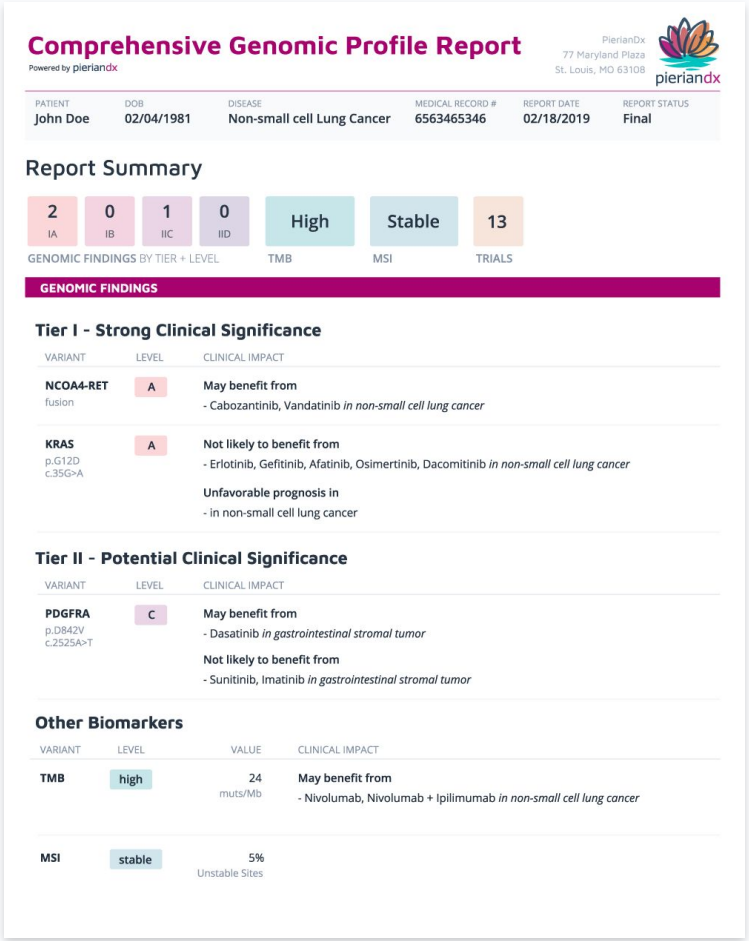

Comprehensive Genomic Profile Report

Powered by pieriandx

PierianDx
77 Maryland Plaza
St. Louis, MO 63108

pieriandx

PATIENT	DOB	DISEASE	MEDICAL RECORD #	REPORT DATE	REPORT STATUS
John Doe	02/04/1981	Non-small cell Lung Cancer	6563465346	02/18/2019	Final

Report Summary

2 IA	0 IB	1 IIC	0 IID	High	Stable	13
GENOMIC FINDINGS BY TIER + LEVEL				TMB	MSI	TRIALS

GENOMIC FINDINGS

Tier I - Strong Clinical Significance

VARIANT	LEVEL	CLINICAL IMPACT
NCOA4-RET fusion	A	May benefit from - Cabozantinib, Vandatinib *in non-small cell lung cancer*
KRAS p.G12D c.35G>A	A	Not likely to benefit from - Erlotinib, Gefitinib, Afatinib, Osimertinib, Dacomitinib *in non-small cell lung cancer* Unfavorable prognosis in - in non-small cell lung cancer

Tier II - Potential Clinical Significance

VARIANT	LEVEL	CLINICAL IMPACT
PDGFRA p.D842V c.2525A>T	C	May benefit from - Dasatinib *in gastrointestinal stromal tumor* Not likely to benefit from - Sunitinib, Imatinib *in gastrointestinal stromal tumor*

Other Biomarkers

VARIANT	LEVEL	VALUE	CLINICAL IMPACT
TMB	high	24 muts/Mb	May benefit from - Nivolumab, Nivolumab + Ipilimumab *in non-small cell lung cancer*
MSI	stable	5% Unstable Sites	

pieriandx

Enabling Comprehensive Genomic Profiling with TruSight™ Oncology 500

Clinically-optimized for Rapid Implementation and Evidence-based Reporting

Rakesh Nagarajan, MD, PhD
Executive Chairman and Founder
PierianDx

Moderated by: Josh Forsythe

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Our Mission

Keep cancer care in the community

By empowering local pathology and cancer centers to provide the same level of advanced testing and precision insights to their patients as major academic centers.

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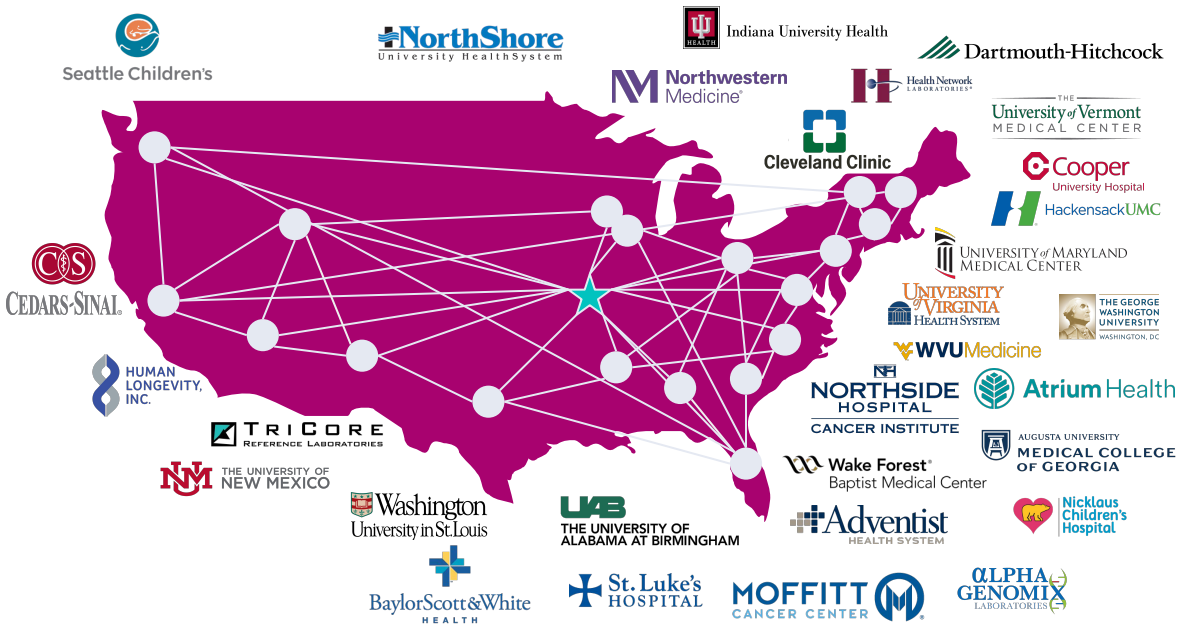
The Network Effect

Wisdom in Every Report



- **Today:** 45+ health systems and laboratories in partner sharing network
- **2019:** PierianDx signs multi-year deal with Illumina to support cancer research and diagnostics
- **2018:** Moffitt 1st to launch TruSight™ Tumor 170 clinically
- **2014:** PierianDx established; Moffitt Cancer Center 1st to go live
- **2011:** PierianDx technology developed at Washington University in St. Louis

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PierianDx Solutions

Technology Enabled Services

Clinical Genomics Workspace

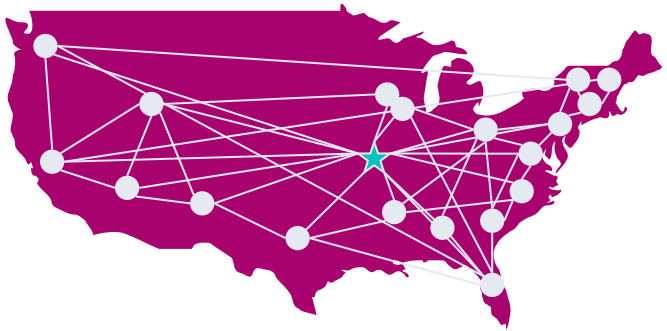
All-in-one informatics and reporting software

Validation & Interpretation Services

Experienced team to **fast-track growth**

Medically Powered Knowledgebase

The largest opt-in content **sharing network**



Laboratory Services

Turnkey, validated assays and informatics

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Agenda

1 Comprehensive Genomic Profiling (CGP)
Trends, Value, and Adoption

2 Assay Content and Performance
TruSight™ Oncology 500

3 Curation Strategies and Approaches
Clinical Management Yield

4 Classification and Interpretation
Evidence-based Reporting

5 Enabling Rapid Clinical Validation and Deployment
LDT Support for RUO Assays

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Comprehensive Genomic Profiling (CGP)

Trends, Value, & Adoption

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Rapid Rise of Precision Medicine

Numbers and Milestones

284 Total # of FDA approved pharmacogenomic biomarkers; 108 in oncology[1]

25 Personalized medicine approvals in 2018 (42% of NMEs)[2]

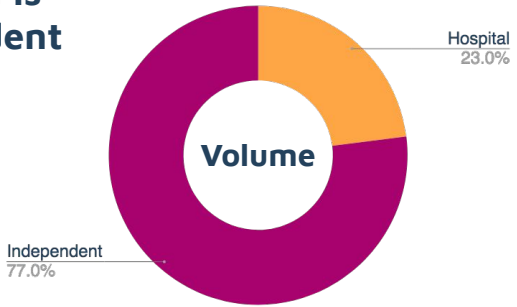
10 Cancer-related personalized medicine approvals in 2018[2]

2nd Approval of a cancer drug (Vitrakvi) indication based on biomarker, not tumor type[2]

3/16/18 CMS announces national coverage policy for NGS diagnostics

Hospitals billing for comprehensive genomic profiling (CGP)[3]

Most of the CGP volume is going to a few independent reference labs.[3]



Sources:
1. FDA - Table of Pharmacogenomic Biomarkers in Drug Labeling. Data as of June 2018
2. Personalized Medicine at FDA: A Progress & Outlook Report, Personalized Medicine Coalition, 2018.
3. Boston Healthcare Associates analysis of claims data 2016.

