

## CLINICALLY RELEVANT RESULTS – ALK, p.G1202R

**Gene Summary – ALK:** This gene encodes a receptor tyrosine kinase, which belongs to the single pass transmembrane region, and an intracellular kinase domain. It plays an important role in cell growth and differentiation. It is frequently found to be rearranged, mutated, or amplified in a series of tumours including anaplastic large cell lymphoma (ALCL), non-small cell lung cancer (NSCLC), and colorectal cancer. Common genetic alterations in this gene, which result in creation of multiple fusion genes, are associated with poor prognosis.

**Interpretation:** ALK (Anaplastic Lymphoma Kinase) encodes a receptor tyrosine kinase. A missense substitution, p.G1202R, was identified in the ALK kinase domain adjoining the crizotinib-binding site and demonstrated to confer resistance to crizotinib in NSCLC (COSMIC, Feb 2018). This mutation is also found in a patient with NSCLC with EML4-ALK fusion, ALK G1202R alteration conferred high resistance to crizotinib (PMID: 28122866). Moreover, the secondary resistant mutation ALK G1202 is susceptible to alectinib in NSCLC patients (NCT03052608, phase 3; NCT02584634, phase 2). This patient's tumor also harbors an EML4-ALK fusion which was identified by RNA-seq. This patient is a candidate for next-generation ALK inhibitors. Clinical correlation is recommended.

## TREATMENT SUMMARY – ALK, p.G1202R

Therapy	Specificity	Mechanism
<a href="#">Avelumab + Lorlatinib</a>	<a href="#">Gene</a>	<a href="#">Anti-PLK Kinase</a>
<a href="#">Lorlatinib</a>	<a href="#">Gene</a>	<a href="#">ALK/ROS</a>
Responsive <input checked="" type="checkbox"/> Non-responsive <input type="checkbox"/>		

## CLINICAL TRIALS – ALK, p.G1202R

Malignancy	Therapy	Title
<a href="#">Non-small cell lung cancer</a>	<a href="#">Avelumab + Lorlatinib</a>	<a href="#">A Phase 1b/2, Open-label, Safety, Efficacy, Pharmacokinetic Study of Avelumab (msb001) in Combination with Crizotinib Or PF-02341434 in Metastatic Non-small Cell Lung Cancer</a>
<a href="#">Non-small cell lung cancer</a>	<a href="#">Lorlatinib</a>	<a href="#">A Phase 3, Randomized, Double-blind, Placebo-controlled Study of Lorlatinib (PF-02341434) Monotherapy Versus Placebo in Crizotinib-resistant ALK-positive Non-small Cell Lung Cancer</a>

# Supporting NGS-Based In Vitro Diagnostics

## A Knowledge Sharing Approach

**Rakesh Nagarajan, MD, PhD**

Executive Chairman and Founder  
PierianDx



Moderated by: Josh Forsythe

# Agenda

1 Large Assays, IVDs: Are We Prepared?

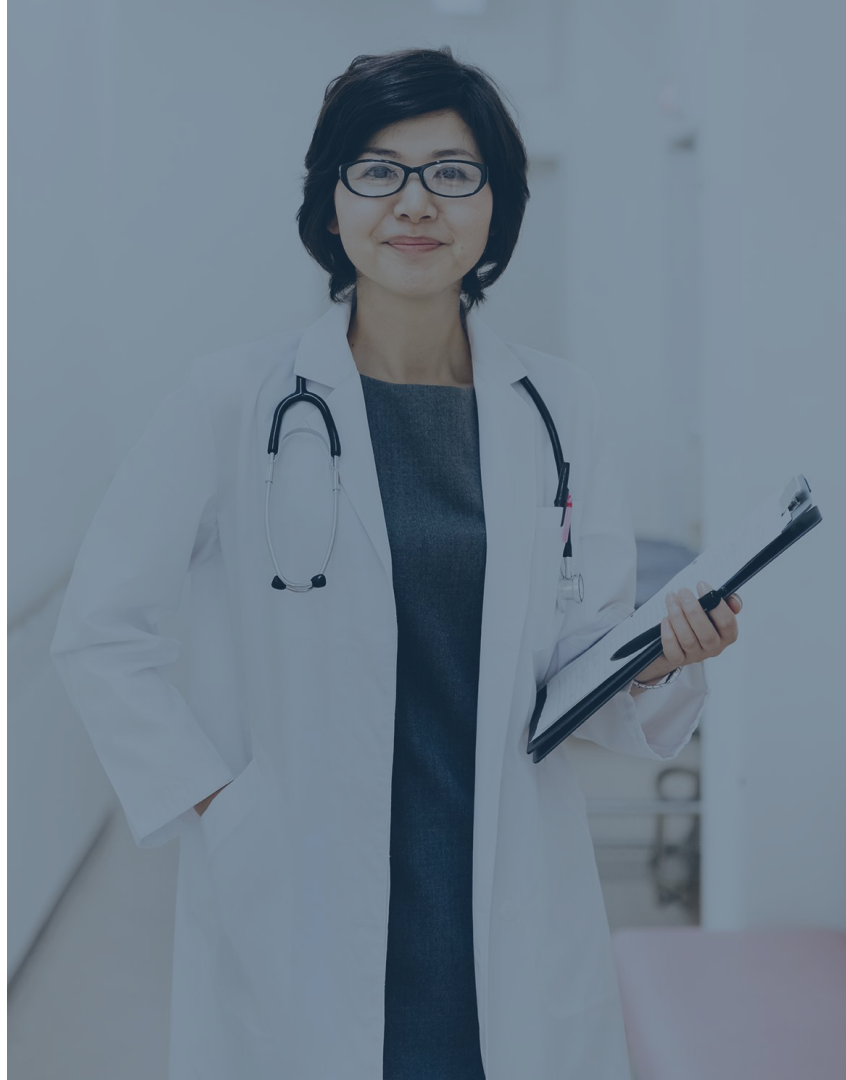
2 First Solving Challenges with LDTs

3 Knowledge Sharing and Inferencing

4 Applied Framework

5 Knowledgebase Benchmarking

6 Achieving More Complete,  
Ready-to-sign-out Reports for IVDs



Larger Assays, IVDs

# **Are We Prepared?**

# More Comprehensive Assays Gaining Ground Drivers Behind Large Assays

## More Genes

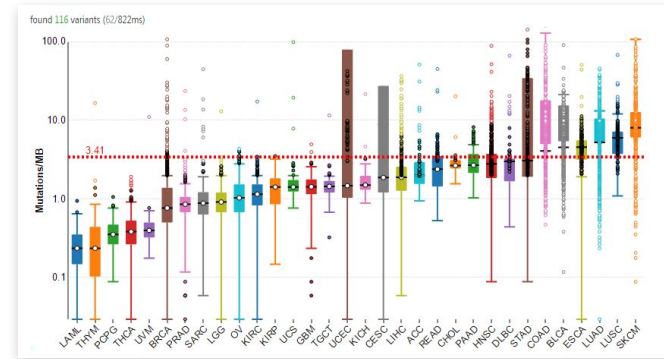
From single gene assays and small targeted hotspot panels to large capture-based assays and exome

## More Variants and Variant Types

From SNVs and indels to copy number variants and gene fusions using both DNA and RNA samples

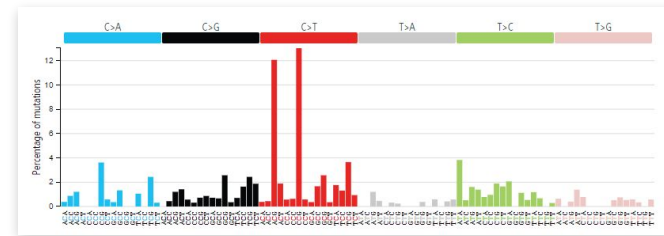
## More comprehensive detection of clinically relevant features

- Tumor mutation load
- Mutational signature
- Confirm mRNA expression of mutations found at DNA level
- Immunoprofiling
- Inherited cancer risk (identified in 20% of cases)



## Mutational Load

(determine candidacy for immunotherapy)



## Mutational Signature

(identify etiology and predict response to therapy)

## HUMAN LEUKOCYTE ANTIGEN (HLA) TYPING

Human leukocyte antigens are cell-surface proteins that play an important part in the body's immune response to foreign substances. HLA typing is also relevant for predicting MHC presentation of specific candidate neoantigens (PMID 24691321, PMID 27563649, PMID 25838375). The subject's MHC class I (A, B, and C) and MHC class II (DR, DQ, and DP) genes, allele groups and subtypes are reported below.

The expression status of a subset of genes have been shown to be important for immunotherapy consideration (PMID 24138885, PMID 27433843, PMID 27377892, PMID 25858804, PMID 24457417, PMID 24782321, PMID 26359337).

