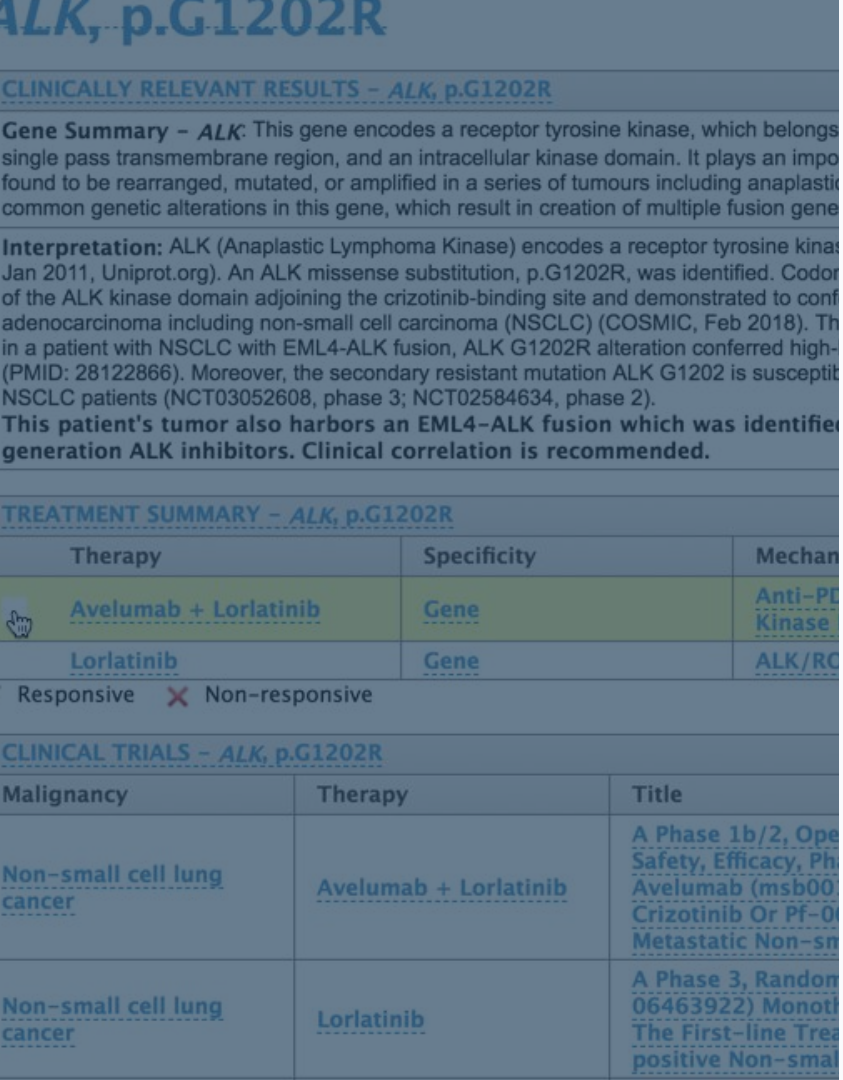


pieriandx

Supporting NGS-Based *In Vitro* Diagnostics

A Knowledge Sharing Approach

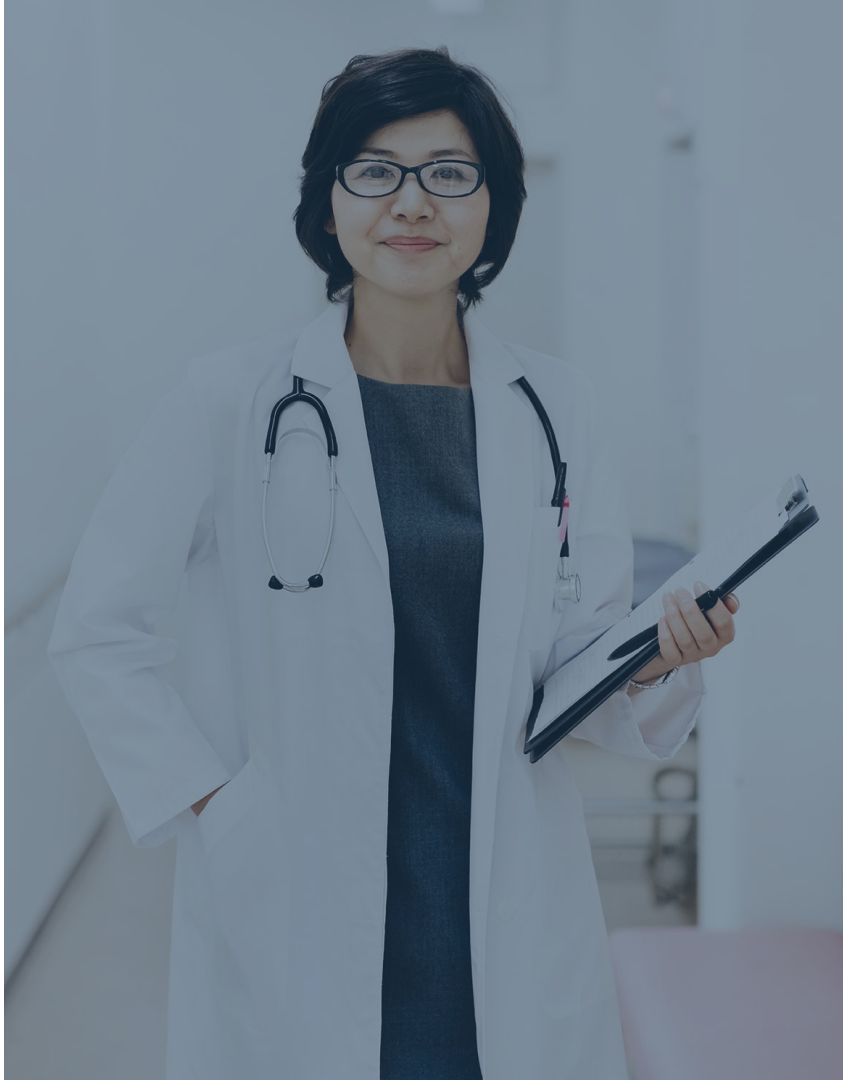
Rakesh Nagarajan, MD, PhD
Executive Chairman and Founder
PierianDx

Moderated by: Josh Forsythe

Supporting NGS-Based IVD Assays

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

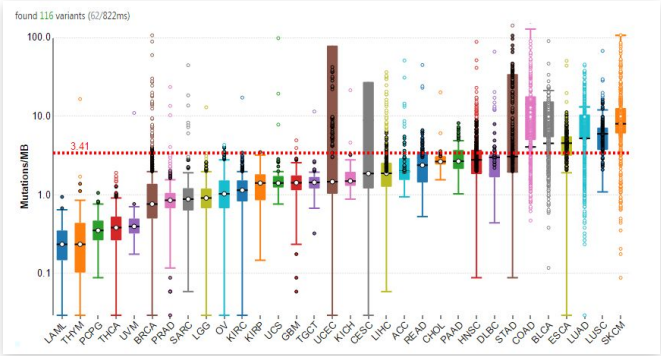
From single gene assays and small targeted hotspot panels to large capture-based assays and exome

More Variants and Variant Types

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

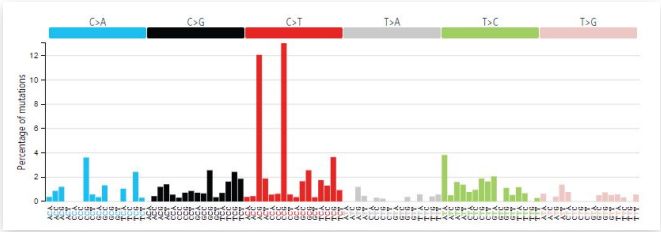
More comprehensive detection of clinically relevant features

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



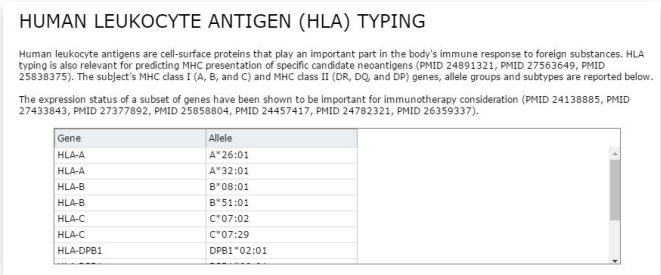
Mutational Load

(determine candidacy for immunotherapy)



Mutational Signature

(identify etiology and predict response to therapy)



HLA Typing

(used for clinical trial enrollment)