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Recent Trends

Biomarker and NGS Testing

Biomarker Testing	NGS Testing
Progressive increase in oncology due to tumor agnostic biomarkers developed to inform targeted and immune therapies	More laboratories performing NGS for common tumor types with approved therapies
Standardized testing algorithms drive biomarker testing for common tumors (NSCLC, CRC, breast) at diagnosis at many healthcare settings	**Tumor agnostic** markers, such as MSI, TMB, and NTRK fusions are significant driver of more NGS testing
	Improving payor coverage with recent Medicare coverage for FDA-approved NGS tests

Approved

Microsatellite Instability, NTRK Fusions


Science Home News Journals Topics Careers

SHARE REPORT

Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade

Dung T. Le[1,2,3], Jennifer N. Durham[1,2,3,*], Kellie N. Smith[1,3,*], Hao Wang[3,*], Bjarne R. Bartlett[2,4,*], Laveet K. Aulakh[2,4], Steve ...

+ See all authors and affiliations

Science 28 Jul 2017:
Vol. 357, Issue 6349, pp. 409-413
DOI: 10.1126/science.aan6733


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Efficacy of Larotrectinib in TRK Fusion–Positive Cancers in Adults and Children

Alexander Drilon, M.D., Theodore W. Laetsch, M.D., Shivaani Kummar, M.D., Steven G. DuBois, M.D., Ulrik N. Lassen, M.D., Ph.D., George D. Demetri, M.D., Michael Nathenson, M.D., Robert C. Doebele, M.D., Ph.D., Anna F. Farago, M.D., Ph.D., Alberto S. Pappo, M.D., Brian Turpin, D.O., Afshin Dowlati, M.D., et al.

Emerging

Tumor Mutational Burden


nature genetics

Letter | Published: 14 January 2019

Tumor mutational load predicts survival after immunotherapy across multiple cancer types

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Single-marker, Tumor-specific to Comprehensive Genomic Profiling

Oncology Testing is Evolving

Current → **Near-Term** → **Long-Term**

Single Markers and Hotspot Panels

Specific patient populations are tested for specific biomarkers using conventional methods (e.g., EGFR PCR for NSCLC)

Limits on tissue availability make this approach less sustainable long-term

Broad NGS Testing

NGS / CGP increasingly dominates conventional methods (e.g., PCR, FISH)

Use of a single test on a single sample to obtain a comprehensive biomarker status of the patient

Multi-Modality

Mix of test methods gives best picture

Possible reflex test patterns with some tests being prioritized because of their ease of use/affordable cost

Some FDA approved; some LDTs

Informatics deployed to create genotypic and phenotypic profile of patient

Source: Boston Healthcare Analysis

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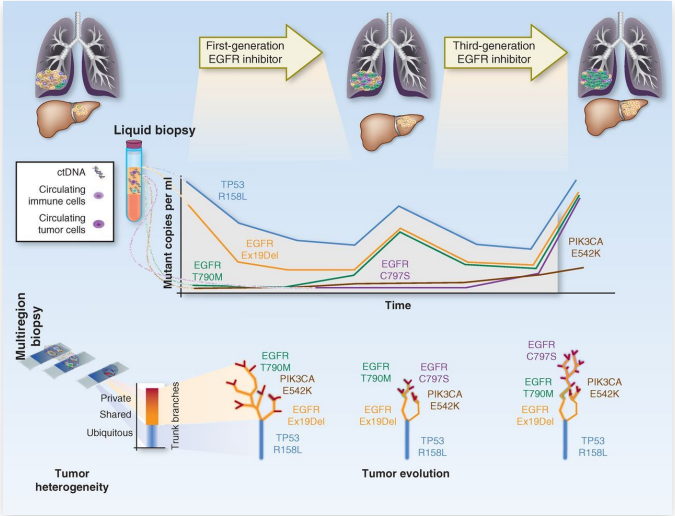
Monitoring Tumor Clonality

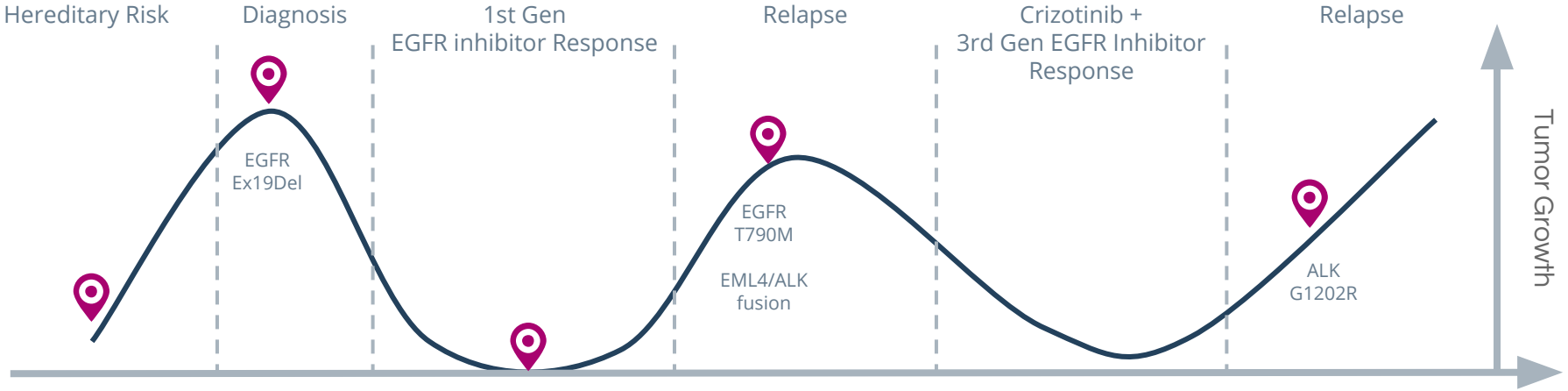
Serial Testing is Vital

Making Cancer a Chronic Disease

Hereditary cancer screening, early stage testing and serial monitoring hold the promise of making cancer a preventable and manageable disease.



Amirouchene-Angelozzi, et al. "Tumor Evolution as a Therapeutic Target". Cancer Discover. Aug. 2017



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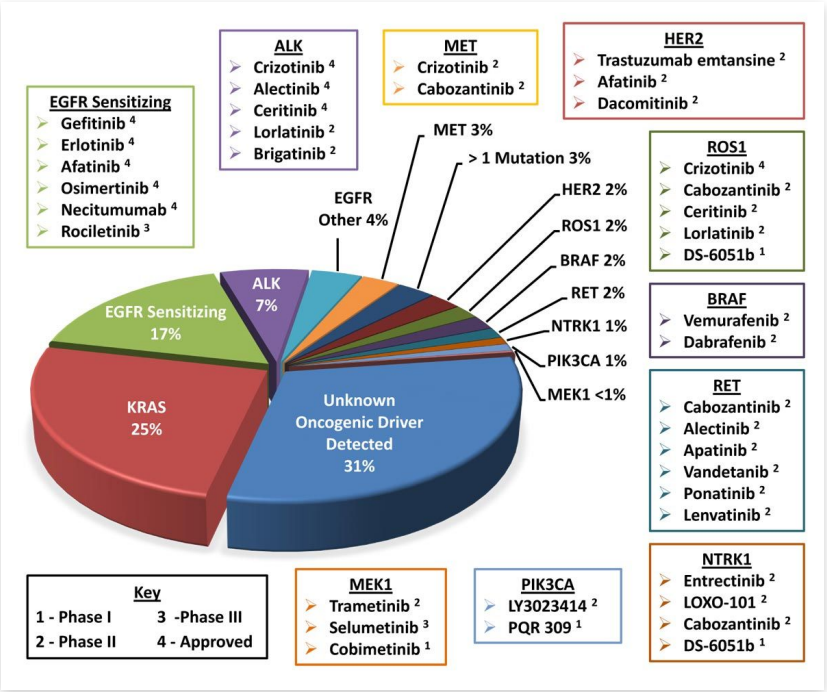
Comprehensive Testing Can Save Time, Sample and Impact Patient Lives Earlier

Too Many Questions for a Small Amount of Tissue



+ TMB, MSI

What if, instead of multiple tests, you could perform a single test that looked at all relevant biomarkers simultaneously?

Frequency of molecular aberrations in various driver oncogenes in lung adenocarcinomas and current available drugs against these oncogenic proteins.

Source: Tsao AS, et al. J Thorac Oncol. 2016;11:613-638.