Gene	Allele
HLA-A	A*26:01
HLA-A	A*32:01
HLA-B	B*08:01
HLA-B	B*51:01
HLA-C	C*07:02
HLA-C	C*07:29
HLA-DPB1	DPB1*02:01

## HLA Typing

(used for clinical trial enrollment)

FDA Approved

# Targeted Therapies Increasing

**VITRAKVI**  
(larotrectinib)  
15 mg/100 mg CAPSULES  
20 mg/mL ORAL SOLUTION

**Inlyta**  
axitinib  
1 mg and 5 mg tablets

**ZYKADIA**  
ceritinib  
150 mg capsules

**AROMASIN**  
exemestane tablets  
25 mg

**Herceptin SC**  
trastuzumab  
subcutaneous

**Caprelsa**  
(vandetanib) Tablets

**ZELBORAF**  
(vemurafenib) tablets

**IBRANCE**  
palbociclib

**Jakafi**  
ruxolitinib (tablets)

**Zytiga**  
(abiraterone acetate)

**Beleodaq**  
(belinostat) for injection  
for intravenous infusion

**ERBITUX**  
CETUXIMAB  
See the difference

**FASLODEX**  
fulvestrant injection

**LENVIMA**  
(lenvatinib) capsules | 10 mg and 4 mg

**Votrient**  
pazopanib tablets (200 mg)

**Odomzo**  
(sonidegib) capsules  
200mg

**Kadcyla**  
trastuzumab emtansine

**Xtandi**  
(enzalutamide)  
capsules

**COTELLIC**  
(cobimetinib) tablets

**IRESSA**  
gefitinib

**Portrazza**  
(necitumumab)

**PERJETA**  
pertuzumab

**Nexavar**  
(sorafenib) tablets

**GIOTRIF**  
(afatinib) tablets

**Bosulif**  
bosutinib  
500 mg tablets

**XALKORI**  
CRIZOTINIB  
250 mg CAPSULES

**imbruvica**  
(ibrutinib)  
560, 420, 280, 140 mg tablets | 70 mg capsules

**Tasigna**  
(nilotinib)

**ICLUSIG**  
(ponatinib) tablets

**SUTENT**  
sunitinib maleate capsules

**Arimidex**  
anastrozole

**COMETRIQ**  
(cabozantinib)

**Tafinlar** + **Mekinist**  
(dabrafenib) (50 mg, 75 mg capsules) + (trametinib) (0.5 mg, 2 mg tablets)

**Arzerra**  
ofatumumab

**Lynparza**  
olaparib

**CYRAMZA**  
ramucirumab injection  
10 mg/mL solution

**TORISEL**  
(temsirolimus) injection

**ALECENSA**  
alelectinib  
150 mg capsules

**Tarceva**  
erlotinib

**AFINITOR**  
(everolimus) tablets

**gleevec**  
imatinib mesylate

**TAGRIS**  
osimertinib



**Stivarga**  
(regorafenib) tablets

**Fareston 60mg**  
(toremifene citrate) Tablets

Larger, More Comprehensive Tests Becoming Available

## IVDs Will be Game-changing

Provider	Assay	Genes
PGDx	PGDx	508
Illumina	TruSight Oncology	500
Memorial Sloan	MSK-Impact	468
Foundation Medicine	F1CDx	324
Illumina	TruSight Tumor	170
ThermoFisher	Oncomine	162

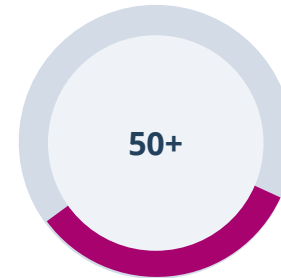
 Sold as kits for RUO/LDT  
 Public plans for IVD



<2011



2011



2017



20??

# Challenges of Time and Accuracy



Tier	Classification (Therapeutic, Prognostic, Diagnostic)	Evidence Level	Evidence
I	<b>Variants of Strong Clinical Significance</b>	<b>A</b>	FDA-approved therapy; Included in professional guidelines
		<b>B</b>	Well-powered studies with consensus from field experts
II	<b>Variants of Potential Clinical Significance</b>	<b>C</b>	FDA-approved therapies for different tumor types or investigational therapies; Multiple small studies w/ consensus
		<b>D</b>	Preclinical trials or a few case reports without consensus
III	<b>Variants of Unknown Clinical Significance</b>		Not observed at a significant allele frequency in the general or specific subpopulation databases, or pan-cancer or tumor-specific variant databases No convincing published evidence of cancer association
IV	<b>Benign or Likely Benign Variants</b>		Observed at significant allele frequency in the general or specific subpopulation databases No existing published evidence of cancer association

Li et al. "Standards and Guidelines for the Interpretation and Reporting of Somatic Variants in Cancer", 2017

# Challenges of Time and Accuracy



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**Ex. TSO-500**

**100s of Variants  
>4 Hours**



First Solving Challenges with LDTs

# PierianDx Background

# Leaders in Clinical Genomics

**Today**

Full suite of software and services

Independent CLIA/CAP “dry lab”

40+ medical center, cancer center,  
health system, and reference lab clients

200+ yrs of clinical genomics experience

**2014**

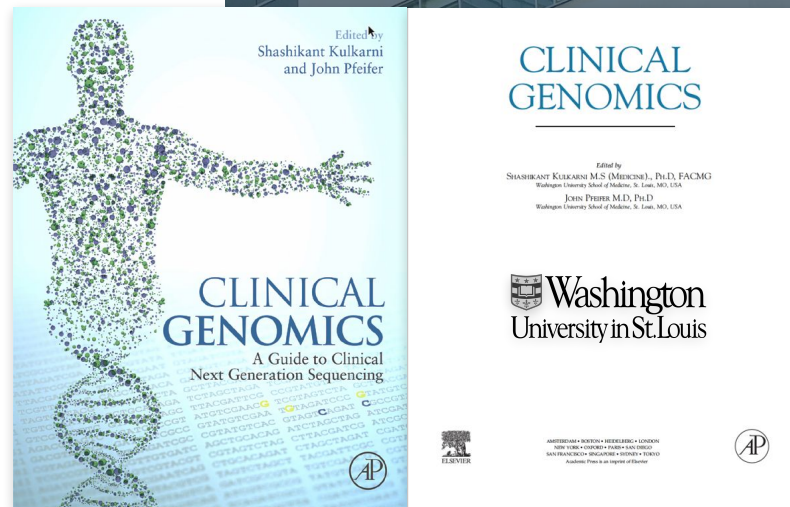
PierianDx est. after ~50 labs visit  
WashU to learn how clinical NGS is  
operationalized.

**2011**

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

**2003**

WashU plays critical role in  
Human Genome Project.



## Complete LDT/IVD Support



```
graph LR; A[Sample Processing Extraction, Library Prep] --> B[Sequencing]; B --> C[Variant Calling, Annotation (Bioinformatic Pipelines)]; C --> D[Data Visualization, QC Analysis]; D --> E[Variant Prioritization]; E --> F[Clinical Interpretation Classification & Reporting]; F --> G[Final Report & Medical Director Sign-out]; G --> H[Data Integration EMR, 3rd Party];
```

The diagram illustrates a clinical genomics workflow and its supporting services. The workflow consists of eight sequential steps, each in a colored box: 1. Sample Processing Extraction, Library Prep (orange), 2. Sequencing (orange), 3. Variant Calling, Annotation (Bioinformatic Pipelines) (dark blue), 4. Data Visualization, QC Analysis (dark blue), 5. Variant Prioritization (dark blue), 6. Clinical Interpretation Classification & Reporting (teal), 7. Final Report & Medical Director Sign-out (teal), and 8. Data Integration EMR, 3rd Party (purple). Below the workflow, two horizontal brackets define service areas: 'Assay Validation Services' covers the first two steps, and 'Interpretation Services' covers the last four steps. A central label 'Clinical Genomics Workspace + Knowledgebase' is positioned above the 'Interpretation Services' bracket. A large orange arrow points from left to right across the bottom of the diagram.

Sample Processing Extraction, Library Prep

Sequencing

Variant Calling, Annotation (Bioinformatic Pipelines)

Data Visualization, QC Analysis

Variant Prioritization

Clinical Interpretation Classification & Reporting

Final Report & Medical Director Sign-out

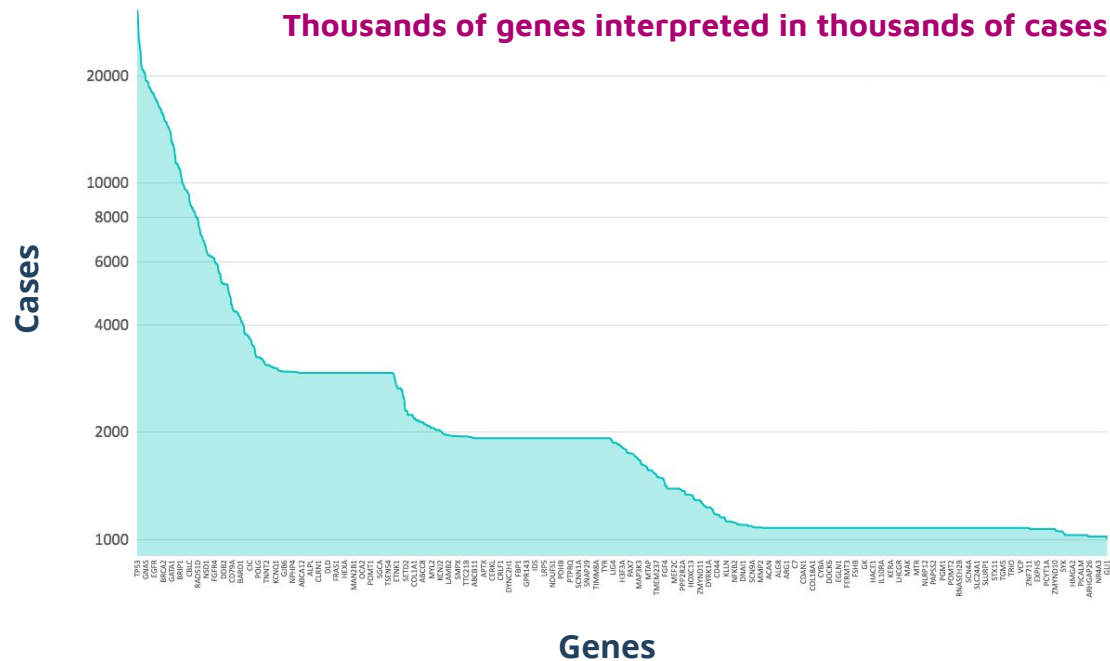
Data Integration EMR, 3rd Party

Assay Validation Services

Interpretation Services

Clinical Genomics Workspace + Knowledgebase

# PierianDx Knowledgebase



## By the Numbers

- 100% clinically focused interpretations
- 1,100+ somatic genes curated
- **~6Mb of sequence coverage**
- 18 million published articles