FDA Approved

Targeted Therapies Increasing

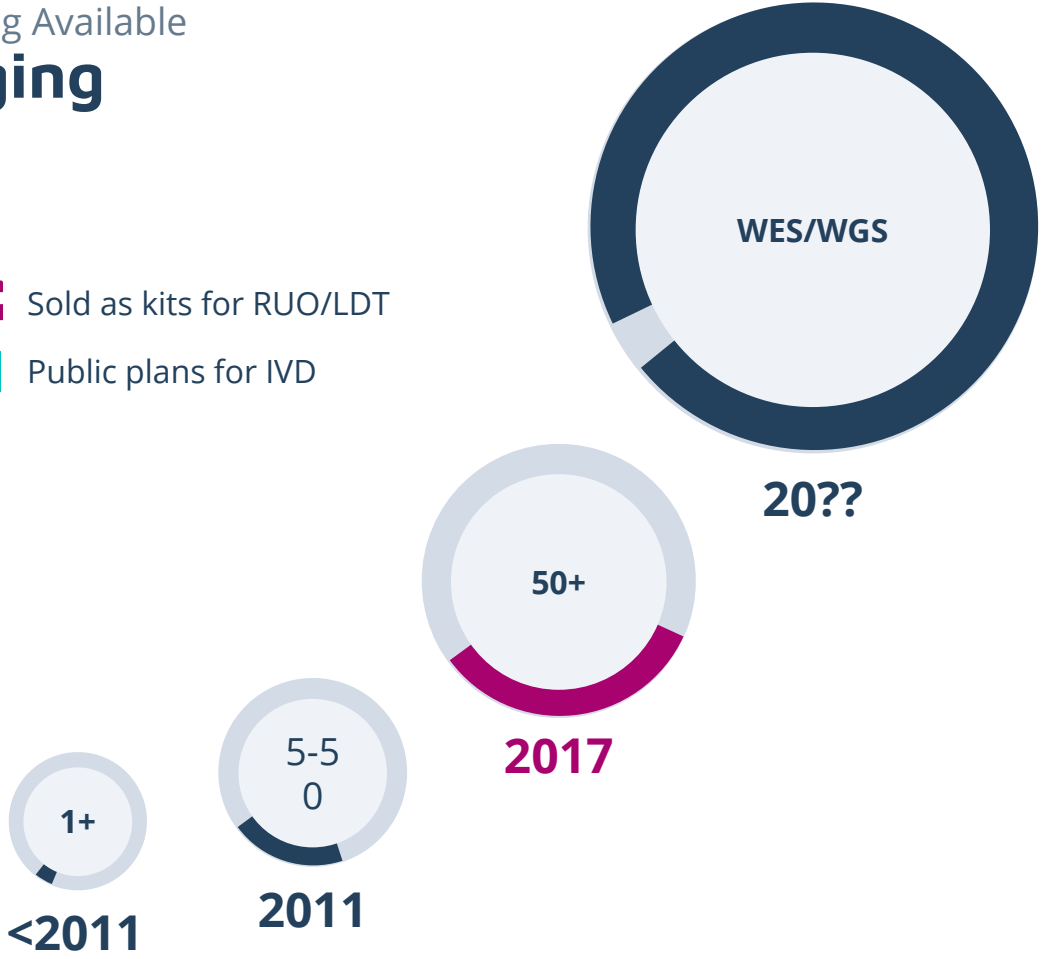
Larger, More Comprehensive Tests Becoming Available

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



Challenges of Time and Accuracy

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
II	**Variants of Potential Clinical Significance**	C D	FDA-approved therapies for different tumor types or investigational therapies; Multiple small studies w/ consensus Preclinical trials or a few case reports without consensus
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Li et al. "Standards and Guidelines for the Interpretation and Reporting of Somatic Variants in Cancer", 2017

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Ex. TSO-500

100s of Variants
>4 Hours

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

First Solving Challenges with LDTs

PierianDx Background

Pioneers in Precision Medicine

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

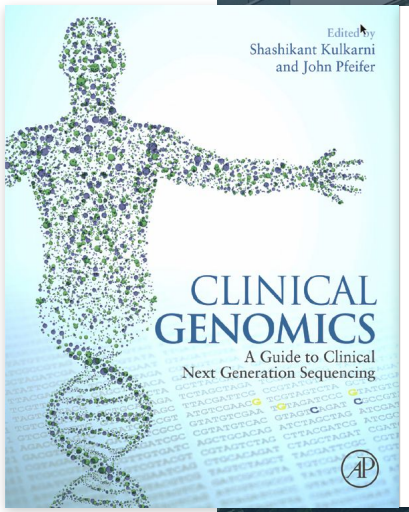
PierianDx est. after ~50 labs visit WashU to learn how clinical NGS is operationalized.

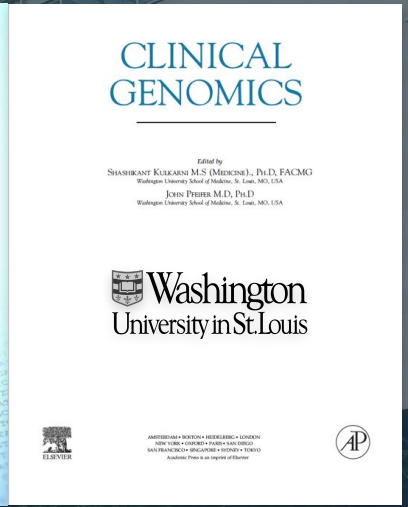
2011

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

2003

WashU plays critical role in Human Genome Project.