Slide courtesy of Illumina

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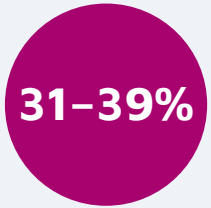
Up to 90% of Patients Who Undergo CGP may have an Actionable Alteration[1-6]

CGP Can Identify Actionable Alterations

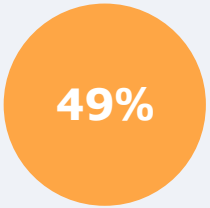
Slide courtesy of Illumina

30%–90% Of patients who undergo Genomic Profiling may have actionable alterations

2 Studies with pediatric solid tumors[4,5]



Prospective Clinical Trials—843 patients with advanced cancers[3]



500 patients with advanced cancer, multiple tumor types[2]

96 patients with multiple tumor types[6]

% of patients found to have an ACTIONABLE genetic alteration, after genomic profiling

1. Stransky N, Cerami E, Schalm S, Kim JL, Lengauer C. The landscape of kinase fusions in cancer. Nat Commun. 2014;5:4846. doi:10.1038/ncomms5846. 2. Boland GM, Piha-Paul SA, Subbiah V, et al. Clinical next generation sequencing to identify actionable aberrations in a phase I program. Oncotarget. 2015;6(24):20099-20110. 3. Massard C, Michiels S, Ferte C, et al. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. Cancer Discov. 2017;7(6):586-595. 4. Harris MH, DuBois SG, Glade Bender JL, et al. Multicenter feasibility study of tumor molecular profiling to inform therapeutic decisions in advanced pediatric solid tumors: the individualized cancer therapy (iCat) study. JAMA Oncol. 2016;2(5):608-615. 5. Parsons DW, Roy A, Yang Y, et al. Diagnostic yield of clinical tumor and germline whole-exome sequencing for children with solid tumors. JAMA Oncol. 2016;2(5):616-624.; 6. Reitsma et al., 2019. Effect of a Collaboration Between a Health Plan, Oncology Practice, and Comprehensive Genomic Profiling Company from the Payer Perspective Journal of Managed Care & Specialty Pharmacy.

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Benefits Presented in a Recent Study

CGP Has Value in Patient Management

Slide courtesy of Illumina

6% → 13%

Increase Targeted Therapy

Utilization projected to double

4% → 54%

Increase Clinical Trial Eligibility

Greater than 1000-fold

$6.6M ↓

Reduce Costs of Non-targeted Therapy

Cost decreases by $6.6M

↓33%

Reduce Adverse Events

Decreased by one-third

Reitsma et al., 2019. Effect of a Collaboration Between a Health Plan, Oncology Practice, and Comprehensive Genomic Profiling Company from the Payer Perspective *Journal of Managed Care & Specialty Pharmacy.*

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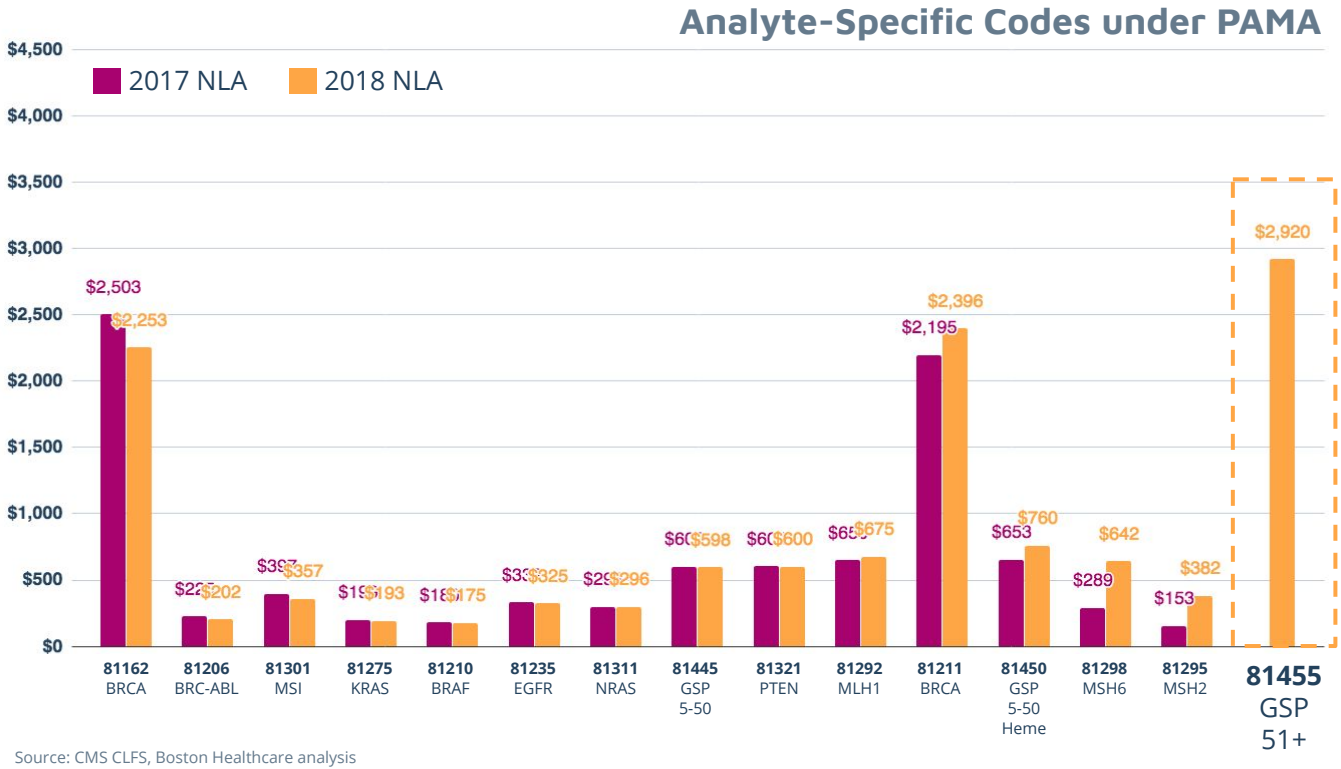
Precedent for Reimbursement



Source: CMS CLFS, Boston Healthcare analysis

National Coverage Determination

March 2018

CMS finalized a National Coverage Determination that covers diagnostic laboratory tests using Next Generation Sequencing (NGS) for patients with advanced cancer.

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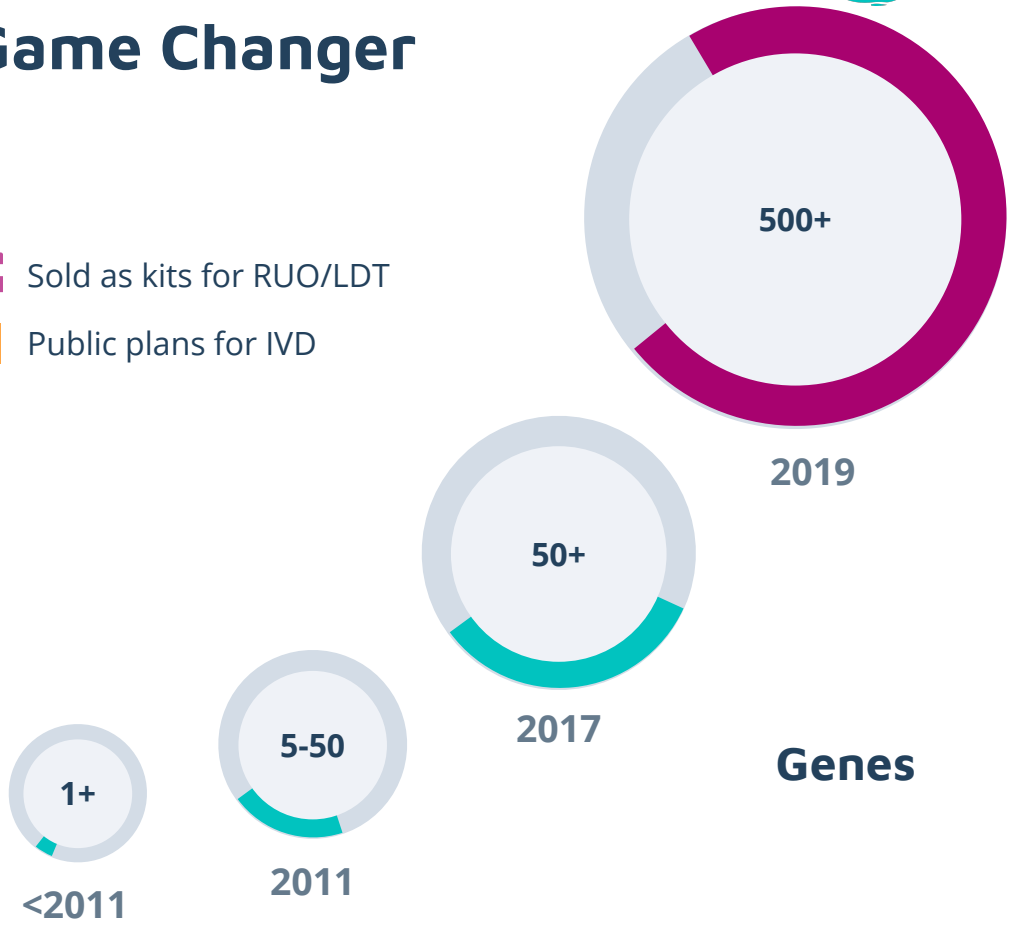
Commercial Pan-cancer Assays

