Classification: *EML4-ALK* rearrangement is classified at Tier 1 based on Level A evidence.

EML4 is involved in microtubule formation (RefSeq, Jan 2015); *ALK* (Anaplastic Lymphoma Kinase) encodes a receptor tyrosine kinase belonging 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 NSCLC (RefSeq, Jan 2011). Abnormal fusion of parts of *EML4* gene with portions of *ALK* gene generates EML4/ALK fusion transcripts, one of the primary mutations associated with NSCLC (RefSeq, Jan 2015).

An EML4/ALK fusion is identified from RNA isolated from this patient's tumor. The protein contains the N-terminal half of EML4 and the intracellular catalytic domain of ALK. 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: 28872581). *EML4/ALK* gene rearrangements are most widely reported in NSCLCs (COSMIC, January 2018; PMID: 26579422). In NSCLC, ALK fusions are biomarkers that predict benefit from targeted therapy such as the ALK kinase inhibitors crizotinib or ceritinib (NCCN, NSCLC, v2.2018).

PierianDx Page 1 of 4

"**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…"

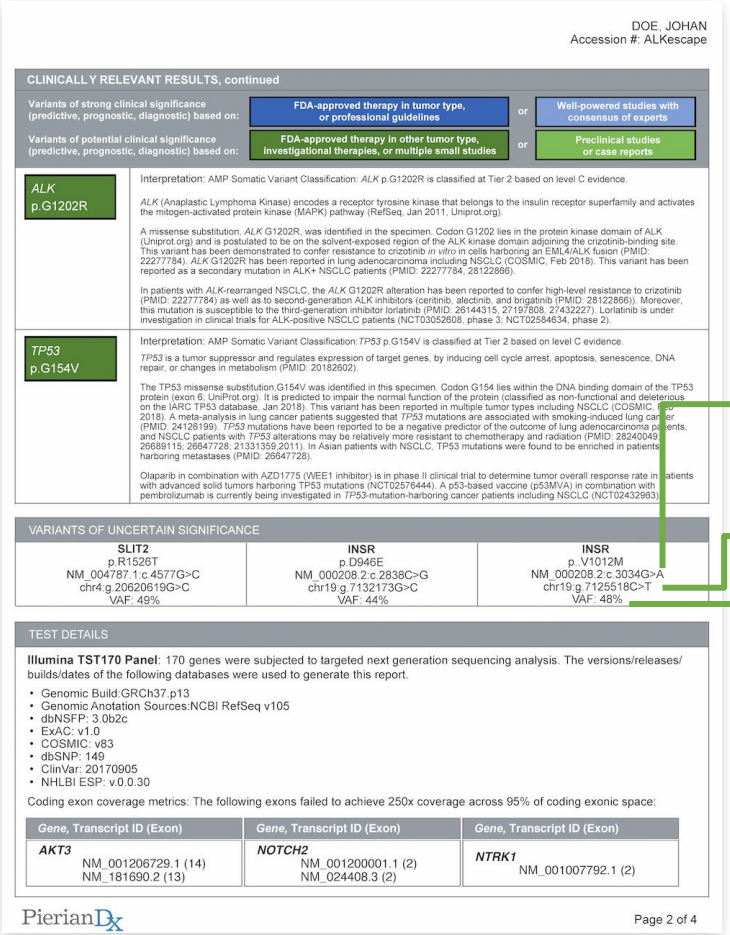
"**It is desirable to include graphs, charts, and tables** to increase the overall clarity of the report."

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

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.



DOE, JOHAN
Accession #: ALKescape

CLINICALLY RELEVANT RESULTS, continued

Variants of strong clinical significance (predictive, prognostic, diagnostic) based on:	FDA-approved therapy in tumor type, or professional guidelines	or	Well-powered studies with consensus of experts
Variants of potential clinical significance (predictive, prognostic, diagnostic) based on:	FDA-approved therapy in other tumor type, investigational therapies, or multiple small studies	or	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, 27197808, 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, 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, 21331359.2011). In Asian patients with NSCLC, TP53 mutations were found to be enriched in patients harboring metastases (PMID: 26647728).