Technology-enabled Services

Complete LDT/IVD Support

Clinical Next Generation Sequencing Workflow

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**

Clinical Genomics Workspace + Knowledgebase

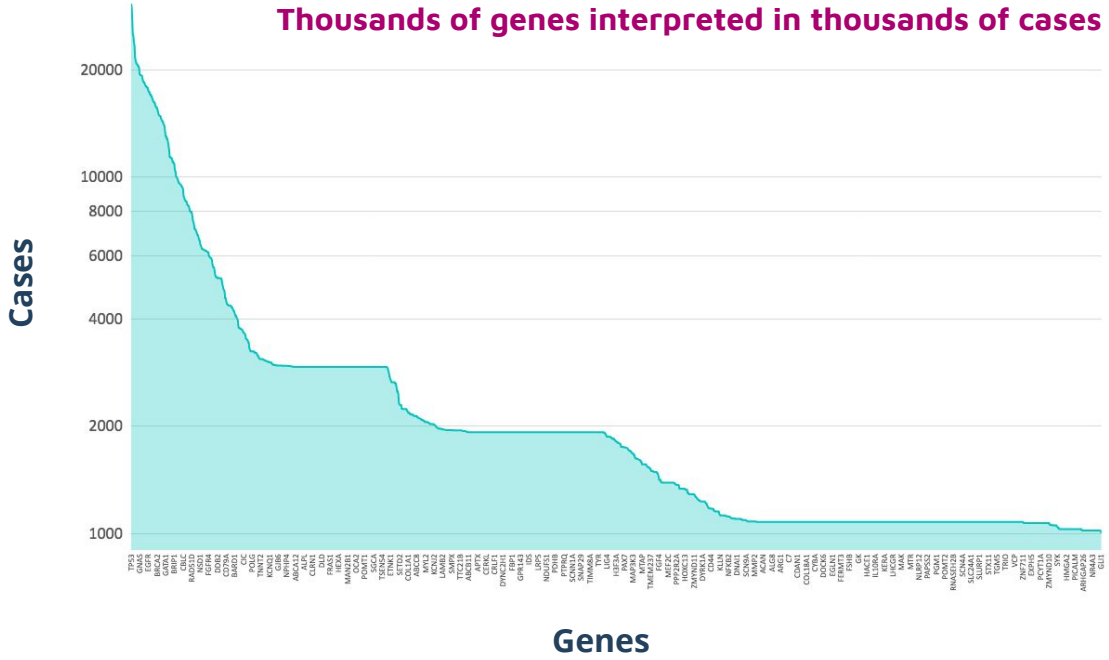
Assay Validation Services

Interpretation Services

Laboratory Services

Fast Facts

PierianDx Knowledgebase



By the Numbers

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

Experience

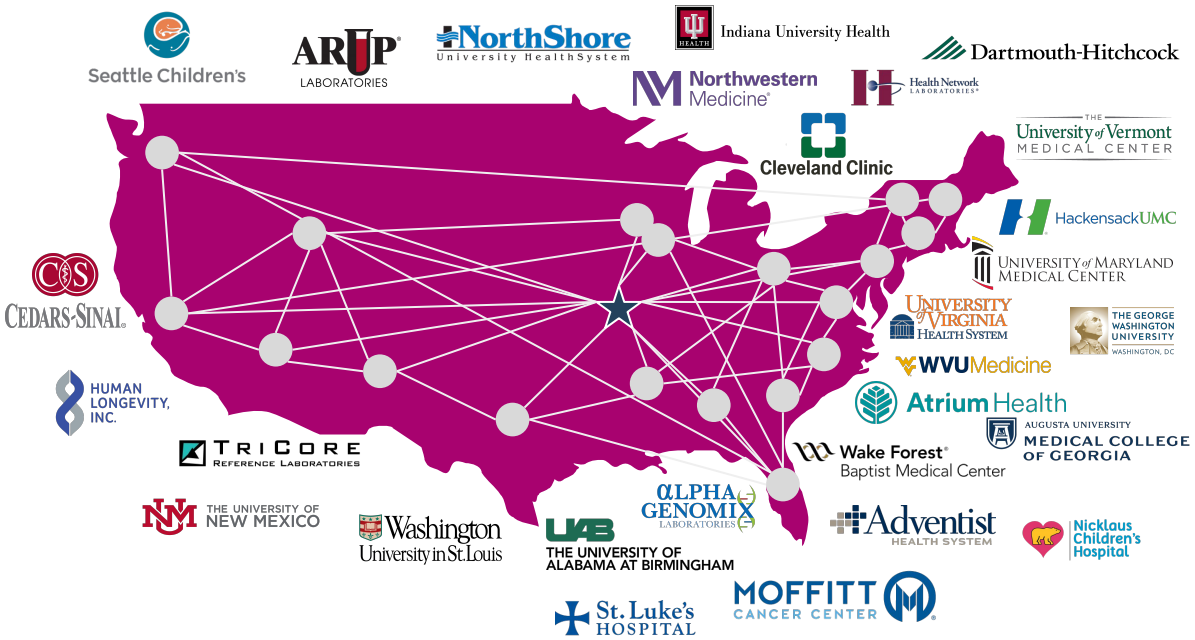
- 40+ PierianDx annotators
- 100 years of combined curation effort
- **Led by board certified molecular genetic pathologist**
- 80+ contributing molecular pathologist customers

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

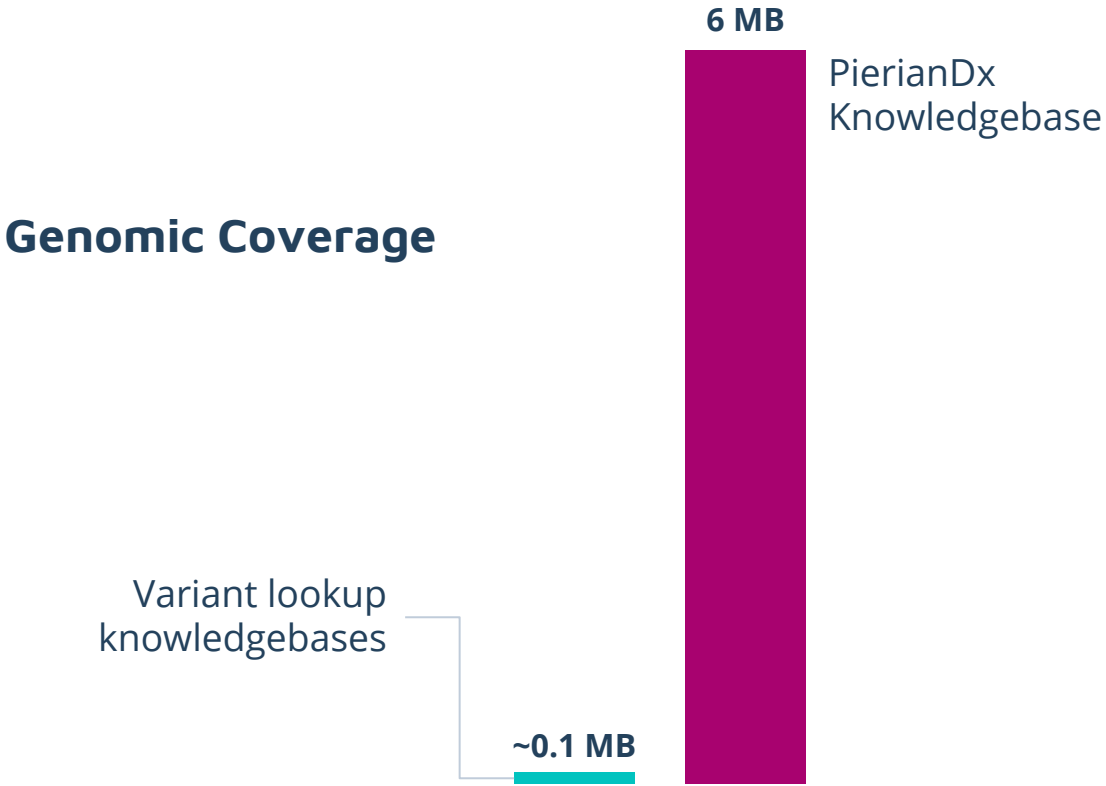
- FDA approved labels
- NCCN, ASCO Guidelines
- Active, recruiting clinical trials

Shared Medical Content

- Clinical interpretations and classifications from partners and PierianDx

More Coverage than a Simple Variant Lookup

Rules Engine and Predicate Model



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

Build + **Gene Models**

+

MAF Databases

Cancer Research Databases

Clinical Variant Databases

Computational Predictions

=

Annotation Rule

Drug Labels

Professional Guidelines

Clinical Trial Registries

Published Literature

Cases

Predicates

Rule Specificity
Disease
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Variant Syntax
Variant Type
Consequence
Copy Number Range