## Experience

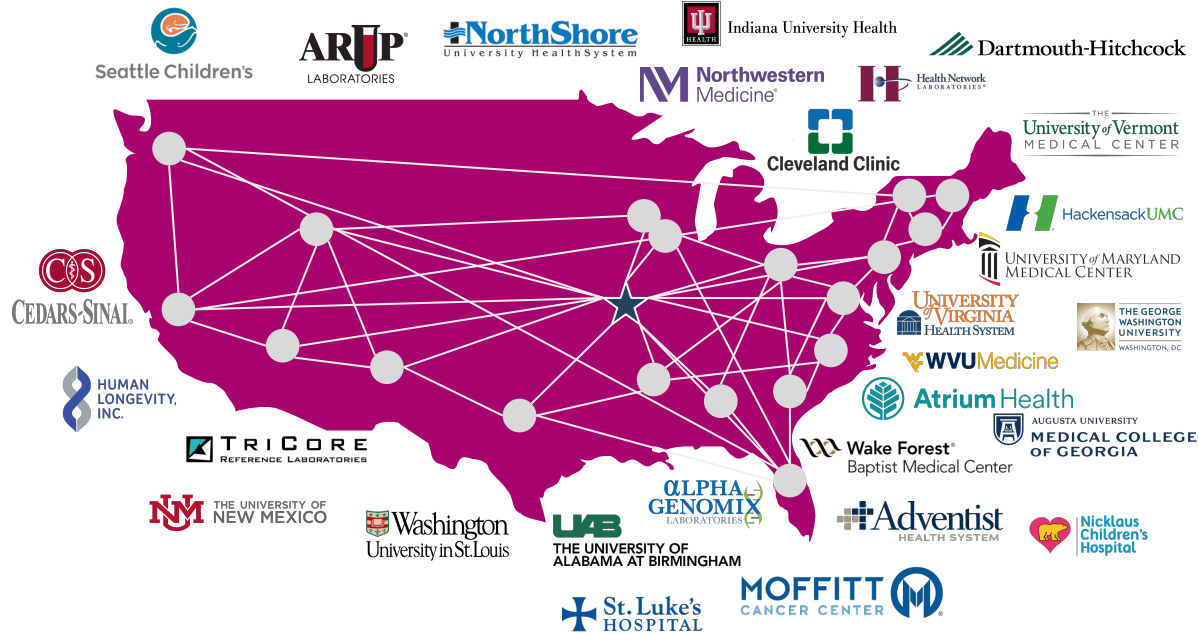
- 40+ PierianDx annotators
- 100 years of combined curation effort
- **Led by board certified molecular genetic pathologist**
- 80+ contributing molecular pathologist customers

# Knowledge Sharing and Inferencing

## **Key Components**

# Largest Clinical Interpretation Sharing Network

## Knowledgebase Sources



## Public Sources-Versioned

- Human genome builds
- Gene RNA protein models
- Pop. frequency databases

## Public Sources - Cleaned

- COSMIC, TCGA, ClinVar
- Emory and Invitae
- PubMed literature

## Highly Curated

- FDA approved labels
- NCCN, ASCO Guidelines
- Active, recruiting clinical trials

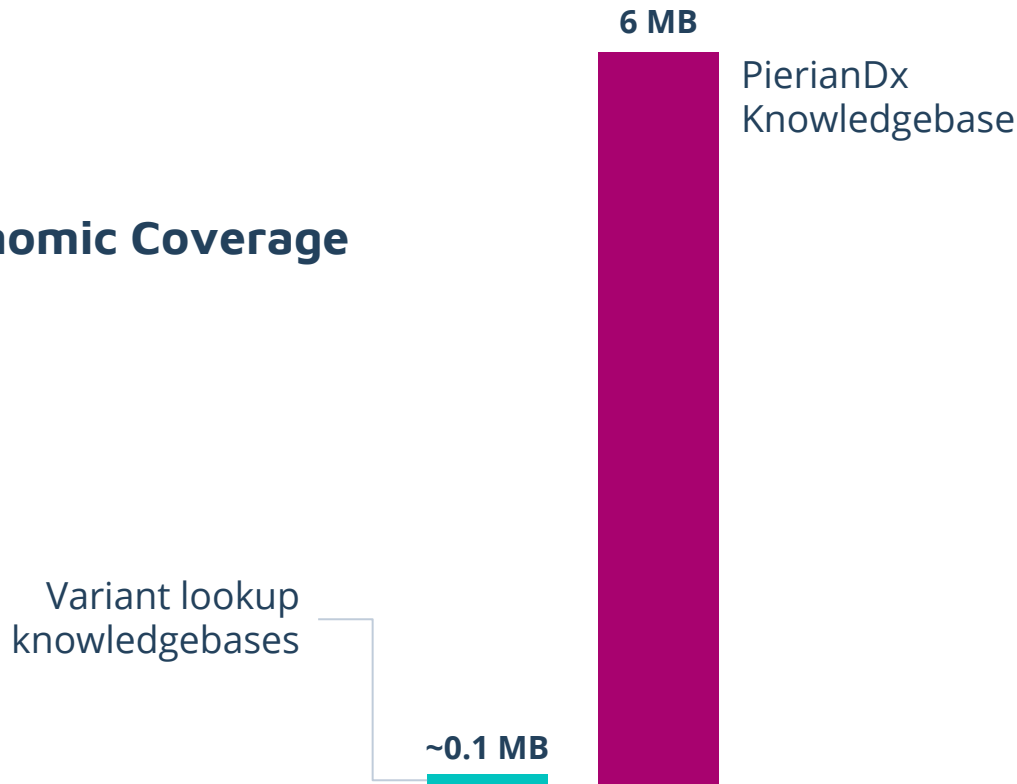
## Shared Medical Content

- Clinical interpretations and classifications from partners and PierianDx

More Coverage than a Simple Variant Lookup

## Rules Engine and Predicate Model

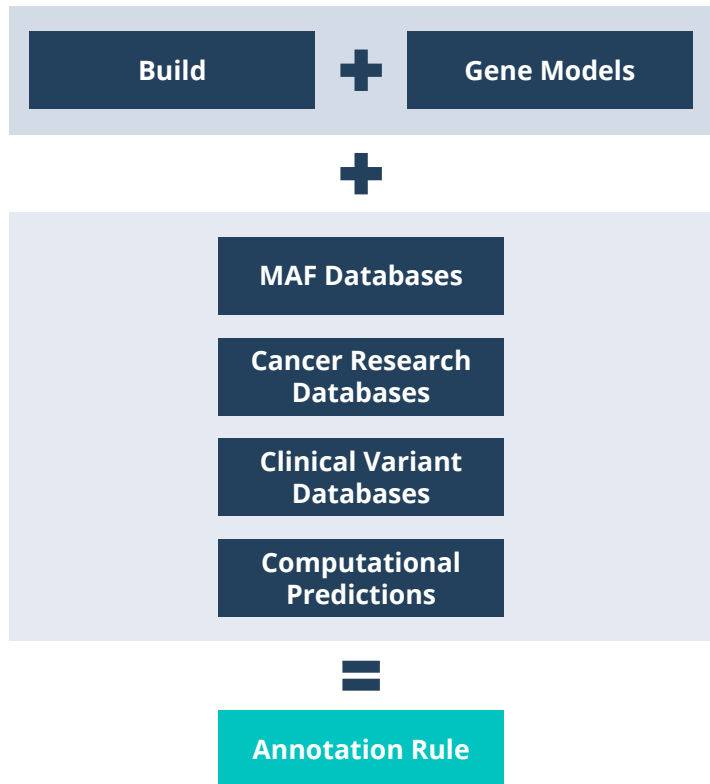
### Genomic Coverage



### Inferences Based On

- HGVS syntax (g-, c-, or p-syntax)
- Genomic, coding, protein coordinates (codon ranges or exon ranges)
- Functional characteristics (frameshift, in frame, truncating)
- Matching known and novel partners for gene fusions (e.g. EML4-ALK vs MLL)
- Limiting CNVs based on a range of CNV gain or loss
- When one or more variants are required to be present within or across genes (e.g. co-occurring EGFR and KRAS mutations in lung cancer)

# Annotation and Curation Source Rules



## Predicates

Rule Specificity	Coordinate Ranges
Disease	Genomic
Gene/Biomarker	mRNA
Variant Syntax	Codon
Variant Type	Exon
Consequence	Domain
Copy Number Range	Partner Gene
	Coordinate Range



## Inferences

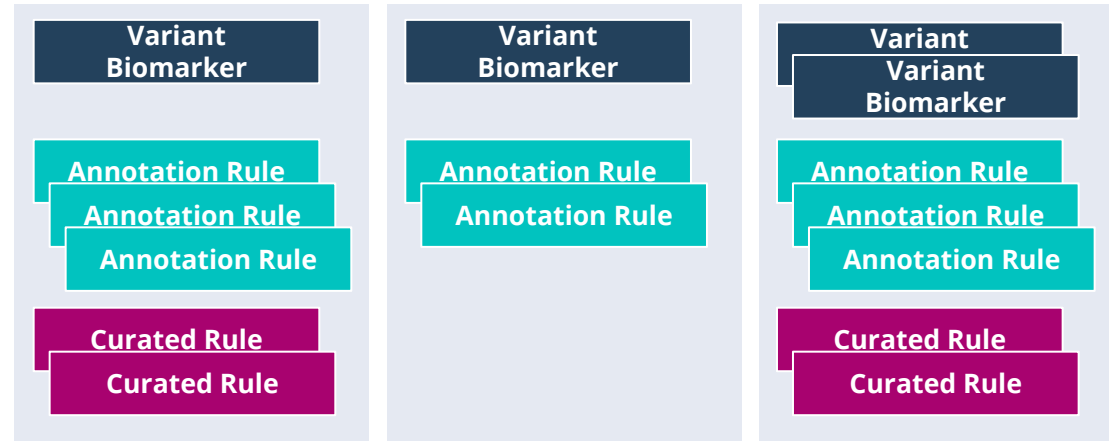
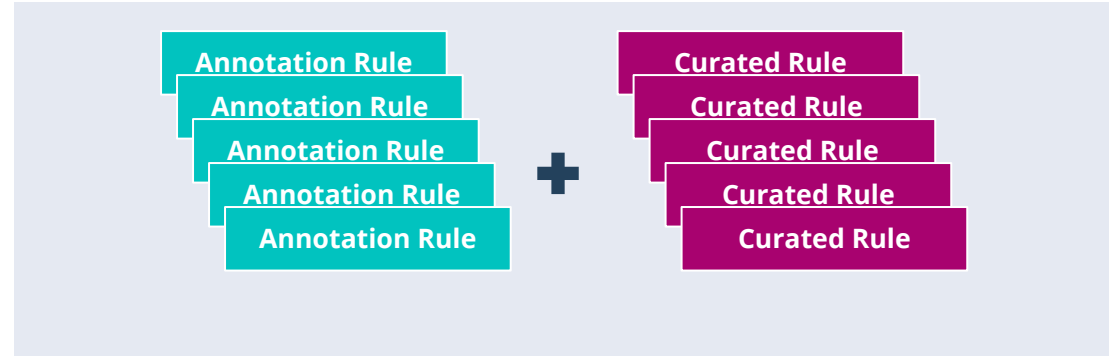
Therapeutic	Drug	Phase
Prognostic	Study Size	Location
Diagnostic	Outcomes	