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	INSR	INSR
p.R1526T	p.D946E	p.V1012M
NM_004787.1:c.4577G>C	NM_000208.2:c.2838C>G	NM_000208.2:c.3034G>A
chr4:g.20620619G>C	chr19:g.7132173G>C	chr19:g.7125518C>T
VAF: 49%	VAF: 44%	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 Anotation Sources:NCBI RefSeq v105
- dbNSFP: 3.0b2c
- ExAC: v1.0
- COSMIC: v83
- dbSNP: 149
- ClinVar: 20170905
- NHLBI ESP: v.0.0.30

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)
AKT3	***NOTCH2***	***NTRK1***
NM_001206729.1 (14)	NM_001200001.1 (2)	NM_001007792.1 (2)
NM_181690.2 (13)	NM_024408.3 (2)	

PierianDx

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Transcript reference sequence

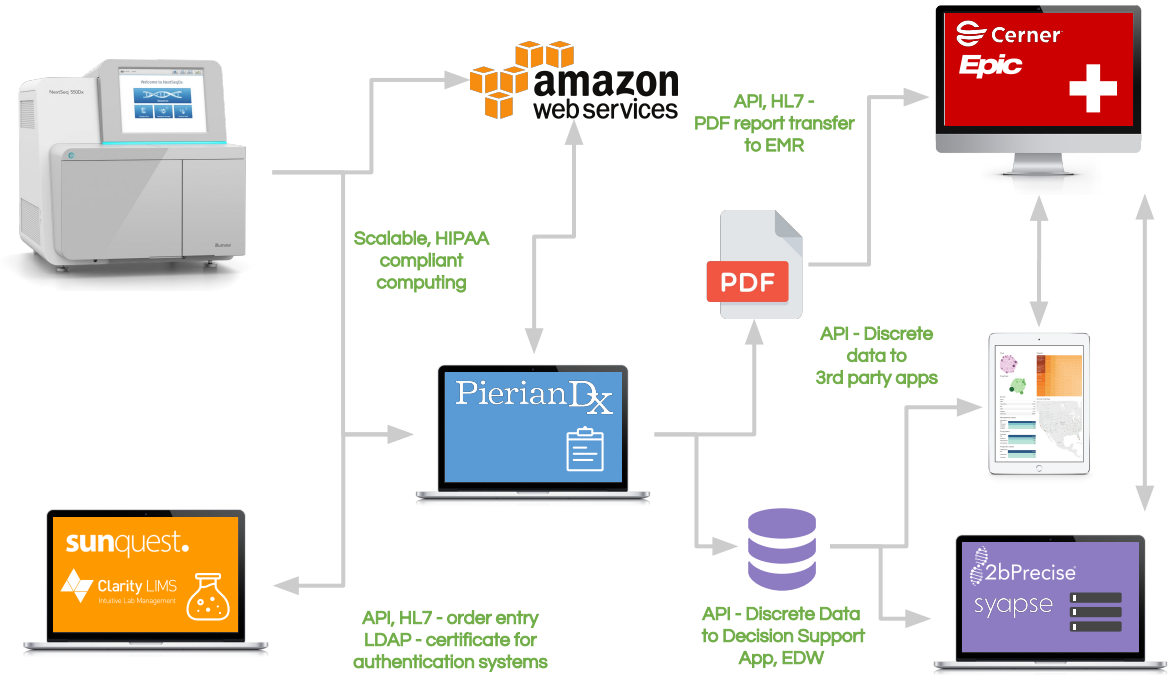
Genomic coordinates

Variant allele fraction (VAF)