Coordinate Ranges
- Genomic
- mRNA
- Codon
- Exon
- Domain

Partner Gene
- Coordinate Range

+

Inferences

Therapeutic
Prognostic
Diagnostic

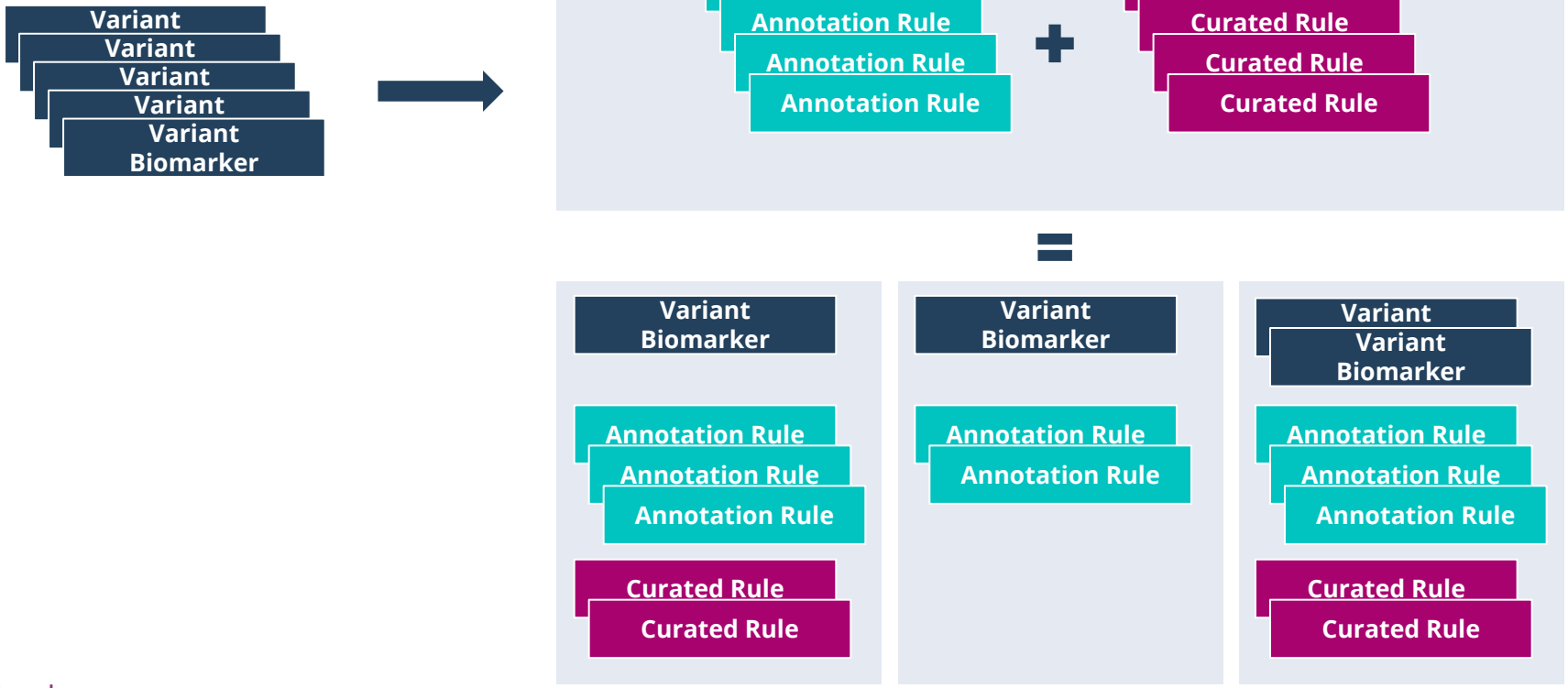
Drug
Study Size
Outcomes

Phase
Location

=

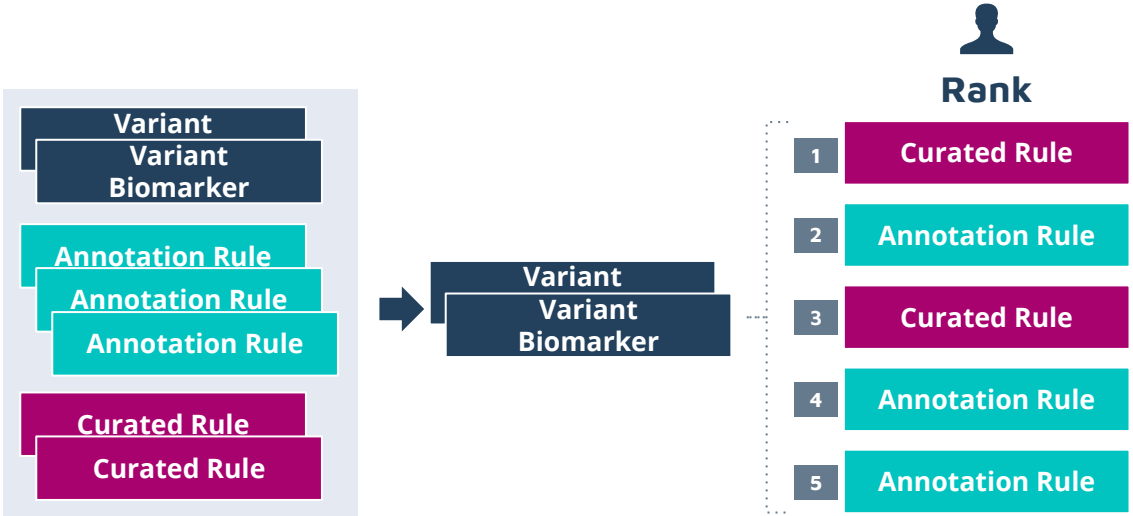
Curated Rule

Biomarker Processing



Rules Engine and Predicate Model Explained

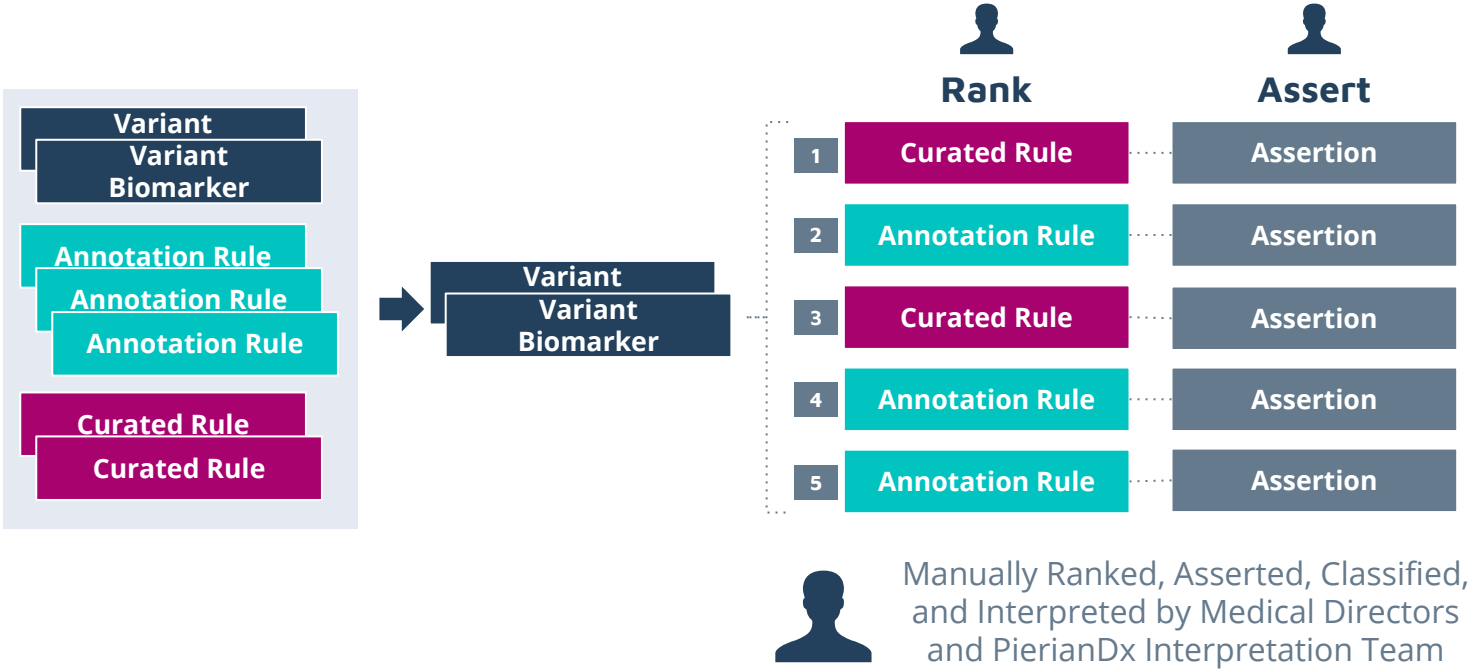
Evidence Reviewing, Ranking, Asserting



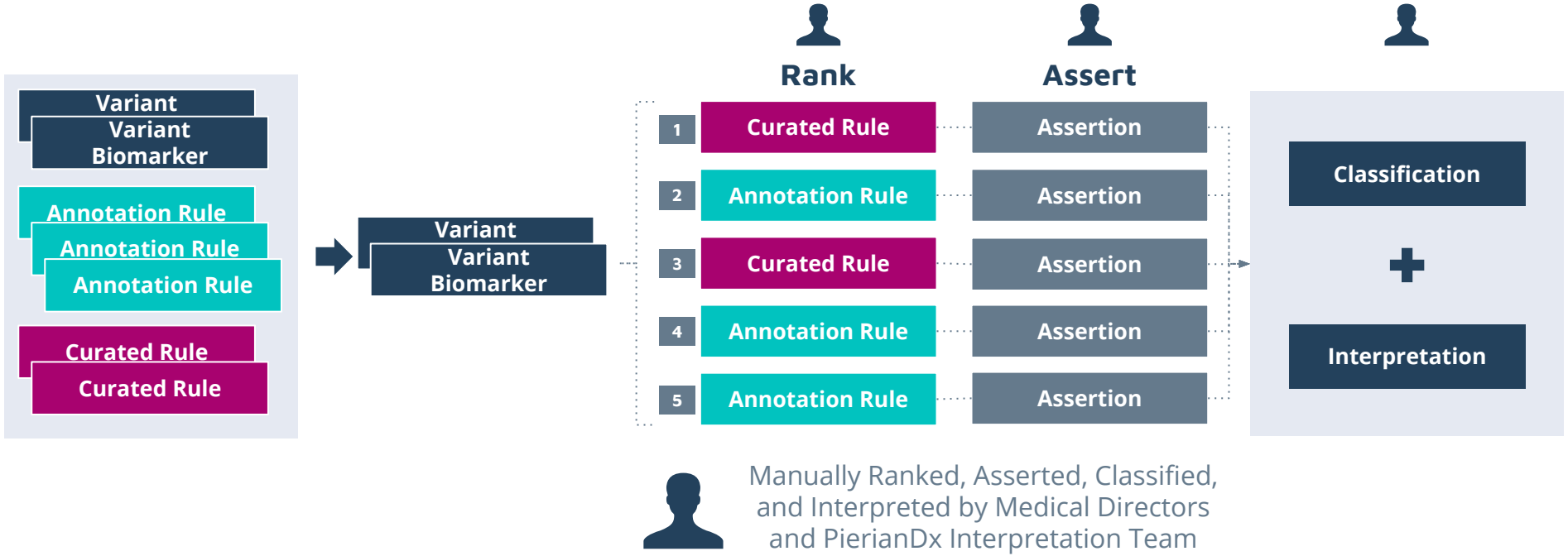
Manually Ranked, Asserted, Classified, and Interpreted by Medical Directors and PierianDx Interpretation Team