In vitro Diagnostics is a Game Changer

Provider	Assay	Genes
PGDx	**PGDx**	500+
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



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Assay Content and Performance

TruSight™ Oncology 500

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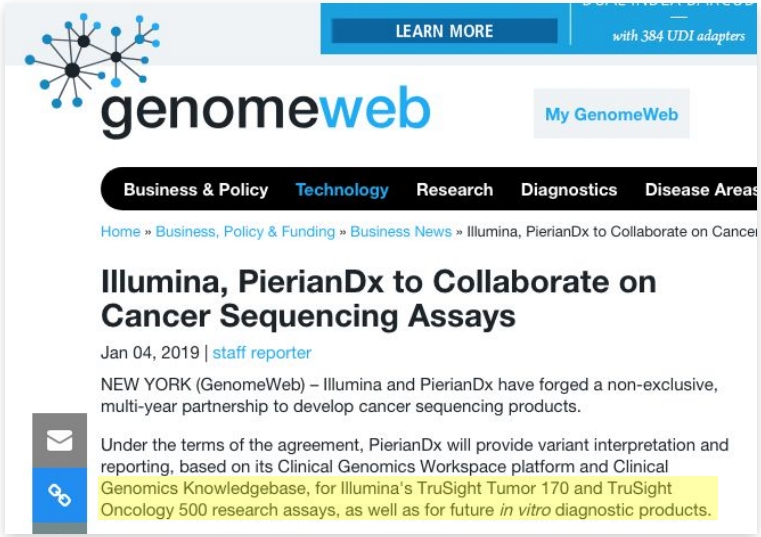
PierianDx Implemented Assays

All Bases Covered



PierianDx Enabling TruSight™ Oncology 500 and TruSight™ Tumor 170

	Assay	Vendor	Variant Types	Input Files
Amplicon	Variant/Fusion Plex	Archer	SNVs, indels, fusions	VCF, BAM
	VariantPlex BRCA1/BRCA2	Archer	SNVs and indels	VCF, BAM, BAI
	TruSight Myeloid	Illumina	SNVs and indels	FASTQ, BAM, VCF
	TruSight™ Tumor 15	Illumina	SNVs and indels	FASTQ, BAM, VCF
	TruSight™ Tumor 26	Illumina	SNVs and indels	FASTQ, BAM, VCF
	TruSeq™ Cancer Amplicon	Illumina	SNVs and indels	FASTQ, BAM, VCF
	BRCA1/BRCA2 (AFP2 assay)	Illumina	SNVs and indels	FASTQ, BAM, VCF
	Oncomine (OCA) v2/3	Thermo Fisher	SNVs, indels, CNVs, fusions	BAM, VCF, BAI
	Ion AmpliSeq™ Cancer HotSpot	Thermo Fisher	SNVs and indels	BAM, VCF, BAI
Hybridization Capture	Agilent probes	Agilent	SNVs, indels, fusions	FASTQ, BAM, VCV
	Agilent/IDT probes	Agilent/IDT	SNVs, indels, fusions	FASTQ, BAM, VCF
	TruSight™ Tumor 170	Illumina	SNVs, indels, CNVs, fusions	BAM, VCFs, CSV
	TruSight™ Oncology 500	Illumina	SNVs, indels, CNVs, fusions, TMB, MSI	FASTQ, BAM, VCF
	TruSight™ Cancer	Illumina	SNVs and indels	FASTQ, BAM, VCF
	Ion AmpliSeq™ Inherited Cancer	Thermo Fisher	SNVs and indels	VCF, BAM, BAI
Haloplex Molecular barcodes/UMIs	Agilent Haloplex Technology	Agilent	SNVs and indels	FASTQ, BAM, VCF
Somatic Fusions	FusionPlex ALK/RET/ROS	Archer	Fusions	FASTQ, BAM
	TruSight™ RNA fusion	Illumina	Fusions	FASTQ, BAM, VCF
Whole Exome Clinical Exome	Agilent SureSelect	Agilent	SNVs and indels	FASTQ, BAM
	TruSight™ One	Illumina	SNVs and indels	FASTQ, BAM, VCF

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Enabling Quality Comprehensive Genomic Profiling

TruSight™ Oncology 500 Content

Single assay: 523 Genes

DNA

Hybrid capture-based NGS assay

Panel size of 1.94 (Mb)

Accurate detection of multiple classes of mutations/biomarkers: SNVs, Indels, MSI, TMB

Unique Molecular Identifiers (UMIs) enable detection of variants present at low VAFs and liquid sample analysis
(under development)

RNA*

55 Genes

Ability to detect fusions (known and novel), splice variants

Some key biomarkers included

Pan-Cancer Biomarkers
NTRK1, NTRK2, NTRK3, MSI (Approved) | TMB (Emerging)

Lung	Melanoma	Colon	Ovarian	Breast	Gastric	Bladder	Myeloid	Sarcoma
AKT1 *ALK* *BRAF* *DDR2* *EGFR* *ERBB2* *FGFR1* *FGFR3* *KRAS* *MAP2K1* *MET* *NRAS* *PIK3CA* *PTEN* *RET* *TP53* TMB	*BRAF* *CTNNB1* *GNA11* *GNAQ* *KIT* *MAP2K1* *NF1* *NRAS* *PDGFRA* *PIK3CA* *PTEN* *TP53*	*AKT1* *BRAF* *HRAS* *KRAS* *MET* *MLH1* *MSH2* *MSH6* *NRAS* *PIK3CA* *PMS2* *PTEN* *SMAD4* *TP53*	*BRAF* *BRCA1* *BRCA2* *KRAS* *PDGFRA* *FOXL2* *TP53*	*AKT1* *AR* *BRCA1* *BRCA2* *ERBB2* *FGFR1* *FGFR2* *PIK3CA* *PTEN*	*BRAF* *KIT* *KRAS* *MET* *MLH1* *PDGFRA* *TP53*	*MSH6* *PMS2* *TSC1*	*ABL1* *ASXL1* *CALR* *CEBPA* *ETV6* *EZH2* *FLT3* *GATA2* *IDH1* *IDH2* *JAK2* *KIT* *MPL* *NPM1* *RUNX1* *SF3B1* *SRSF2* *TP53*	*ALK* *APC* *BRAF* *CDK4* *CTNNB1* *ETV6* *EWSR1* *FOXO1* *GLI1* *KIT* *MDM2* *MYOD1* *NAB2* *NF1* *PAX3* *PAX7* *PDGFRA* *PDGFRB* *SDHB* *SDHC* *SMARCB1* *TFE3* *WT1*

https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-500-data-sheet-1170-2018-010.pdf

** The products to evaluate DNA + RNA variants are the TruSight™ Oncology 500 DNA + TruSight™ Oncology 170 RNA bundles (PN: 20028215 / 20028216 / 20032626 / 20032627)*

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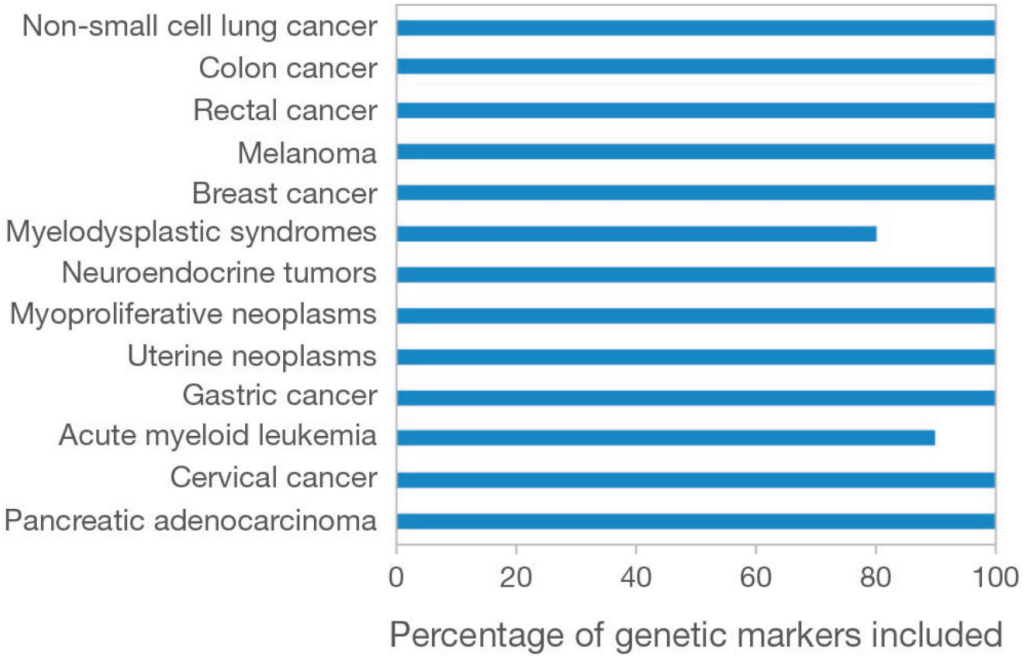
Content Aligned to Guidelines and Clinical Trials



100% coverage of guidelines for 11 tumor types

For each cancer type, the percentage of genetic markers in current guidelines that are included in the gene panel is indicated.