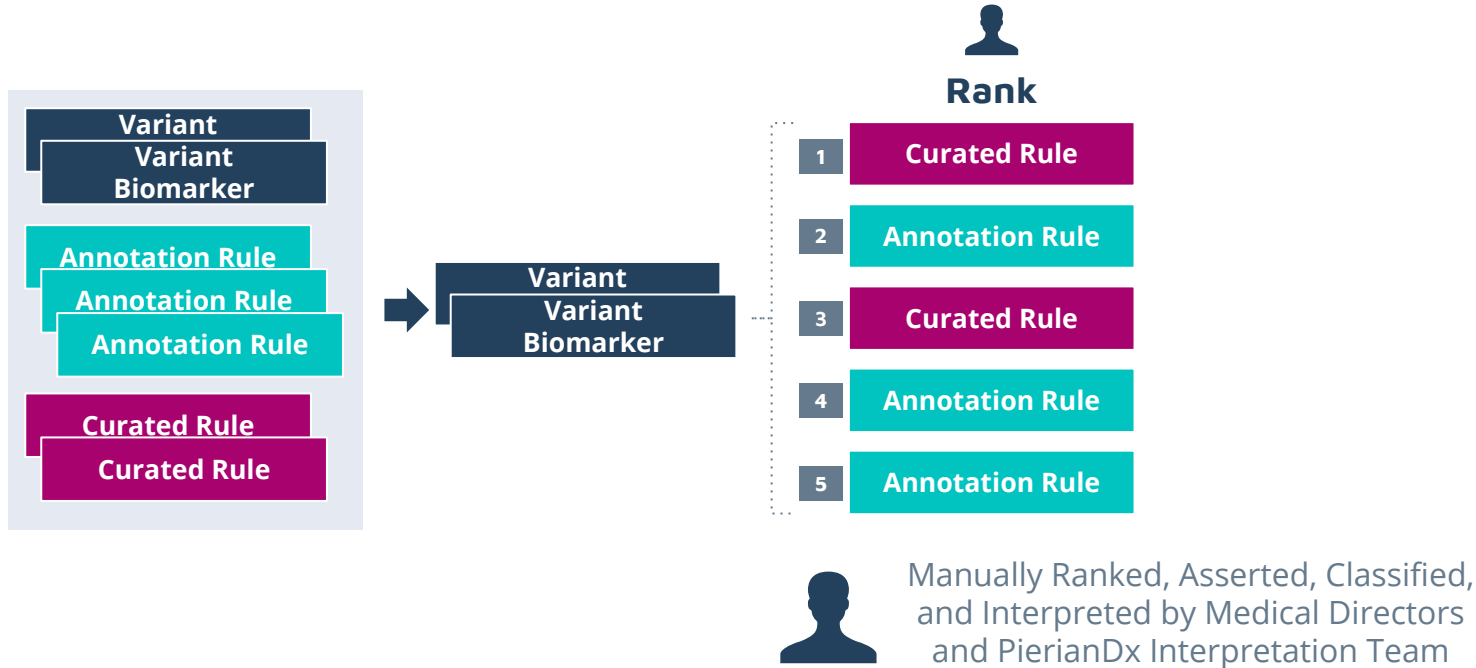
**Curated Rule**



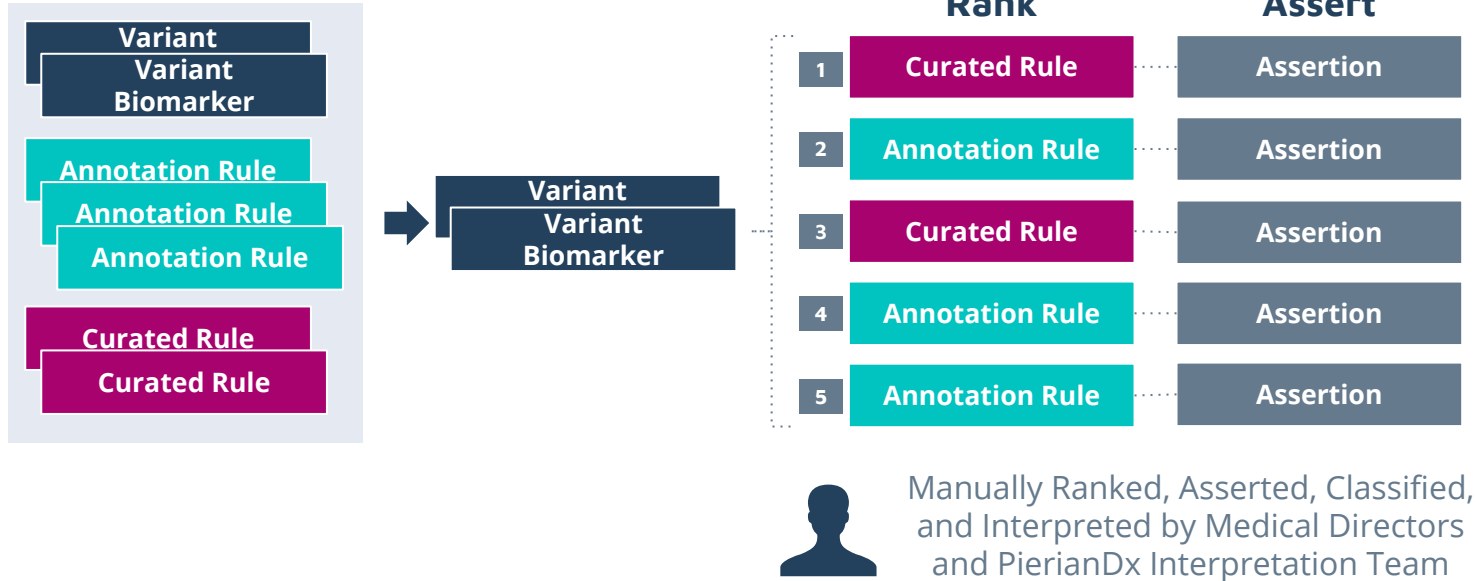
# Biomarker Processing



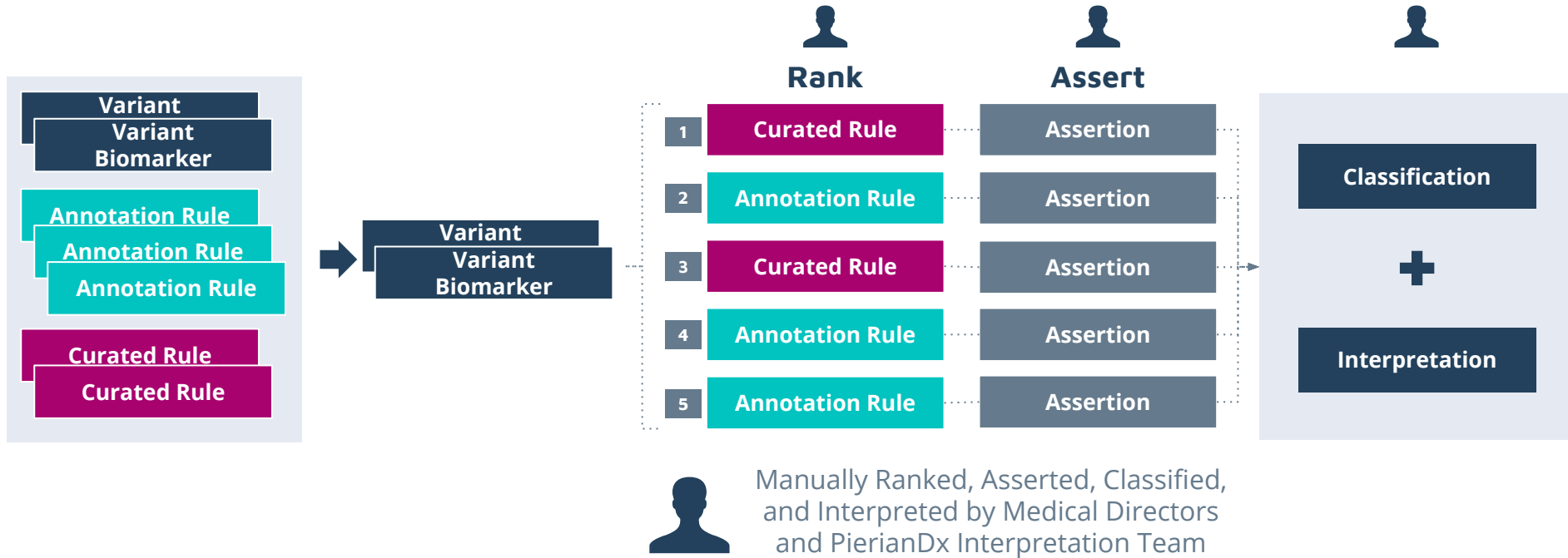
# Evidence Reviewing, Ranking, Asserting



# Evidence Reviewing, Ranking, Asserting



# Evidence Reviewing, Ranking, Asserting



Data Science + Human Acumen

# Applied Framework



# Somatic Variant Classification Scheme

Tier	Classification (Therapeutic, Prognostic, Diagnostic)	Evidence Level	Evidence
I	Variants of Strong Clinical Significance	A	FDA-approved therapy; Included in professional guidelines
		B	Well-powered studies with consensus from field experts
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# Driven (largely) by Population Frequency Databases