Rules Engine and Predicate Model Explained

Evidence Reviewing, Ranking, Asserting



Evidence Reviewing, Ranking, Asserting



Data Science + Human Acumen

Applied Framework

AMP/CAP/ASCO Consensus Guidelines

Somatic Variant Classification Scheme

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
II	**Variants of Potential Clinical Significance**	**C** **D**	FDA-approved therapies for different tumor types or investigational therapies; Multiple small studies w/ consensus Preclinical trials or a few case reports without consensus
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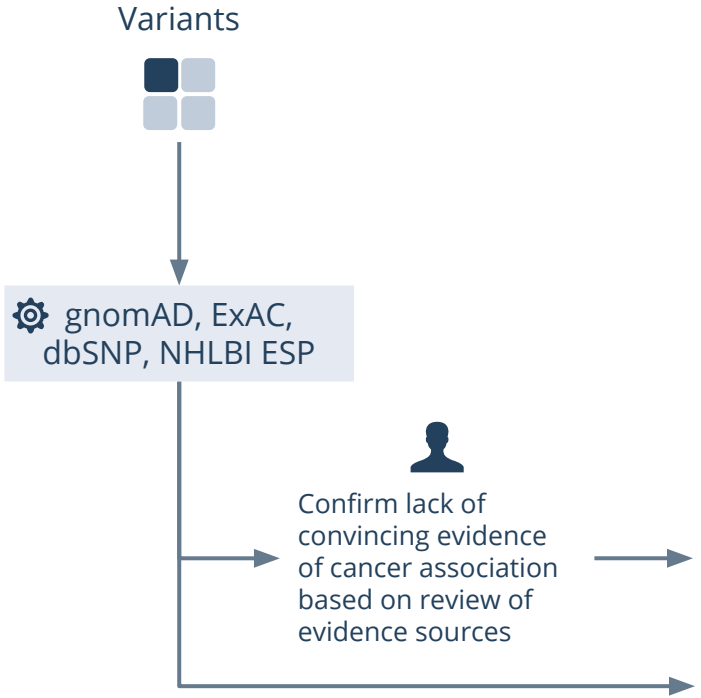
Tier III and Tier IV Classifications

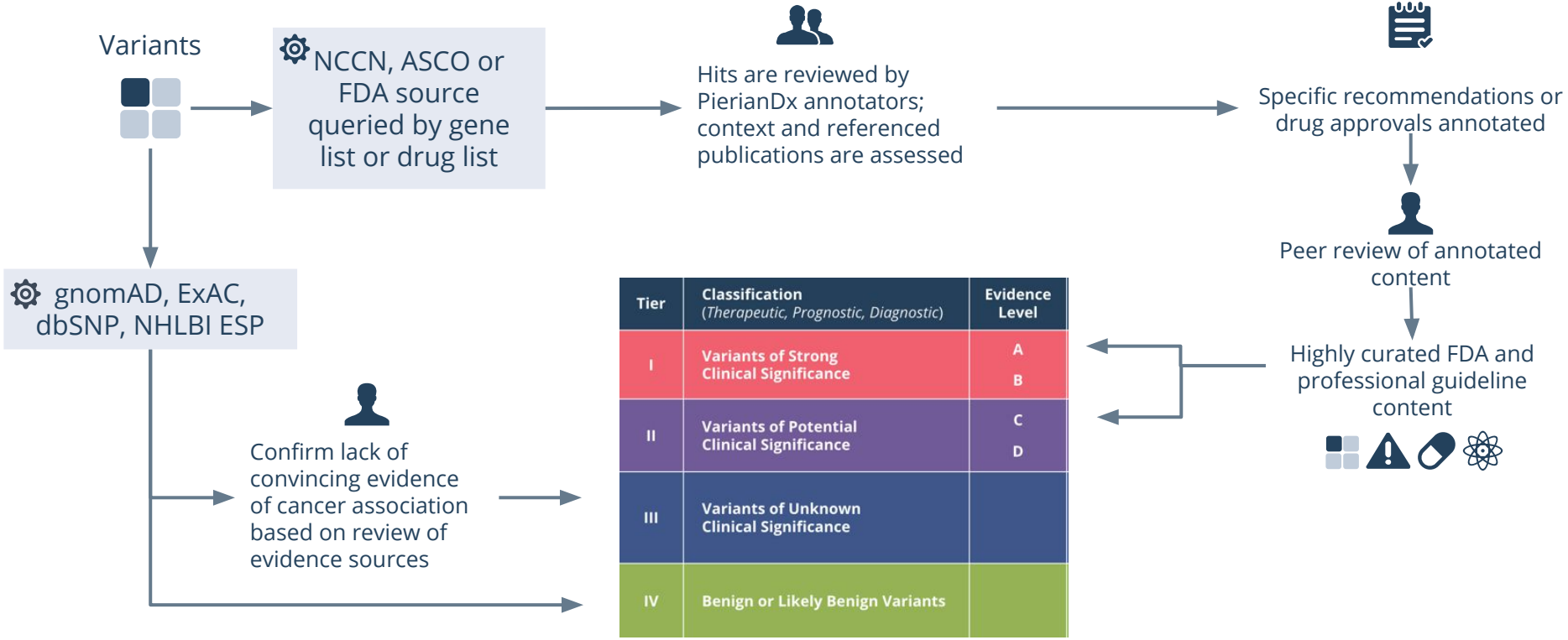
Driven (largely) by Population Frequency Databases



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	

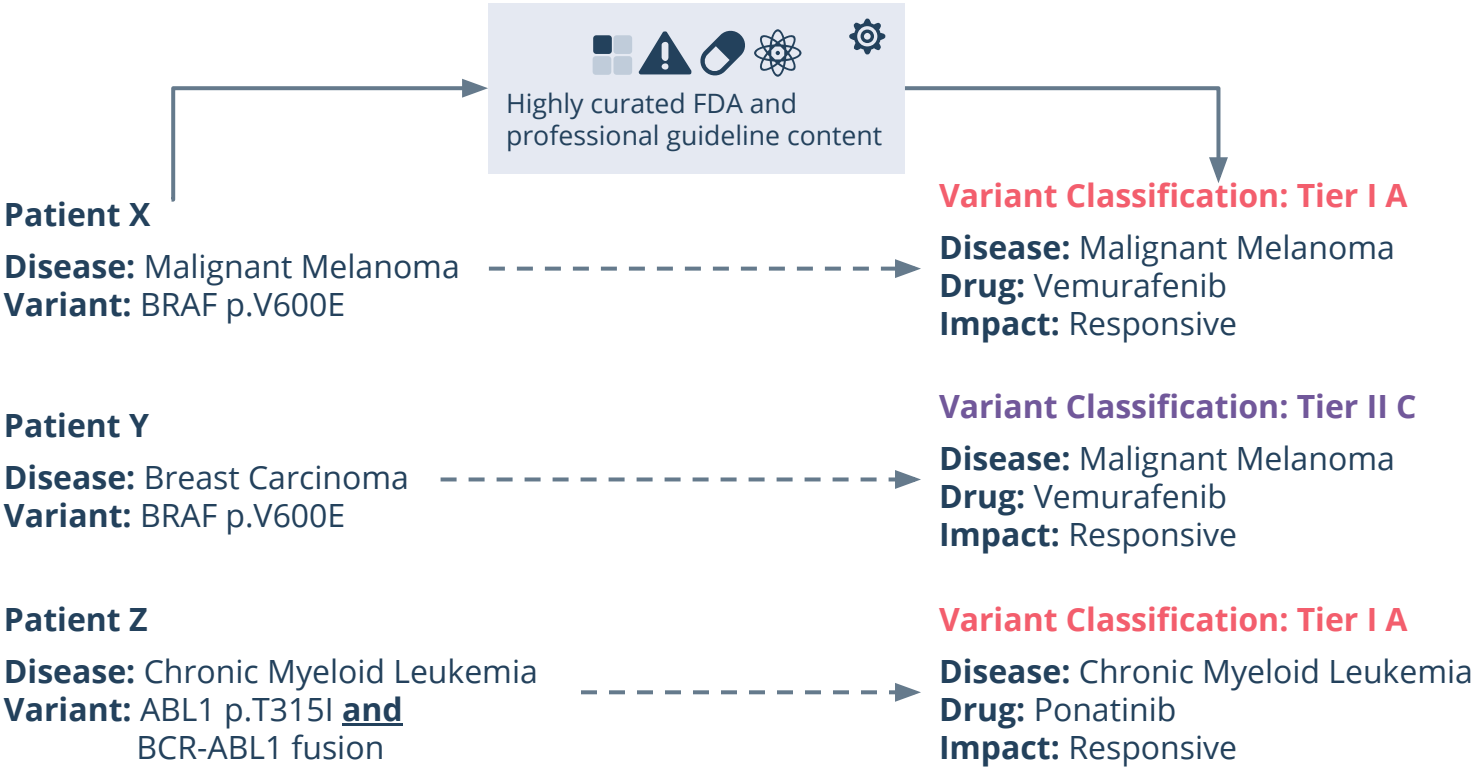
Tier IA and Tier IIC Classifications

Driven by FDA and Professional Guidelines



FDA and Professional Guideline-based

Automated Inference Example



Tier I Classification Today

Combination of Automated and Manual Processes

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

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

Tier II Classification Today

Combination of Automated and Manual Processes

Tier	Classification (*Therapeutic, Prognostic, Diagnostic*)	Evidence Level	Evidence
I	**Variants of Strong Clinical Significance**	A	FDA-approved therapy; Included in professional guidelines
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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