1233 Clinical trials (US-based)

https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-500-data-sheet-1170-2018-010.pdf

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Expanding Utility

Addressing Needs of Today and Tomorrow

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Home » Sponsors » Genomics: Clinical Implementation » Illumina Details NCI Effort to Validate NGS Oncology Panel for Liquid Biop

Illumina Details NCI Effort to Validate NGS Oncology Panel for Liquid Biopsies

Feb 08, 2019 | Molika Ashford

Premium

NEW YORK (GenomeWeb) – After launching the tissue-directed TruSight Oncology 500 panel late last year, Illumina has now cemented a partnership that, along with potential others, will validate the firm's comprehensive sequencing assay for an eventual liquid biopsy application.

Researchers from the Molecular Characterization Laboratory at the National Cancer Institute's Frederick National Laboratory said last month that they plan to use TSO 500 to support multiple clinical studies, assessing liquid biopsy samples from up to 7,000 subjects.

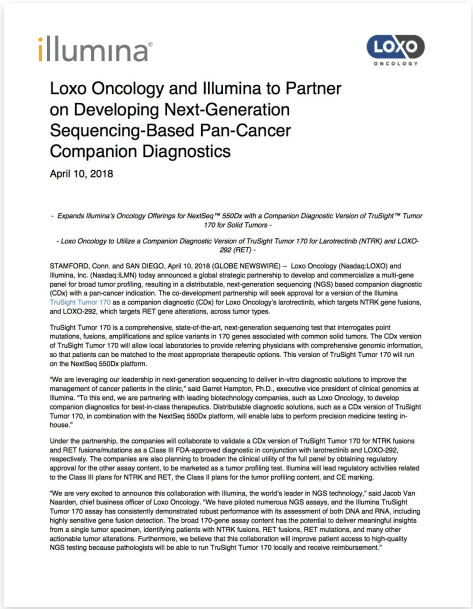
The effort will include validation of a liquid biopsy version of the TSO 500 kit, as well as a number of analyses focused on variant detection in rare tumor types and on blood-tissue mutation concordance.

In an email, an Illumina representative said this week that the first customer shipment for its TSO 500 tissue assay was in early January. "We had a very successful early access program, and now we see strong momentum for the assay in tissue," the firm said.

According to Illumina, a robust interest in liquid biopsy application is following suit, which means that Frederick National Lab is not necessarily the only group that will be working to prove the technology. There may be other "potential collaborators" ahead of the launch of an official TS Oncology 500 cfDNA kit, the company's spokesperson wrote.

In its announcement of the FNL collaboration, Illumina projected a sense of caution regarding liquid biopsy, stating that it is "still in its infancy and requires additional validation to more fully demonstrate clinical utility." Responding to questions this week, however, the company clarified that these comments are specific to the question of large assays like TSO 500, and not directed at the larger field.

Although blood-based sequencing assays are now on the market for both research and clinical customers, most profile smaller panels of genes if not single genes or mutations. Guardant Health has launched its own 500-gene assay but has limited marketing of that panel to the research community thus far. Baltimore-based Personal Genome Diagnostics has also developed 500-plus-gene assays for both tissue and blood, with plans to advance FDA-approved kit versions.



illumina

LOXO ONCOLOGY

Loxo Oncology and Illumina to Partner on Developing Next-Generation Sequencing-Based Pan-Cancer Companion Diagnostics

April 10, 2018

- Expands Illumina's Oncology Offerings for NextSeq™ 550Dx with a Companion Diagnostic Version of TruSight™ Tumor 170 for Solid Tumors -

- Loxo Oncology to Utilize a Companion Diagnostic Version of TruSight Tumor 170 for Larotrectinib (NTRK) and LOXO-292 (RET) -

STAMFORD, Conn. and SAN DIEGO, April 10, 2018 (GLOBE NEWSWIRE) -- Loxo Oncology (Nasdaq:LOXO) and Illumina, Inc. (Nasdaq:ILMN) today announced a global strategic partnership to develop and commercialize a multi-gene panel for broad tumor profiling, resulting in a distributable, next-generation sequencing (NGS) based companion diagnostic (CDx) with a pan-cancer indication. The co-development partnership will seek approval for a version of the Illumina TruSight Tumor 170 as a companion diagnostic (CDx) for Loxo Oncology's larotrectinib, which targets NTRK gene fusions, and LOXO-292, which targets RET gene alterations, across tumor types.

TruSight Tumor 170 is a comprehensive, state-of-the-art, next-generation sequencing test that interrogates point mutations, fusions, amplifications and splice variants in 170 genes associated with common solid tumors. The CDx version of TruSight Tumor 170 will allow local laboratories to provide referring physicians with comprehensive genomic information, so that patients can be matched to the most appropriate therapeutic options. This version of TruSight Tumor 170 will run on the NextSeq 550Dx platform.

"We are leveraging our leadership in next-generation sequencing to deliver in-vitro diagnostic solutions to improve the management of cancer patients in the clinic," said Garret Hampton, Ph.D., executive vice president of clinical genomics at Illumina. "To this end, we are partnering with leading biotechnology companies, such as Loxo Oncology, to develop companion diagnostics for best-in-class therapeutics. Distributable diagnostic solutions, such as a CDx version of TruSight Tumor 170, in combination with the NextSeq 550Dx platform, will enable labs to perform precision medicine testing in-house."

Under the partnership, the companies will collaborate to validate a CDx version of TruSight Tumor 170 for NTRK fusions and RET fusions/mutations as a Class III FDA-approved diagnostic in conjunction with larotrectinib and LOXO-292, respectively. The companies are also planning to broaden the clinical utility of the full panel by obtaining regulatory approval for the other assay content, to be marketed as a tumor profiling test. Illumina will lead regulatory activities related to the Class III plans for NTRK and RET, the Class II plans for the tumor profiling content, and CE marking.

"We are very excited to announce this collaboration with Illumina, the world's leader in NGS technology," said Jacob Van Naarden, chief business officer of Loxo Oncology. "We have piloted numerous NGS assays, and the Illumina TruSight Tumor 170 assay has consistently demonstrated robust performance with its assessment of both DNA and RNA, including highly sensitive gene fusion detection. The broad 170-gene assay content has the potential to deliver meaningful insights from a single tumor specimen, identifying patients with NTRK fusions, RET fusions, RET mutations, and many other actionable tumor alterations. Furthermore, we believe that this collaboration will improve patient access to high-quality NGS testing because pathologists will be able to run TruSight Tumor 170 locally and receive reimbursement."

Bristol-Myers Squibb

illumina

Press Release

Bristol-Myers Squibb and Illumina Announce Strategic Collaboration to Develop and Commercialize Companion Diagnostics for Bristol-Myers Squibb's Oncology Immunotherapies

Expands Illumina's Oncology Offerings for NextSeq™ 550Dx

A Diagnostic Version of the Illumina TruSight™ Oncology 500 Assay Will Measure Potentially Predictive Genomic Biomarkers, Including Tumor Mutation Burden

CATEGORY: PARTNERING NEWS

FRIDAY, APRIL 13, 2018 4:15 PM EDT

NEW YORK & SAN DIEGO--(BUSINESS WIRE)--Bristol-Myers Squibb Company (NYSE: BMY) and Illumina, Inc. (NASDAQ: ILMN) today announced a collaboration that will utilize Illumina's next-generation sequencing (NGS) technology to develop and globally commercialize in-vitro diagnostic (IVD) assays in support of Bristol-Myers Squibb's oncology portfolio. The companies plan to develop a diagnostic version of the Illumina TruSight Oncology 500 assay to measure potentially predictive genomic biomarkers, including Tumor Mutation Burden (TMB). Illumina's TruSight Oncology 500 assay is being developed to detect most of the known biomarkers for oncology therapeutics, including TMB and Microsatellite Instability for immunotherapies.

"Through our deep understanding of cancer biology and emerging research, we recognize the importance for physicians to know each patient's biomarker status to help fight their cancer in a more personalized way," said Saurabh Saha, M.D., Ph.D., Senior Vice President, Global Head of Translational Medicine, Bristol-Myers Squibb. "We are excited to partner with

1/5

Illumina partners with NCI to assess TruSight™ Oncology 500 in liquid biopsy samples.

Illumina partnering on companion diagnostic development for *in vitro* diagnostic use.

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Analysis of TruSight™ Oncology 500 and TruSight™ Tumor 170 Assays

PierianDx *In Silico* Analytical Validation

Analyses are analogous to analytical validation for laboratory developed tests

	DNA/RNA (SNVs, Indels, Fusions, Splice Variants)
Accuracy	✓
Analytical Sensitivity	✓
Analytical Specificity	✓
Precision and Reproducibility	✓
Sequencing and Quality Metrics	✓
Coverage Metrics	✓
TMB and MSI (DNA-only)	✓
Final Filters for Variants	✓

Goal

Determine analytical performance of the assay and **optimize for clinical use** in PierianDx's Clinical Genomics Workspace

Samples

Multiple reference standards and FFPE clinical samples were included

Results

Demonstrated **excellent performance** using the Illumina provided secondary analysis pipeline deployed within PierianDx's Clinical Genomics Workspace, providing the foundation for any clinical laboratory to perform analytical and diagnostic validation using appropriate methods-based and diagnostic samples.