Variants



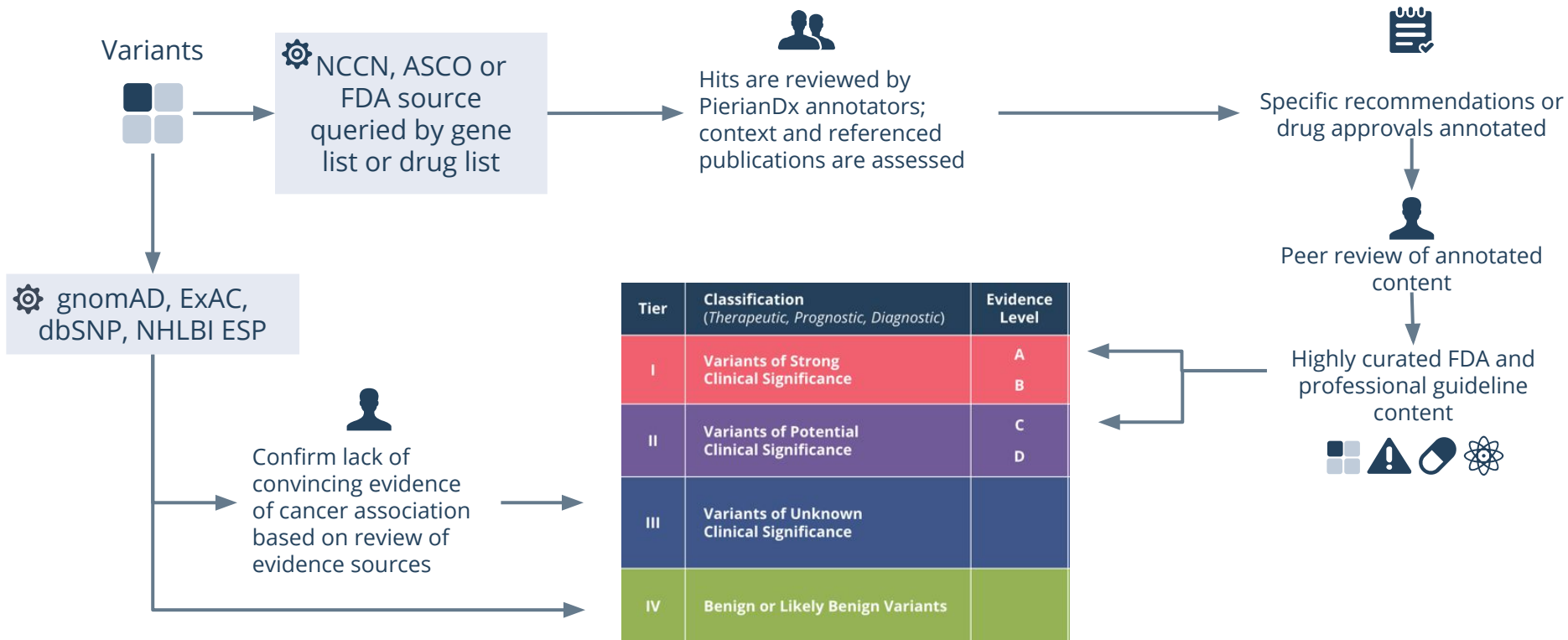
gnomAD, ExAC,  
dbSNP, NHLBI ESP



Confirm lack of  
convincing evidence  
of cancer association  
based on review of  
evidence sources

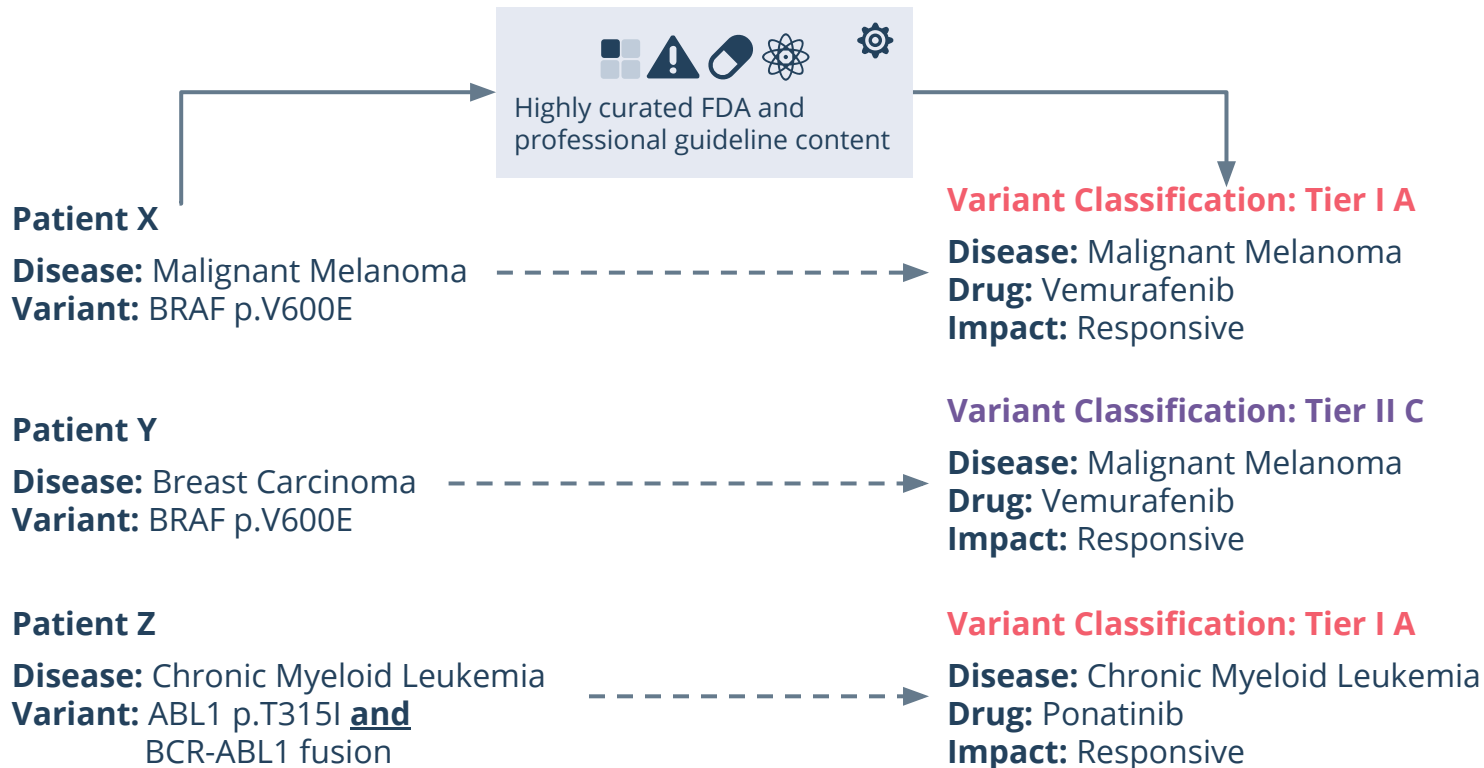
Tier	Classification (Therapeutic, Prognostic, Diagnostic)	Evidence Level
I	Variants of Strong Clinical Significance	A B
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# Driven by FDA and Professional Guidelines







# Automated Inference Example



# Combination of Automated and Manual Processes

Tier	Classification (Therapeutic, Prognostic, Diagnostic)	Evidence Level	Evidence
I	Variants of Strong Clinical Significance	A  B 	FDA-approved therapy; Included in professional guidelines Well-powered studies with consensus from field experts



## Literature Search Tool

**Search by:** Gene, variant syntax, disease drug





**Filter by:** Clinical relevance, study type, variant type, consequence, amino acid range

## Shared Medical Content

Classifications and Interpretations from molecular pathologists and clinical geneticists within the PierianDx network



# Combination of Automated and Manual Processes

Tier	Classification (Therapeutic, Prognostic, Diagnostic)	Evidence Level	Evidence
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## Clinical Trials

Software and human curation to identify the latest detail on recruiting trials.



## Review Evidence from Prior Cases

## Variant Frequency by Classification

[illegible]

# Breast Cancer

# Lung Cancer

## Conflicting Classifications/Interpretations

## Variant Frequency by Classification



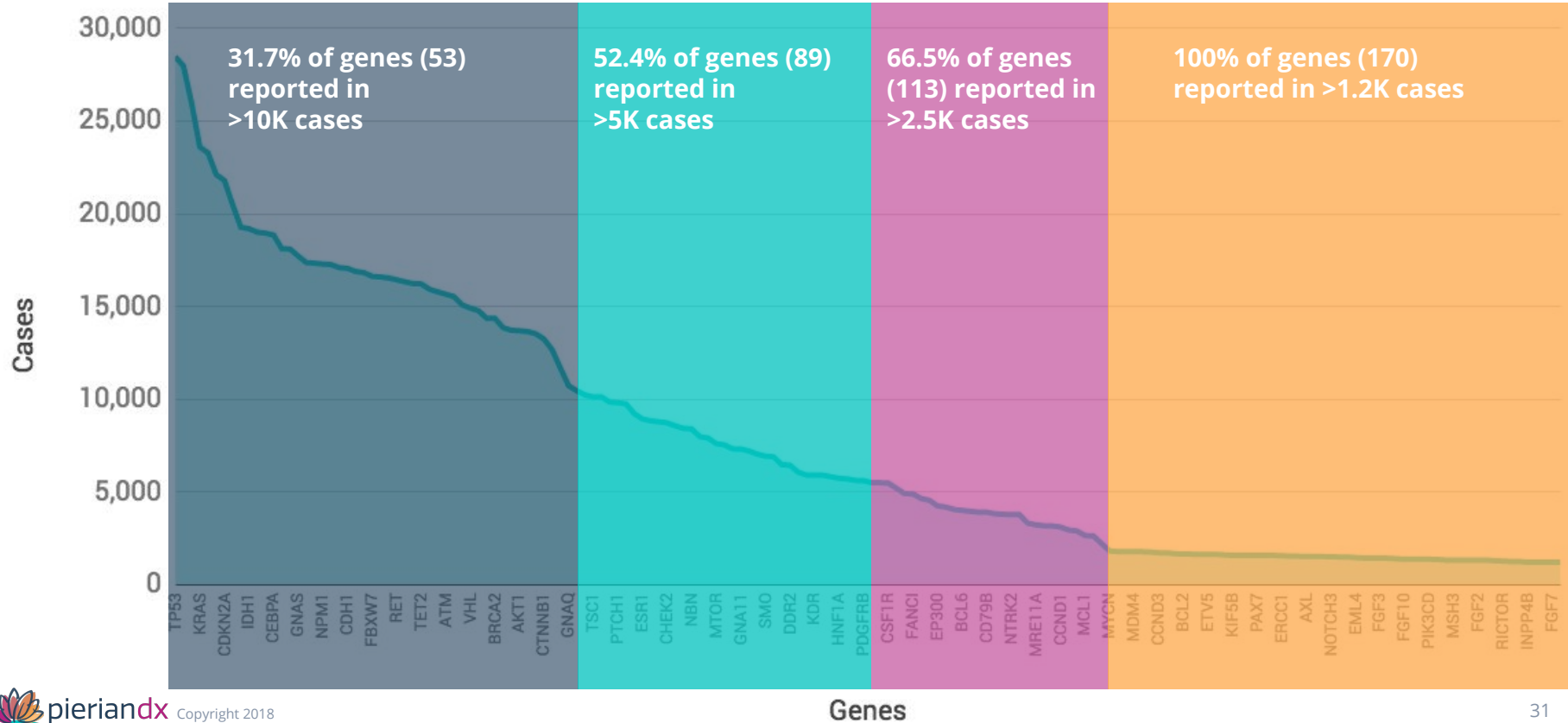
## Conflicting Classifications

[illegible]