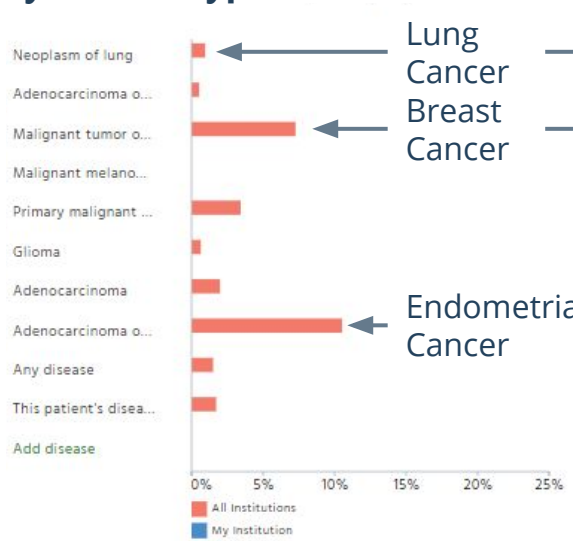
Clinical Trials

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

Shared Classifications and Interpretations

Review Evidence from Prior Cases

Variant Frequency by Tumor Type



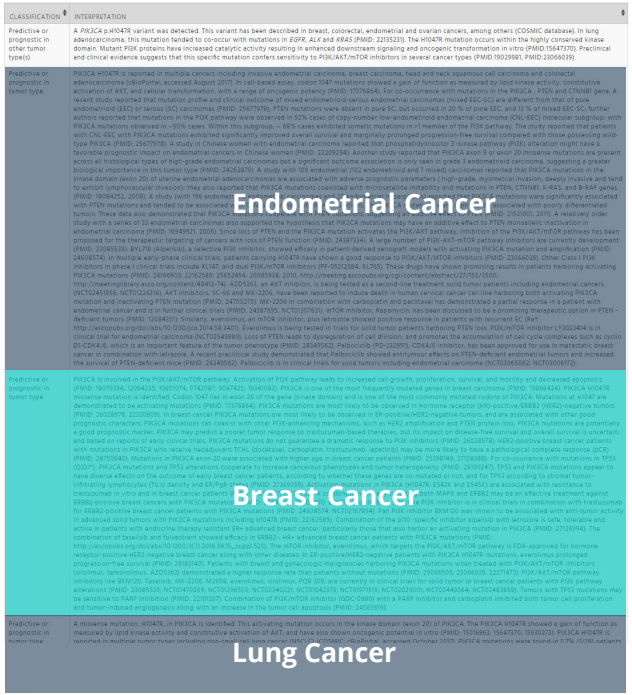
Variant Frequency by Classification

Lung Cancer

Breast Cancer

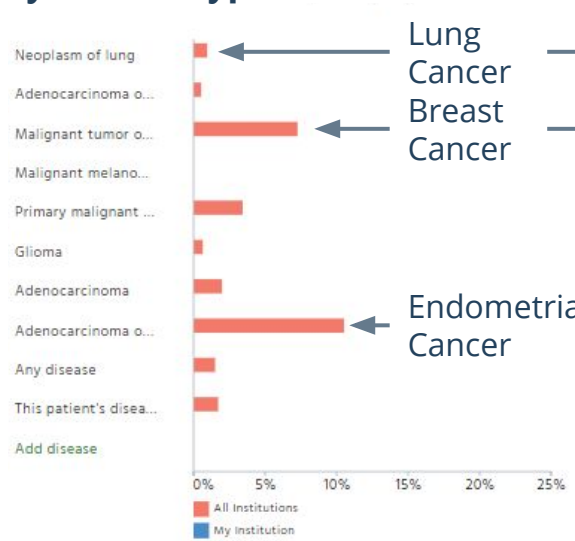
Endometrial Cancer

Clinical Interpretations



Conflicting Classifications/Interpretations

Variant Frequency by Tumor Type



Lung Cancer

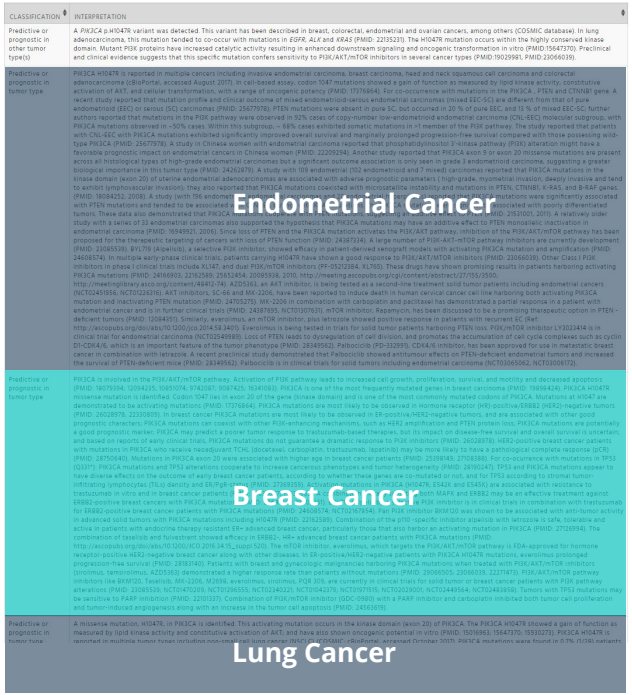
Breast Cancer

Endometrial Cancer

Variant Frequency by Classification

Conflicting Classifications

Clinical Interpretations

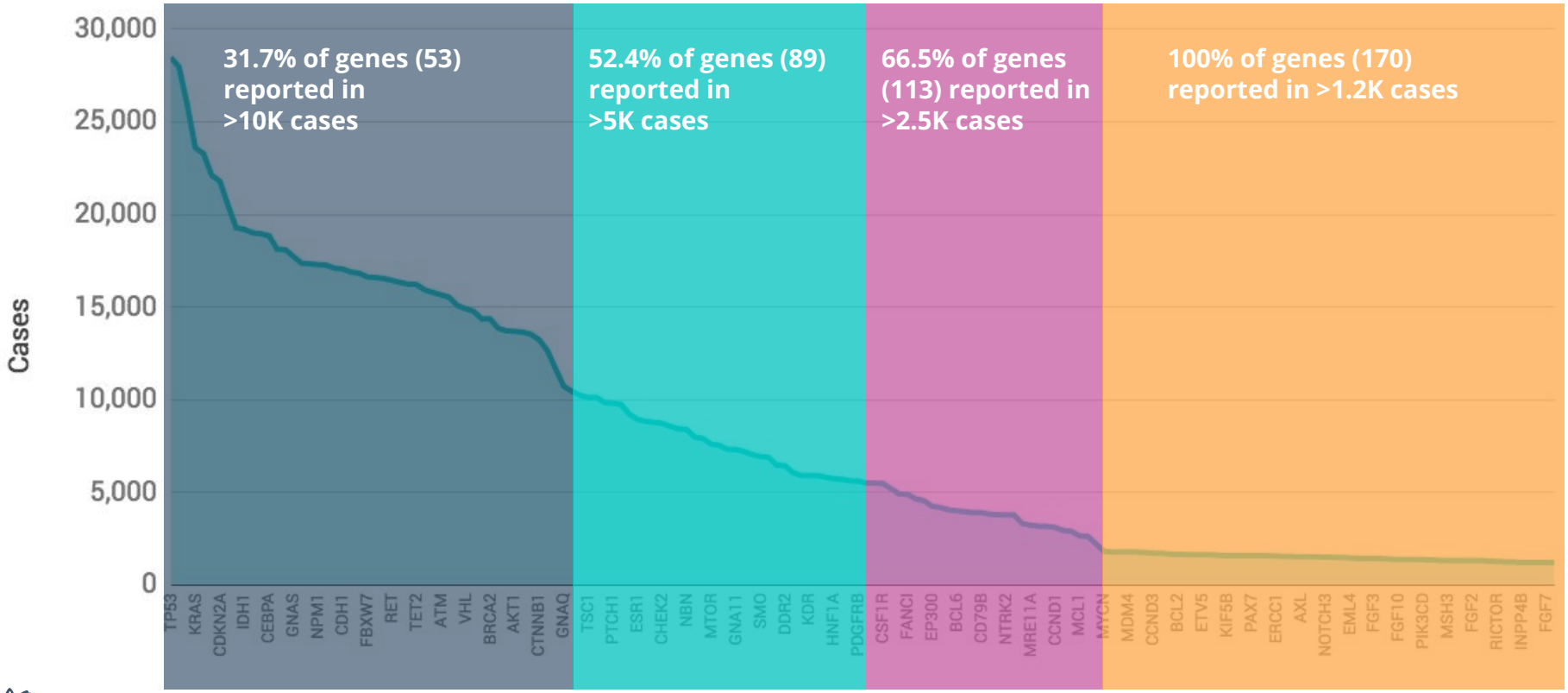


Comprehensive and Accurate

KB Performance

Breadth of Content

Illumina TruSight Tumor 170 Evaluation



Knowledgebase Performance Benchmarking

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

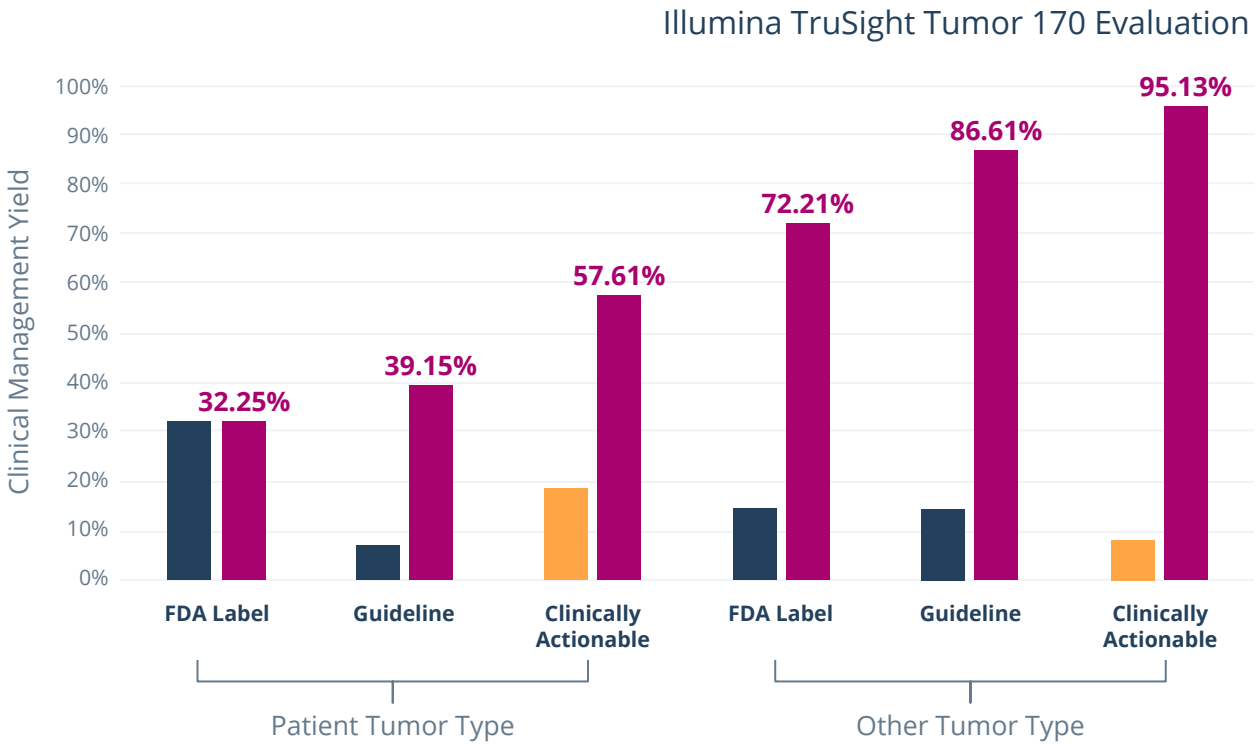
True Negative: Draft **Tier III** or **Tier IV** classification remained within **Tier III** or **Tier IV** after sign-out

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



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

Genome, Public, Clinical