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Accuracy (DNA)

1. Sensitivity with Reference Standard

Objective: Establish sensitivity for a reference standard across the entire TruSight™ Oncology 500 coding region (+2 bp) by using whole exome sequencing data (100x coverage)

Reference Sample: HD301 from Horizon Discovery

Results:

- SNVs: **99.8%**
  1/3 false-negative present at low frequency in BAM though it was embedded between homopolymer regions
- Indels: **100.0%**

2. Sensitivity w/ Known Variants in Ref. Standards and Clinical Samples

Objective: Concordance for "evaluate-able" targets between TruSight™ Oncology 500 and orthogonal methodology.

Reference standard samples: HD300, HD301, HD753, SeraCare AF10

Clinical samples: 12 FFPE samples across colon, lung, uterine, endometrial, brain, thyroid tumors

Results:

- SNVs: **100.0%** on reference standards (Reagent Lots 3B and 5); **100%** on clinical samples
- Indels: **100%** on reference samples; **100%** on clinical samples

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Analytical Sensitivity and Specificity (DNA)

Sensitivity

Objective: Define the lower limit of detection (LLOD), defined as the lowest concentration of analyte that the TruSight™ Oncology 500 DNA assay can consistently detect with acceptable precision. For this assay, the LLOD will be expressed in terms of variant allele frequency (VAF) for a particular depth of sequencing coverage (100x).

Samples: 7 FFPE clinical samples; 3 reference samples

Results:

- SNVs: Sensitivity **100% >= 3% VAF** at 100x coverage
- Indels: Sensitivity **100% >= 3% VAF** at 100x coverage

Specificity

Objective: Determine the probability that the assay will not detect a sequence variation when none are present (according to a gold standard comparison composed of verified variants).

Samples: 12 DNA samples across ovary, lung, liver, uterus, prostate, colon, and liver tumors

Results:

- SNVs: Specificity of **100%**
- Indels: Specificity of **100%**

All variants detected in the experimental sample set were detected in the 50-100.0% VAF bin, indicating that no somatic variants were present in these samples as was expected based on the benign nature of the selected samples.

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Accuracy (RNA)

Sensitivity with Known Verified Variants in Ref Standards and Clinical Samples

Objective: Determine concordance for evaluate-able targets between the TruSight™ Tumor 170 RNA assay and orthogonal methodology

Clinical samples: 11 FFPE clinical samples.

Reference standard samples: 8 cell lines, 3 FFPE-ized cell lines

Results:

- *Reference Standards:* Sensitivity of **100.0% for fusions** and **100.0% for splice variants**
- *FFPE-ized Cell Line Samples:* Sensitivity of **100.0% for fusions** and **100.0% for splice variants**
- *FFPE Clinical Samples:* Sensitivity of **100.0% for fusions** and **100.0% for splice variants**

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Analytical Sensitivity and Specificity (RNA)

Sensitivity

Objective: Define the lower limit of detection (LLOD), defined as the lowest concentration of analyte that the TruSight™ Tumor 170 assay can consistently detect with acceptable precision.

Samples: 8 cell lines (and 31 mixtures of these samples), 3 FFPE-ized cell lines (11 mixtures of these samples)

Results:

- Fusions: Sensitivity of **100.0%** in reference standards and clinical FFPE samples at > 5 copies per ng RNA input
- Splice Variants: Sensitivity of **100.0%** in reference standards and clinical FFPE samples at > 5 copies per ng RNA input

Specificity

Objective: Determine the probability that the assay will not detect a sequence variation when none are present (according to a gold standard comparison composed of verified variants).

Samples: 29 RNA Samples (including 4 cell lines, 2 FFPE-ized cell lines, and 23 FFPE tissue samples)

Results:

- Fusions: Specificity of **96.49%**
- Splice Variants: Specificity of **100%**

2/55 false-positive fusions detected were a PDGFRB/FIP1L1 fusion and a FLI1/SPATA32 fusion

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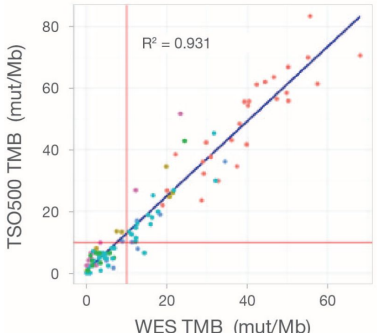
TMB and MSI (DNA)

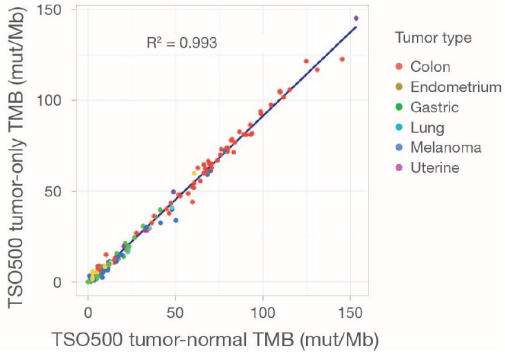
Tumor Mutational Burden

At low value range (10 mut/Mb threshold):

- Positive percent agreement: **94.7%**
- Negative percent agreement: **96.1%**

https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-500-data-sheet-1170-2018-010.pdf



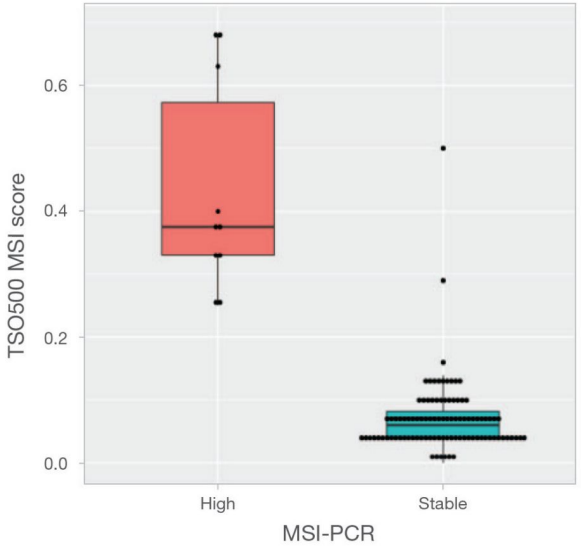


Microsatellite Instability

Threshold of >=40 marker sites to ensure 95% detection of MSI samples

- Specificity: **100%**
- Detection rate: **100%**

https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-500-data-sheet-1170-2018-010.pdf



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Curation Strategies and Approaches

Clinical Management Yield

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AMP/CAP/ASCO Variant Classification

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
III	**Variants of Unknown Clinical Significance**		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	**Benign or Likely Benign Variants**		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

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

How to maintain rapid turnaround while achieving highest accuracy?

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

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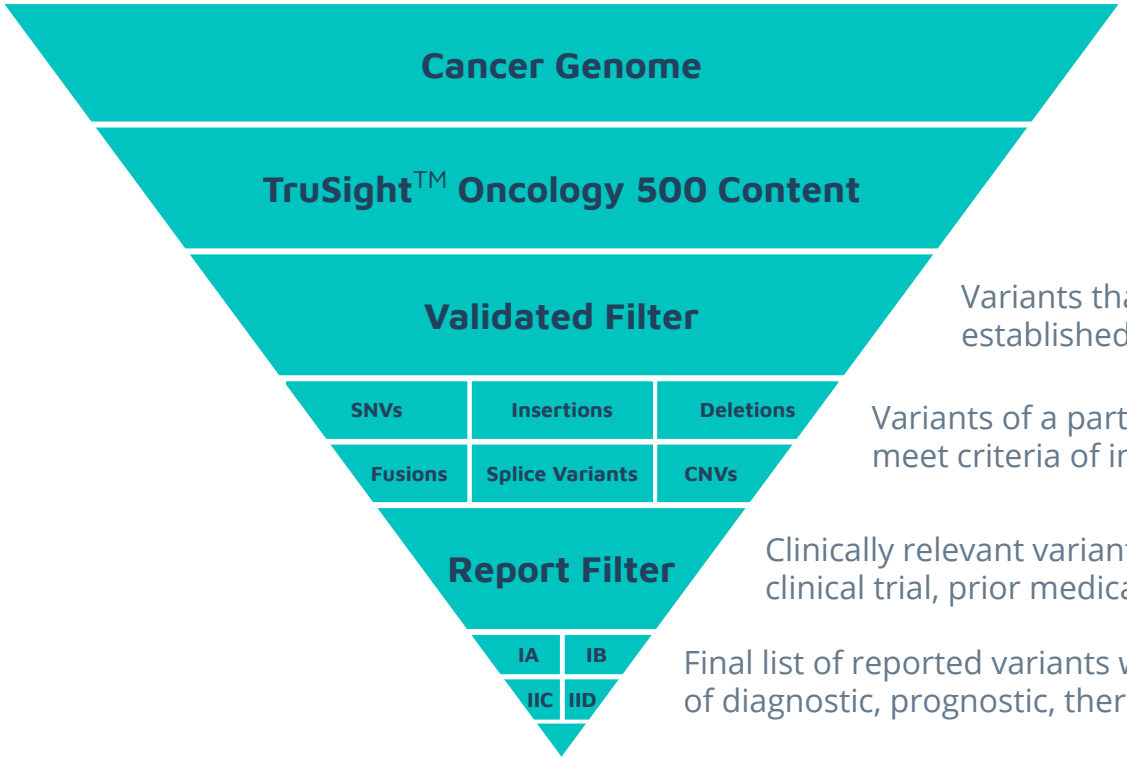
Validated and Intuitive Filtering