Comprehensive and Accurate  
**KB Performance**

# Breadth of Content

Illumina TruSight Tumor 170 Evaluation



# Classification Accuracy

Illumina TruSight Tumor 170 Evaluation

Statistical Measure	Percentage
Specificity	99.99%
Sensitivity	93.77%
Positive Predictive Value	98.79%
Accuracy	99.95%

## Draft vs. Signed-out Classification

**True Positive:** Draft **Tier I** or **Tier II** classifications remained within **Tier I** or **Tier II** after sign-out

**True Negative:** Draft **Tier III** or **Tier IV** classification remained within **Tier III** or **Tier IV** after sign-out

**False Positive:** Draft **Tier I** or **Tier II** classification changed to **Tier III** or **Tier IV** after sign-out

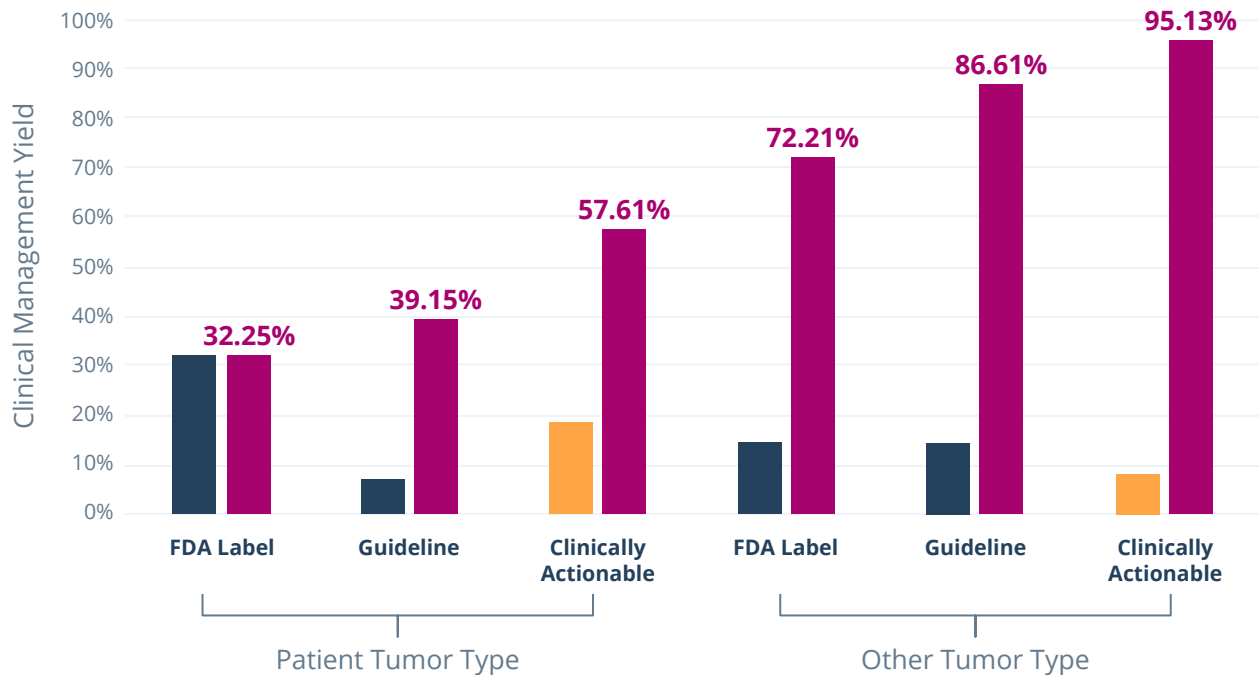
**False Negative:** Draft **Tier III** or **Tier IV** classification changed to **Tier I** or **Tier II** after sign-out



# Knowledgebase Performance Benchmarking

## Clinical Management Yield

Illumina TruSight Tumor 170 Evaluation



### Summary

Substantially greater yield than FDA labels and guideline content alone (68.15% vs. 95.13%)

26.98% of cases had clinically relevant variants that could not be inferred by FDA or guideline content alone.z

68.15%

Sum of  
FDA Labels/Guidelines

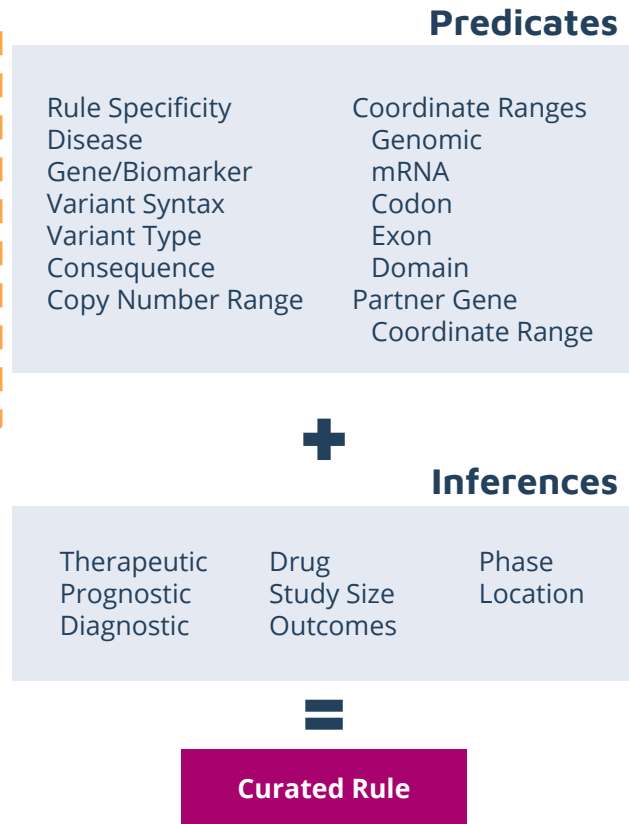
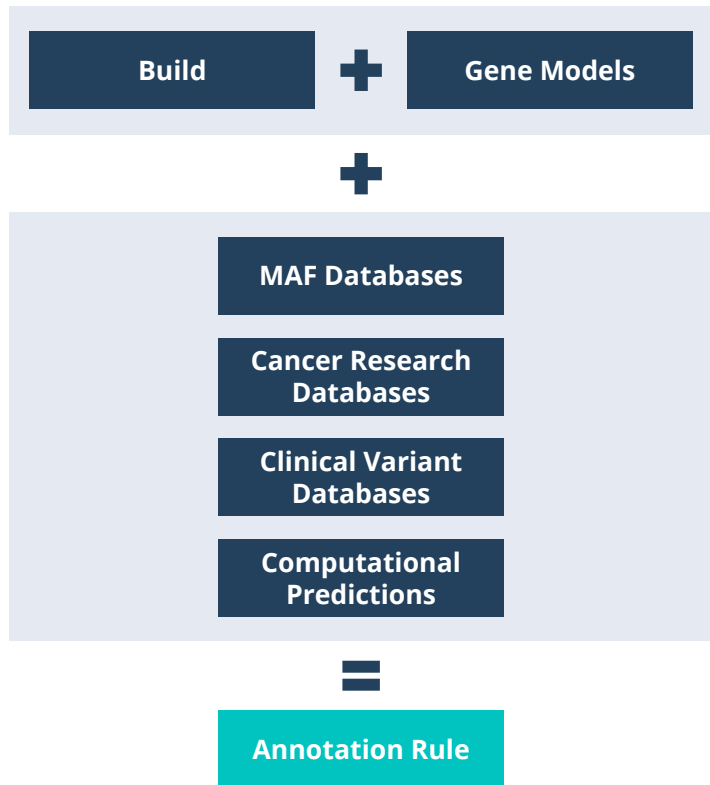
95.13%

Total Clinical  
Management Yield

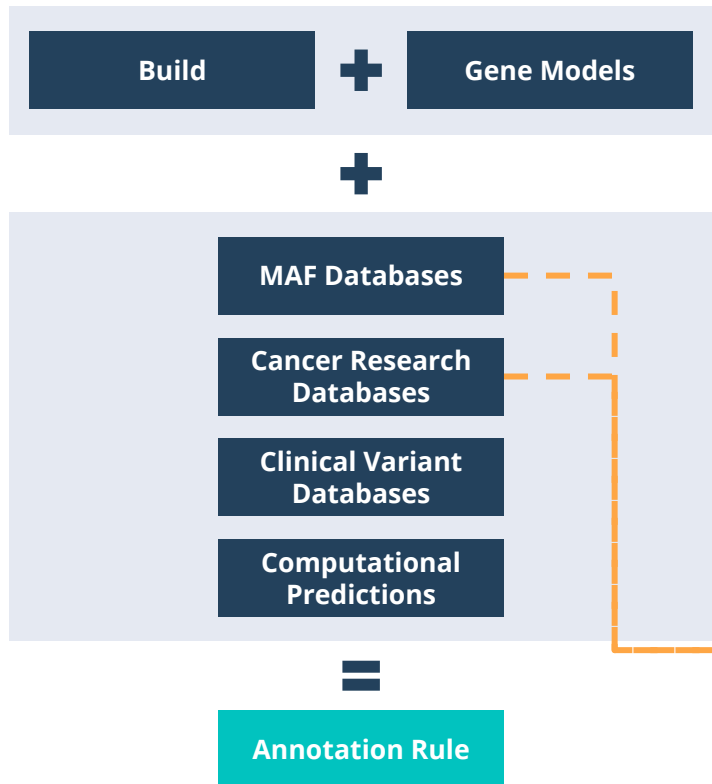
More Complete, Ready-to-sign-out Reports