Leverage and Extend Sources

Build + **Gene Models**

+

MAF Databases

Cancer Research Databases

Clinical Variant Databases

Computational Predictions

=

Annotation Rule

Drug Labels

Professional Guidelines

Clinical Trial Registries

Published Literature

Cases

Extend Curated Sources

Predicates

Rule Specificity
Disease
Gene/Biomarker
Variant Syntax
Variant Type
Consequence
Copy Number Range

Coordinate Ranges
- Genomic
- mRNA
- Codon
- Exon
- Domain

Partner Gene
- Coordinate Range

+

Inferences

Therapeutic
Prognostic
Diagnostic

Drug
Study Size
Outcomes

Phase
Location

=

Curated Rule

Genome, Public, Clinical

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Cases

Extend Curated Sources

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

Predicates

Rule Specificity
Disease
Gene/Biomarker
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Partner Gene
- Coordinate Range

+

Inferences

Therapeutic
Prognostic
Diagnostic

Drug
Study Size
Outcomes

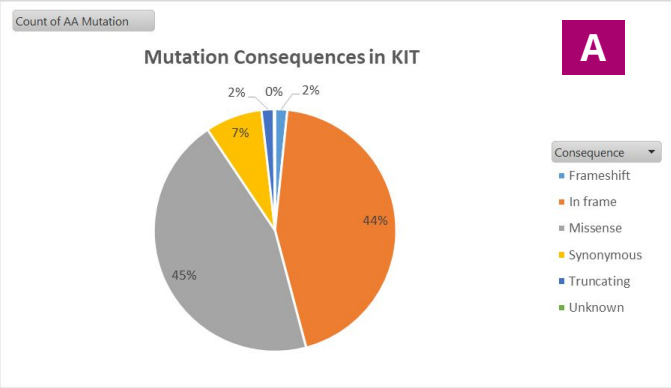
Phase
Location

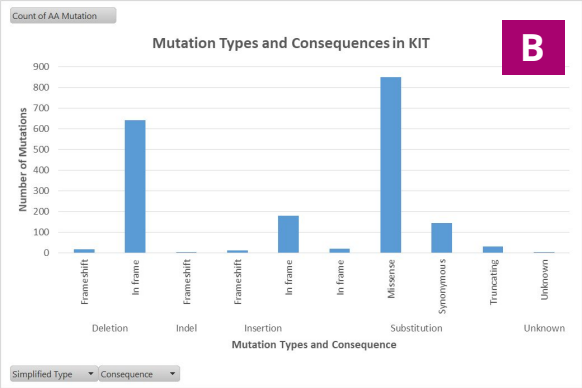
=

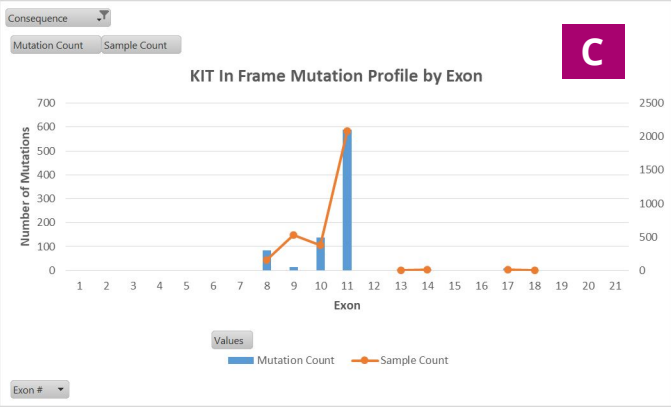
Curated Rule

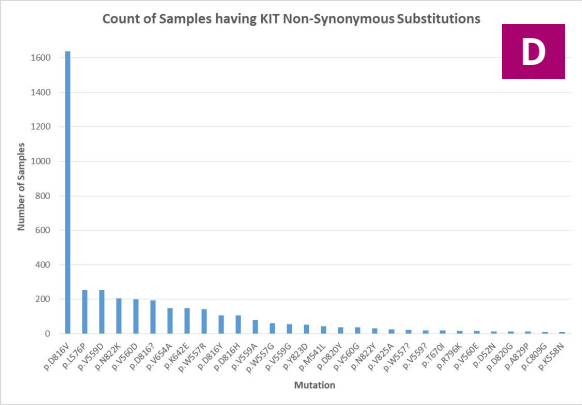
Identification of Biomarkers to Review Proactively