Rapidly Identify Clinically Relevant Variants



Variants that meet quality control parameters established during assay validation

Variants of a particular class or that meet criteria of interest

Clinically relevant variants, e.g. FDA label, NCCN guideline, clinical trial, prior medical interpretation

Final list of reported variants with clinical impact: e.g. of diagnostic, prognostic, therapeutic relevance

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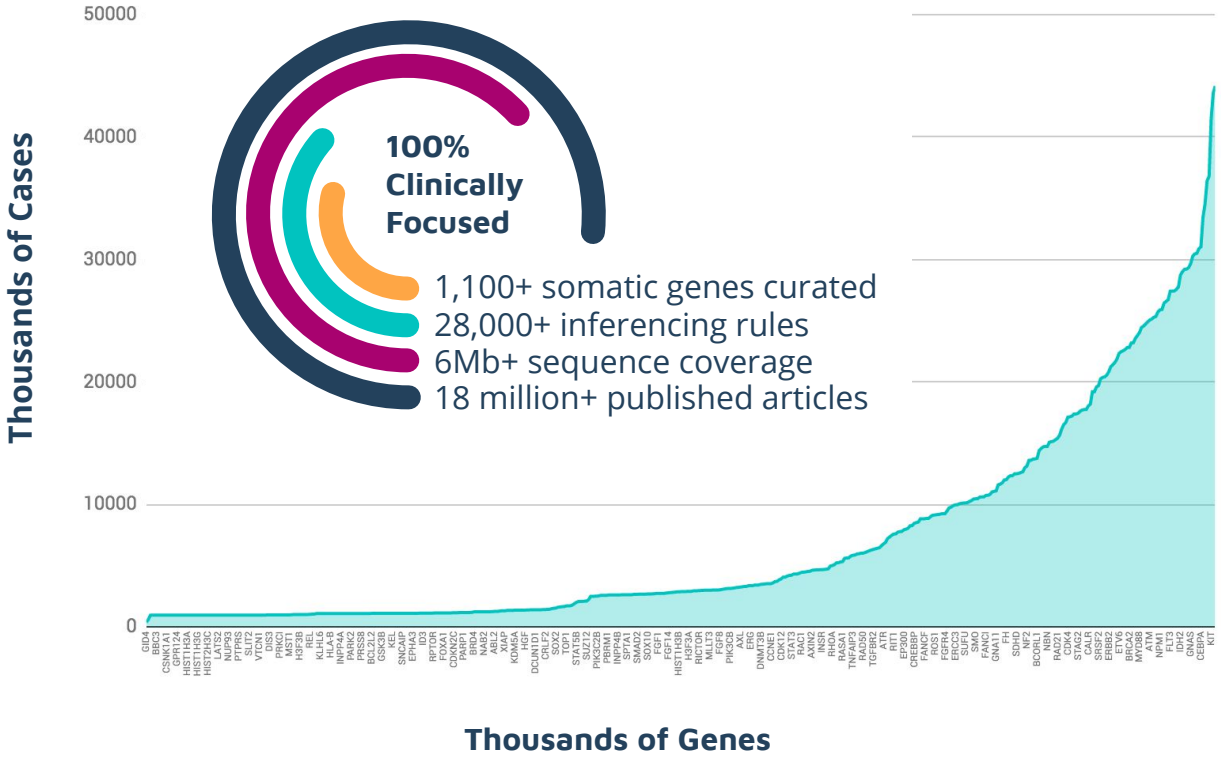
Most Accurate, Comprehensive, and Up-to-Date

PierianDx Knowledgebase



Knowledge Sources

Expertly Curated Assertions

- FDA approved labels
- NCCN, ASCO guidelines
- Active, recruiting trials
- 2H/2019: ESMO guidelines, EMA drug labels, EU clinical trials

Shared Medical Content

- Medically signed-out interpretations
- 10s of thousands of cases

Public Data Sources

- PubMed literature
- Population frequencies
- TCGA
- ClinVar
- COSMIC
- Emory
- Invitae
- dbNSFP
- SNOMED-CT

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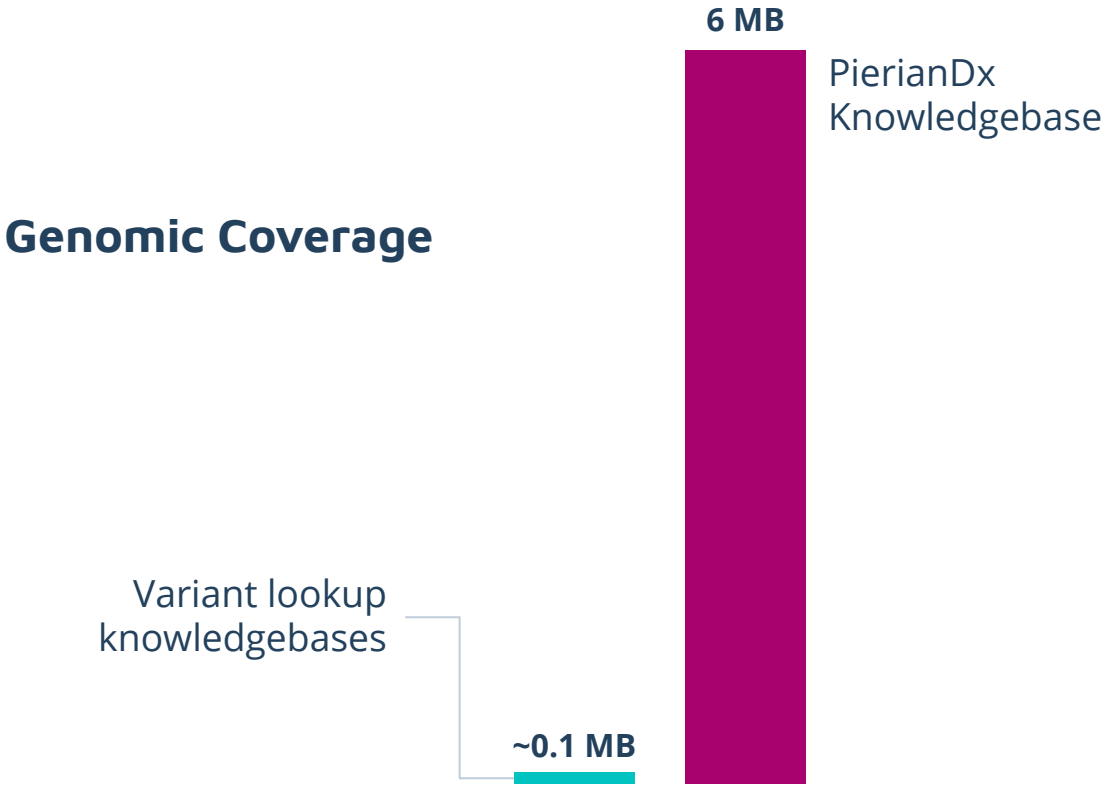
More Comprehensive than a Variant Lookup

Rules-based Engine



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)

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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	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

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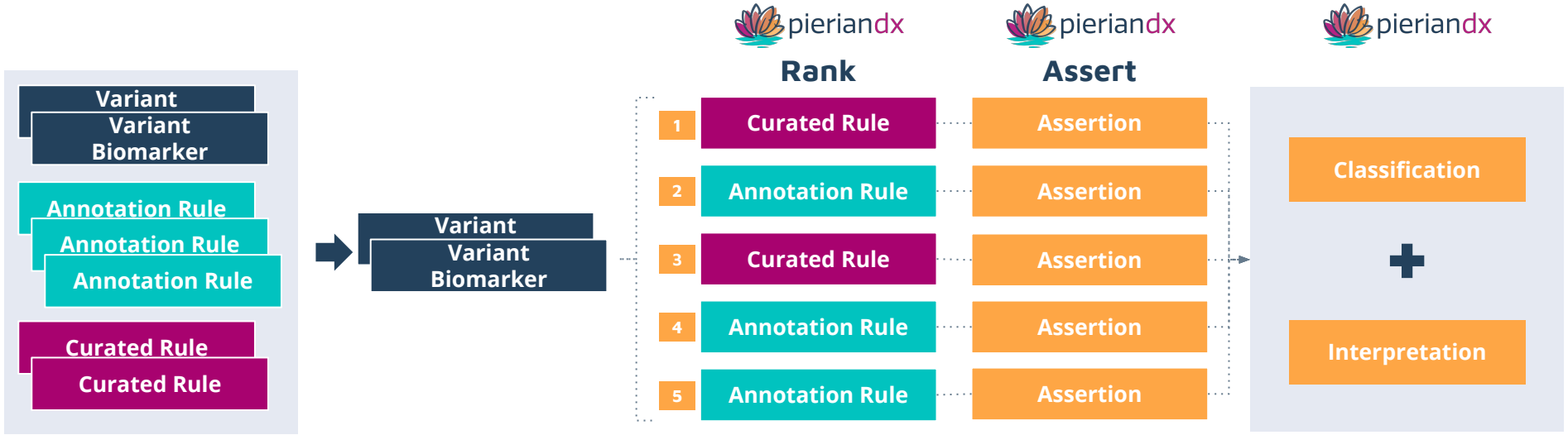
Evidence Reviewing, Ranking, Asserting