# **Support for IVDs**

# Leverage and Extend Sources



# Leverage and Extend Sources



## Extend Curated Sources

Profile gene-variant frequencies in tumor types to identify biomarkers to pre-classify and interpret

## Predicates

Rule Specificity	Coordinate Ranges
Disease	Genomic
Gene/Biomarker	mRNA
Variant Syntax	Codon
Variant Type	Exon
Consequence	Domain
Copy Number Range	Partner Gene
	Coordinate Range



## Inferences

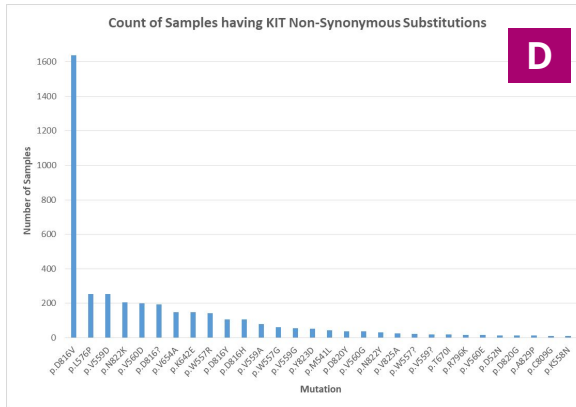
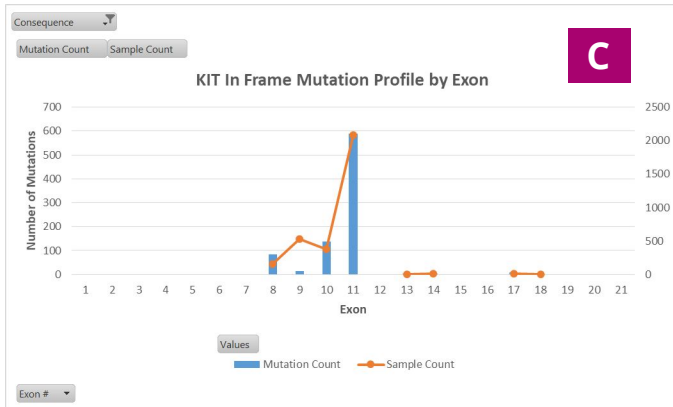
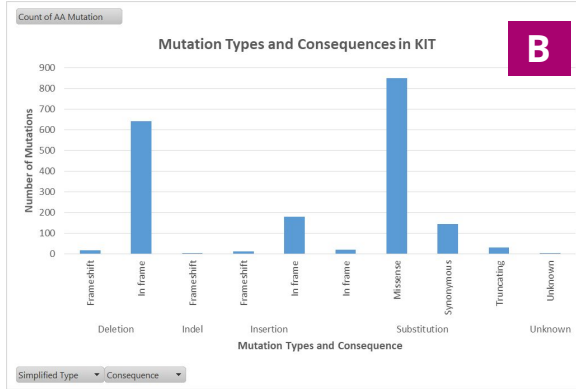
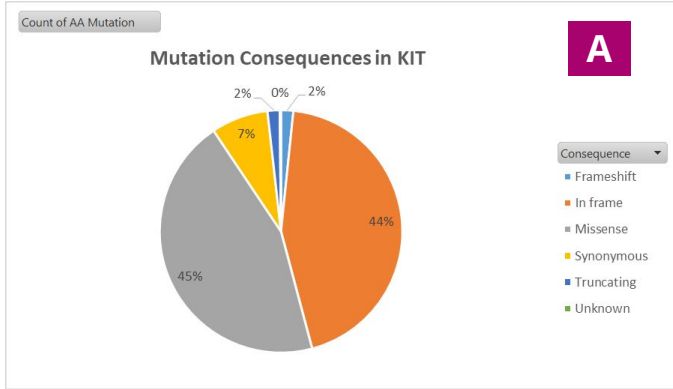
Therapeutic	Drug	Phase
Prognostic	Study Size	Location
Diagnostic	Outcomes	



Curated Rule

# Identification of Biomarkers to Review Proactively

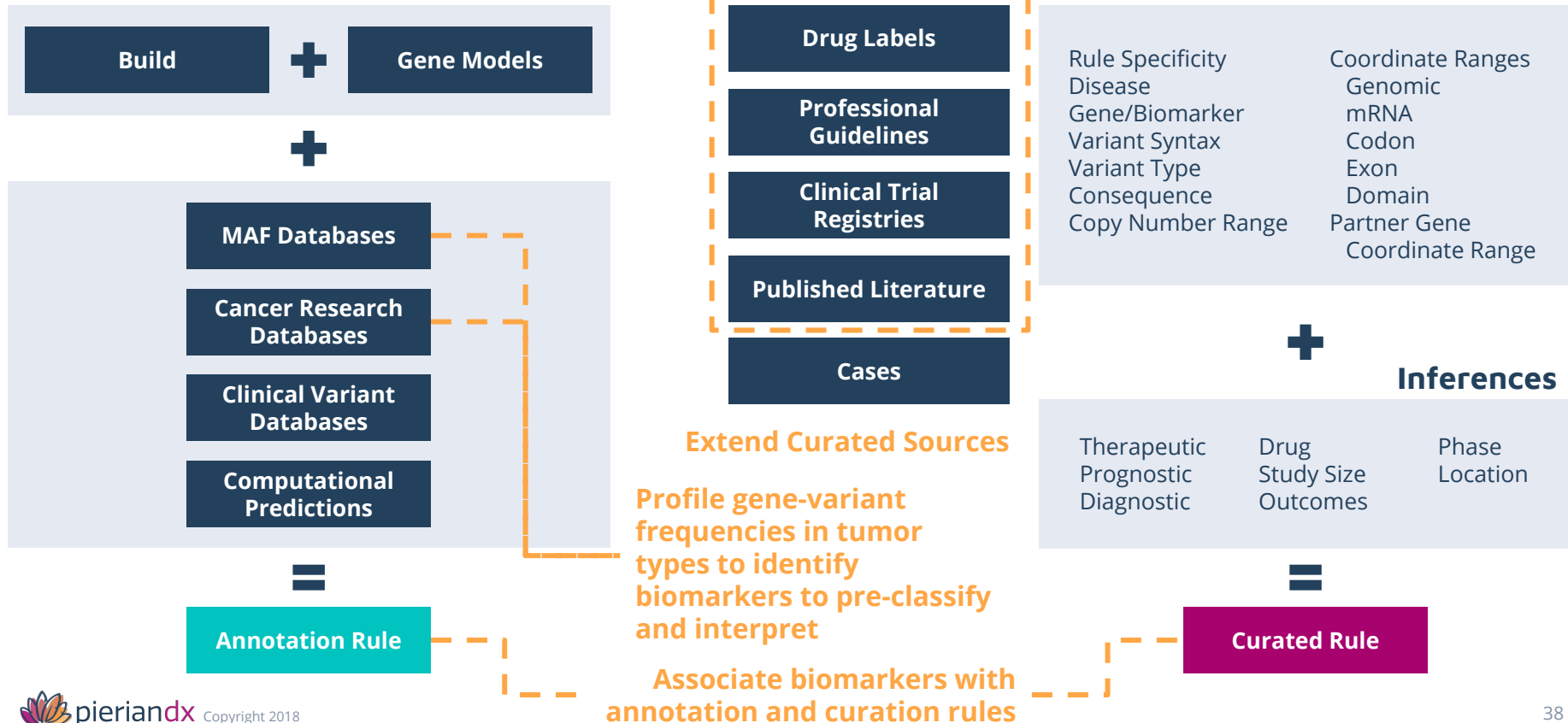
## KIT Mutations Example



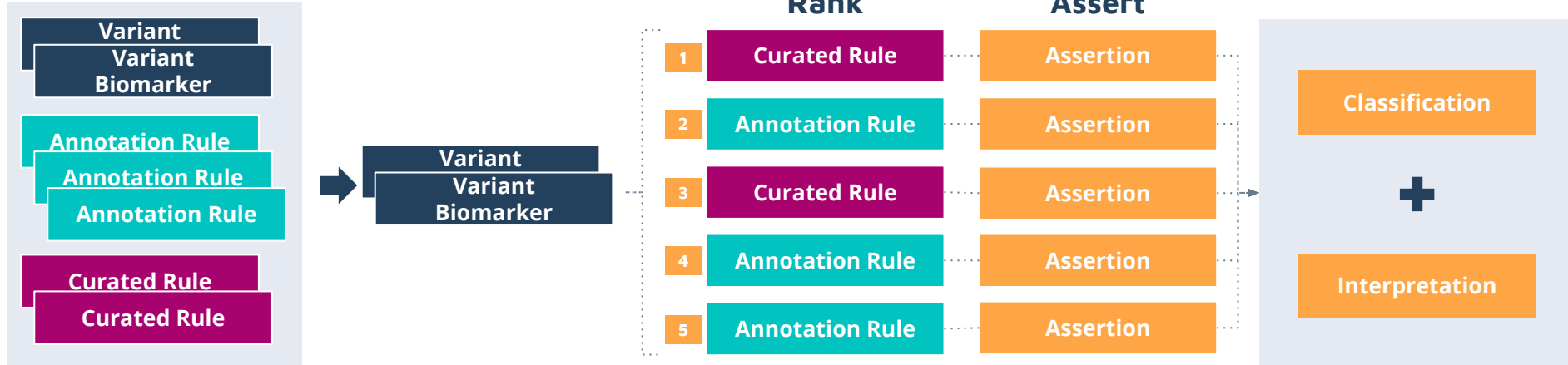
- 44% of KIT mutations from COSMIC maintain reading frame
- Deletions and insertions account for mutations maintaining reading frame; deletions 80%
- In frame mutations are clustered in exons 8-11
- Mutation hotspots outside these regions are present as missense substitutions

Rules at an exon level using consequence predicates and rules targeting hotspots may be used to capture the clinical intent driving FDA labels, guidelines, and other levels of evidence.