Genome build

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

HealthIT Integration



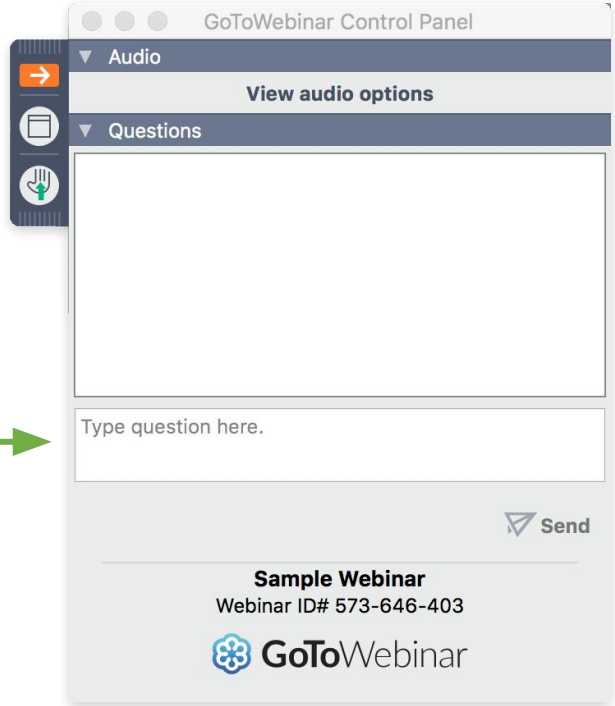
"Ideally, the report should be in a **format that enables integration with an electronic health record**.

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



Type questions here

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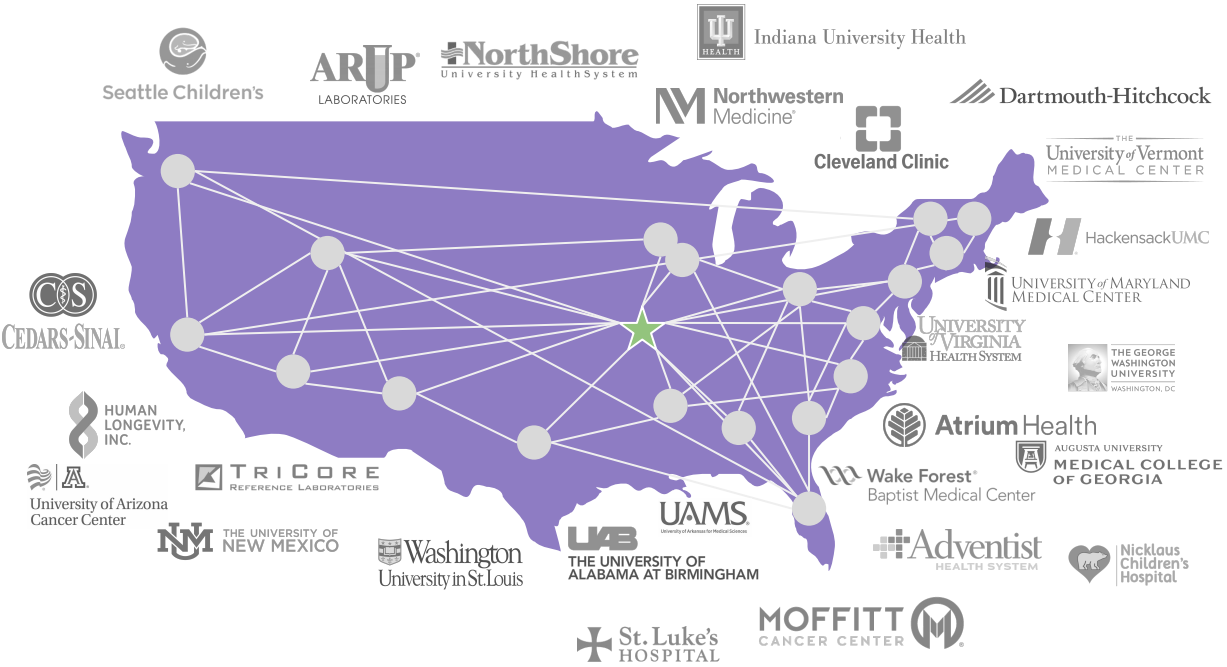
Let's Work Together

The Pierian Partnership



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