KIT Mutations Example









A. 44% of KIT mutations from COSMIC maintain reading frame

B. Deletions and insertions account for mutations maintaining reading frame; deletions 80%

C. In frame mutations are clustered in exons 8-11

D. 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.

Genome, Public, Clinical

Leverage and Extend Sources

Build + Gene Models

+

MAF Databases

Cancer Research Databases

Clinical Variant Databases

Computational Predictions

=

Annotation Rule

Drug Labels

Professional Guidelines

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Cases

Extend Curated Sources

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

Associate biomarkers with annotation and curation rules

Predicates

Rule Specificity
Disease
Gene/Biomarker
Variant Syntax
Variant Type
Consequence
Copy Number Range

Coordinate Ranges
- Genomic
- mRNA
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Partner Gene
- Coordinate Range

+

Inferences

Therapeutic
Prognostic
Diagnostic

Drug
Study Size
Outcomes

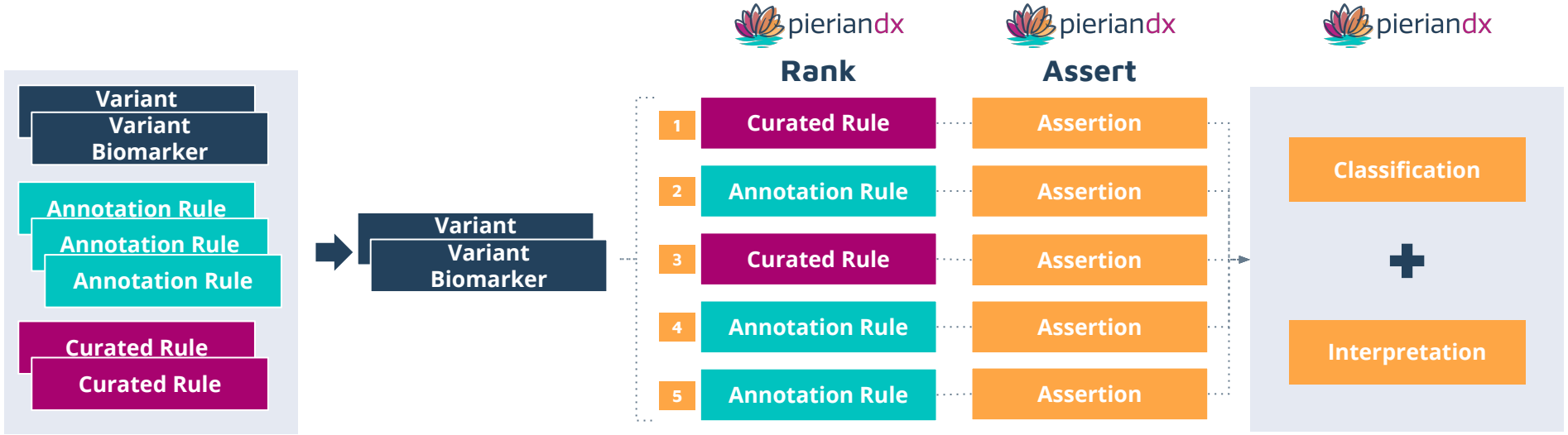
Phase
Location

=

Curated Rule

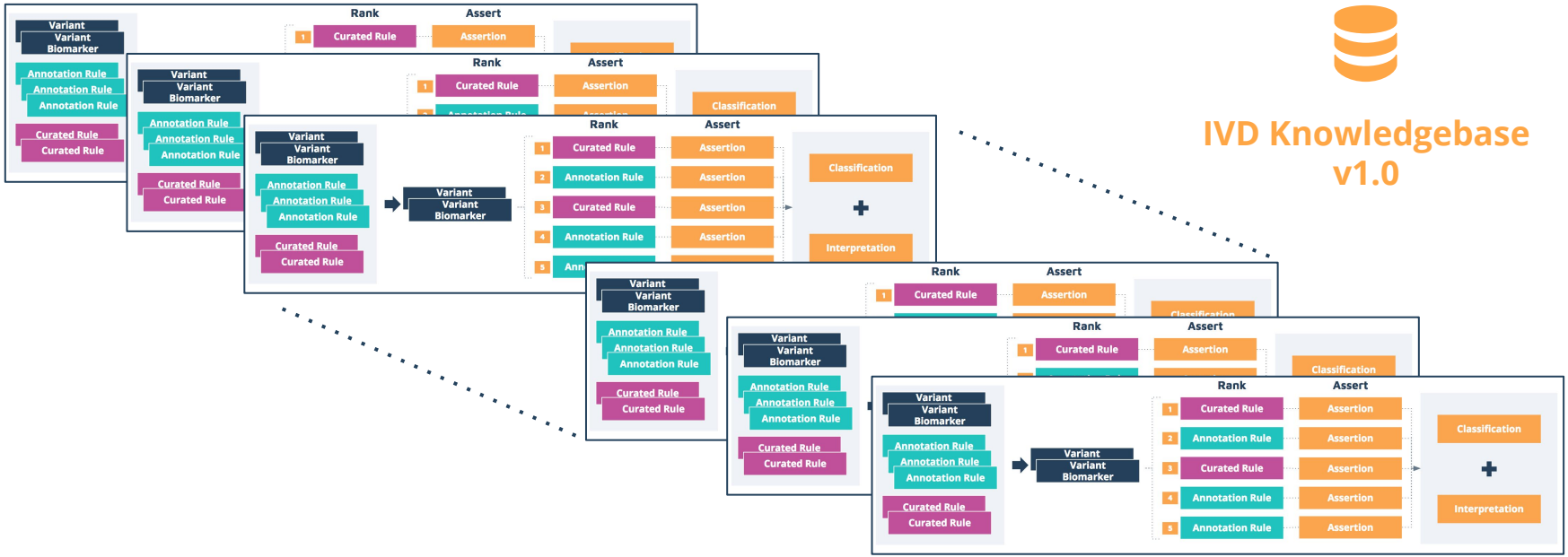
Rules Engine and Predicate Model Explained

Evidence Reviewing, Ranking, Asserting



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

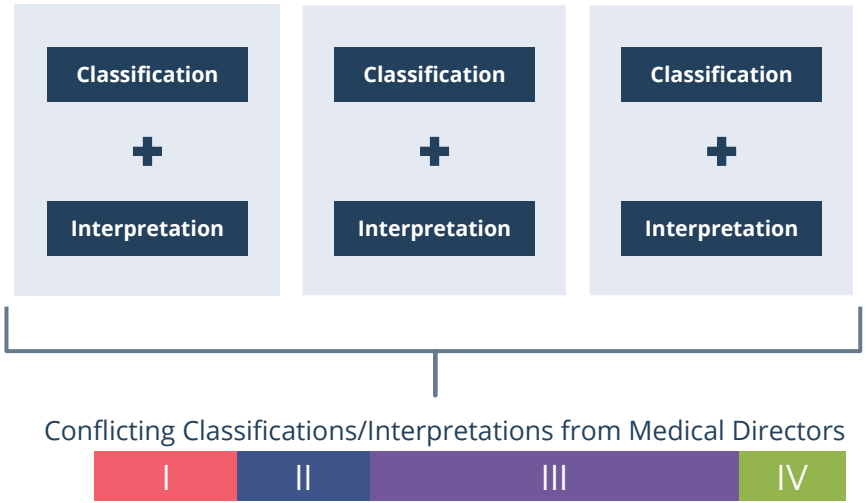
Evidence Reviewing, Ranking, Asserting



Consensus Building

Proactively Assess and Resolve Conflicts

Variant p.XXXX



Consensus Building

Proactively Assess and Resolve Conflicts

Variant p.XXXX

Classification + Interpretation

Classification + Interpretation

Classification + Interpretation

Conflicting Classifications/Interpretations from Medical Directors

I | II | III | IV

I

VS

Assess

PierianDx Curation Team

Resolve

Knowledgebase Clinical Expert Panel

Proactively Draft Consensus Interpretations

Variant p.XXXX (v2.0)

Classification

+

Interpretation

VS

Assess

PierianDx Curation Team

Resolve

Knowledgebase Clinical Expert Panel

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**