# Leverage and Extend Sources

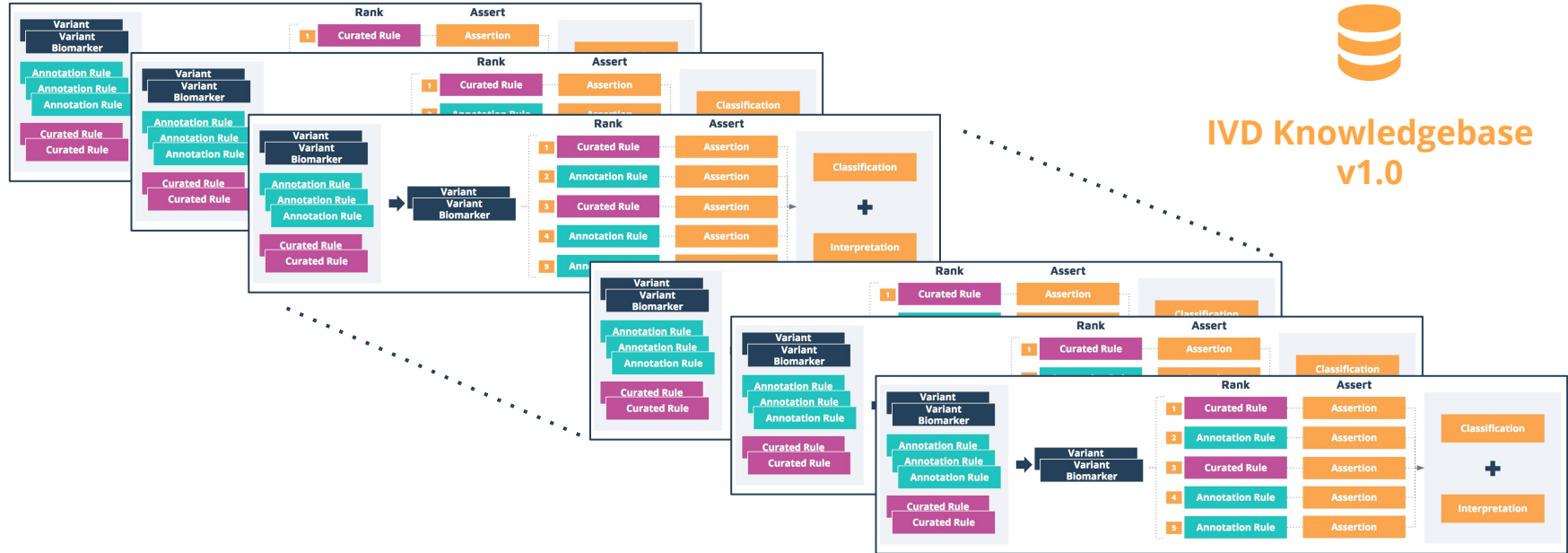


# Evidence Reviewing, Ranking, Asserting



Manually, ranked, asserted, classified, and interpreted proactively by PierianDx

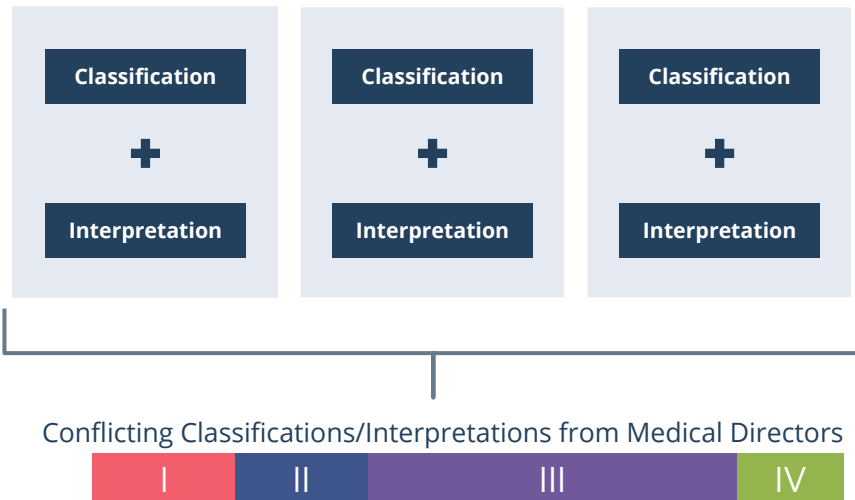
# Evidence Reviewing, Ranking, Asserting





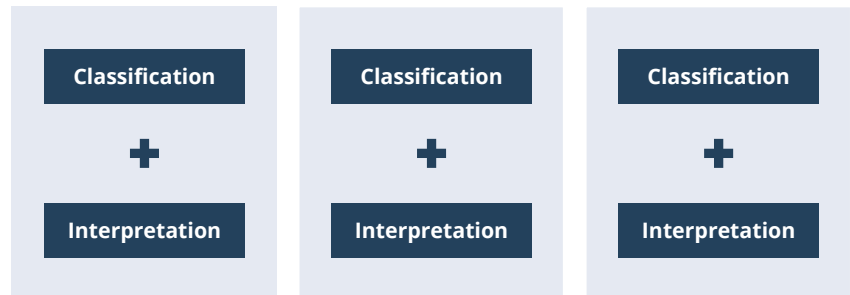
# Proactively Assess and Resolve Conflicts

Variant p.XXXX

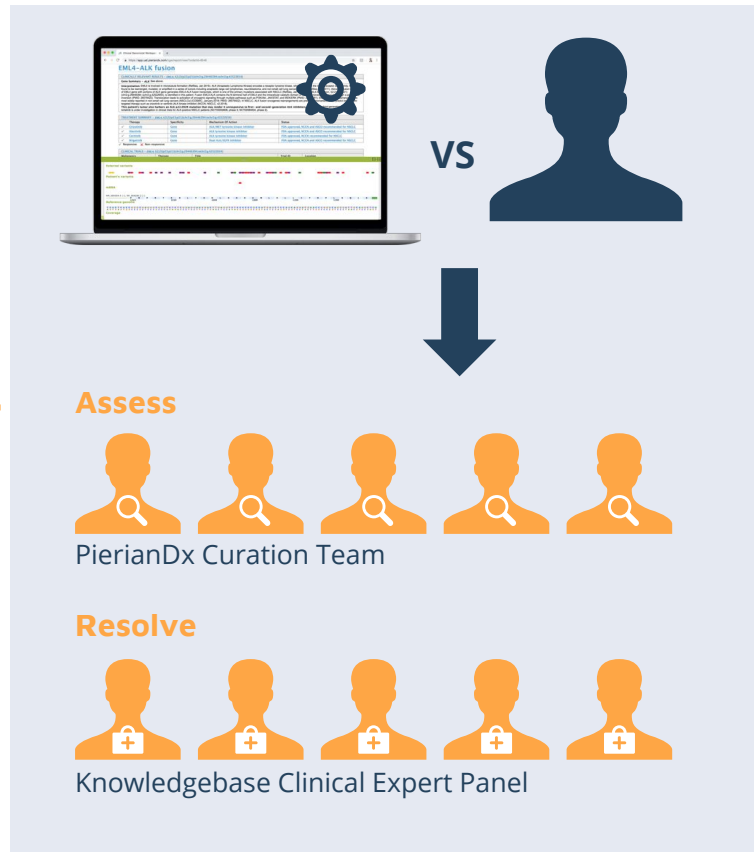


# Proactively Assess and Resolve Conflicts

Variant p.XXXX

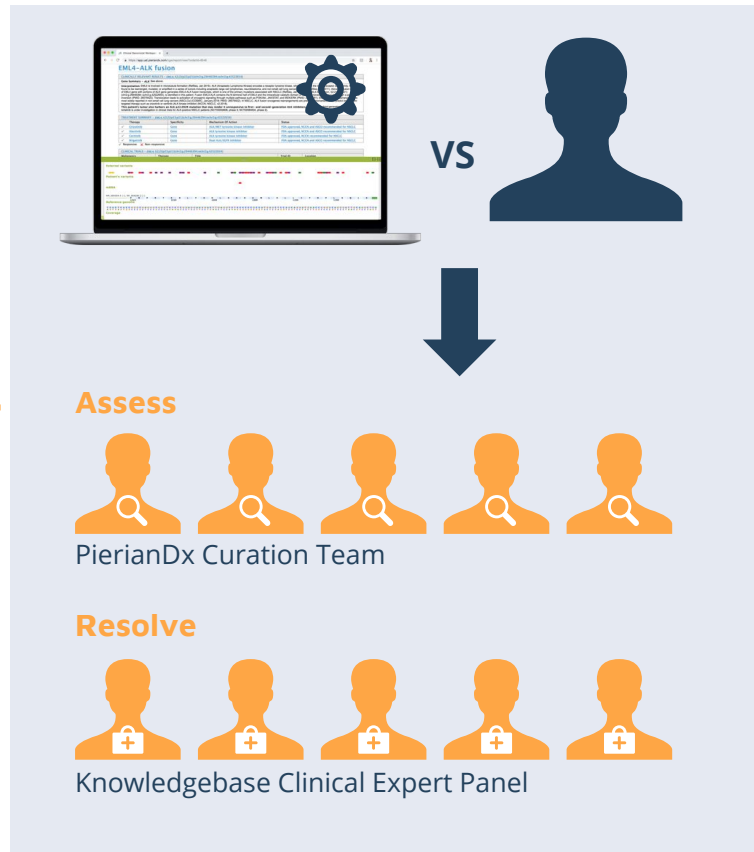
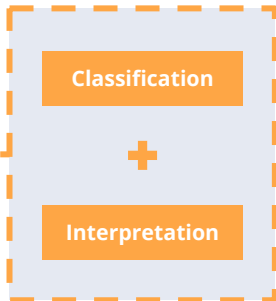


Conflicting Classifications/Interpretations from Medical Directors



# Proactively Draft Consensus Interpretations

Variant p.XXXX (v2.0)



## Conclusion

# Leadership in LDT/IVD Support

- PierianDx is taking a leadership position in the cancer community
- Key differentiators in sharing network and inferencing concepts
- Facilitating consensus-based knowledgebase for IVD
- Knowledgebase will continually be informed, trained by community
- **Reports will continually become more comprehensive, automated, and ready-to-sign-out**