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

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PierianDx Customer Reported Genes and Curated Content

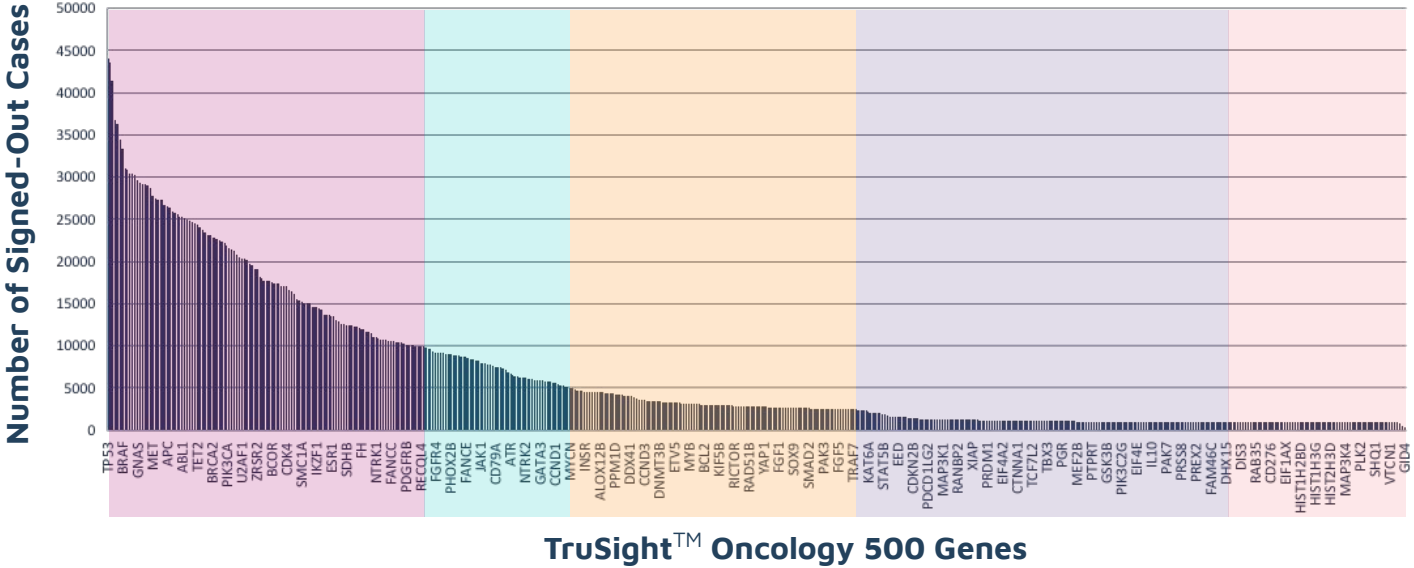
24.1% of genes (126 genes) reported in > 10,000 cases

35.9% of genes (188 genes) reported in > 5000 cases

57.7% of genes (302 genes) reported in > 2500 cases

86% of genes (450 genes) reported in > 1000 cases

100% of genes (523 genes) reported in > 350 cases



TruSight™ Oncology 500 Genes

PierianDx Curated Information for 523 Gene Set

- 47 drug labels
- 38 guidelines
- 1223 clinical trials

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Classification Accuracy

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

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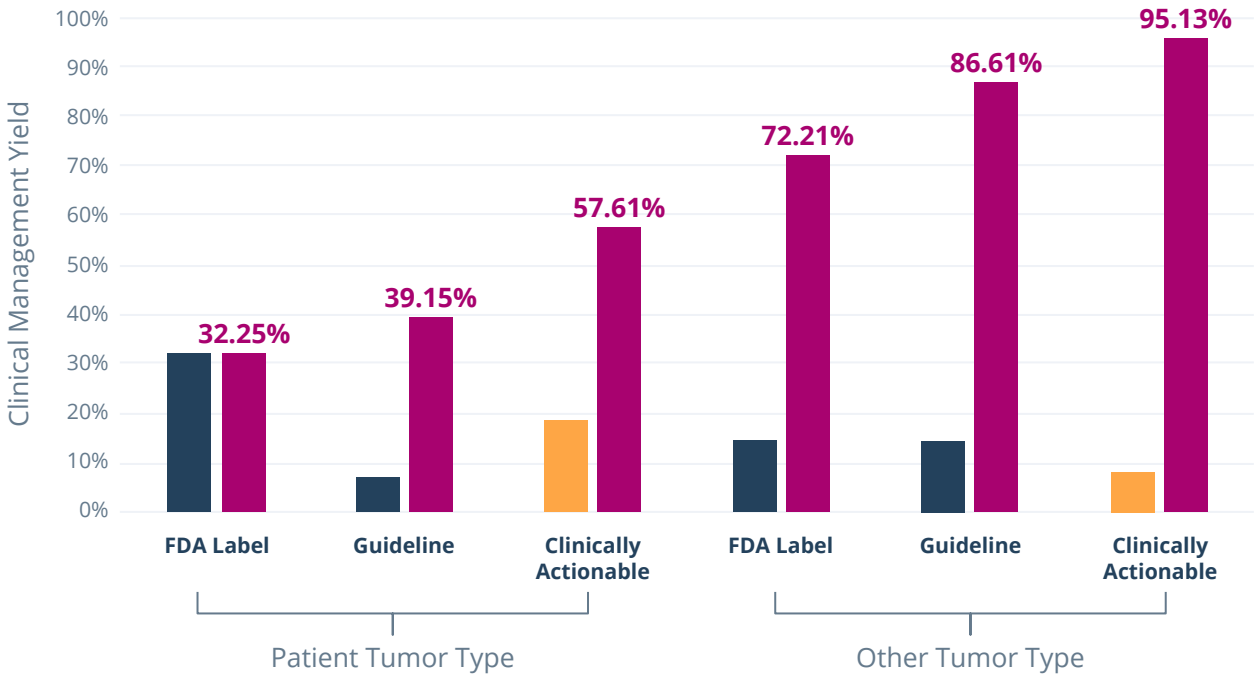
Clinical Management Yield



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.

68.15%	Sum of FDA Labels/Guidelines
26.98%	Sum of Non-FDA Labels/Guidelines
95.13%	Total Clinical Management Yield

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Variant Classification and Interpretation

Evidence-based Reporting

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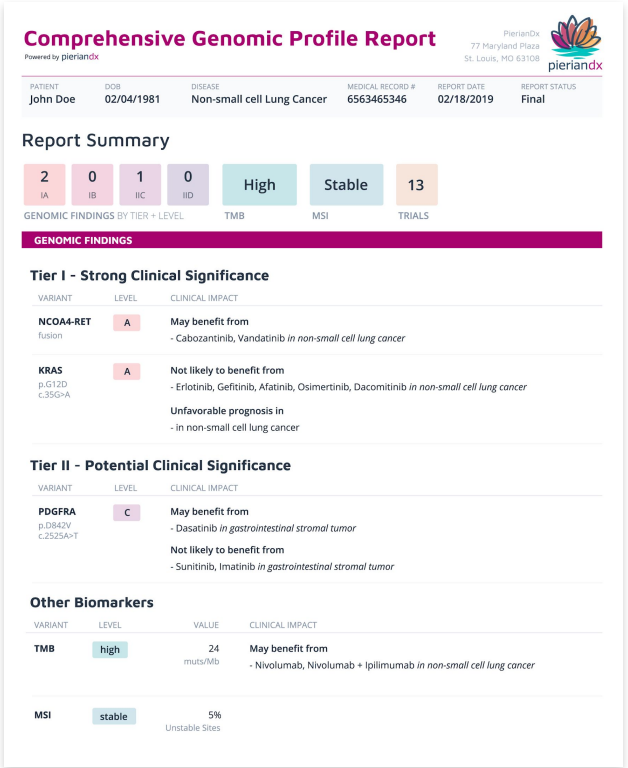
Evidence-based Reporting

PierianDx Clinical Reporting and Sign-out

Select Report Pages



Comprehensive Genomic Profile Report

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PierianDx
77 Maryland Plaza
St. Louis, MO 63108

PATIENT	DOB	DISEASE	MEDICAL RECORD #	REPORT DATE	REPORT STATUS
John Doe	02/04/1981	Non-small cell Lung Cancer	6563465346	02/18/2019	Final

Report Summary

2	0	1	0	High	Stable	13
IA	IB	IIC	IID	TMB	MSI	TRIALS

GENOMIC FINDINGS BY TIER + LEVEL

GENOMIC FINDINGS

Tier I - Strong Clinical Significance

VARIANT	LEVEL	CLINICAL IMPACT
NCOA4-RET fusion	A	May benefit from - Cabozantinib, Vandatinib *in non-small cell lung cancer*
KRAS p.G12D c.35G>A	A	Not likely to benefit from - Erlotinib, Gefitinib, Afatinib, Osimertinib, Dacomitinib *in non-small cell lung cancer* Unfavorable prognosis in - in non-small cell lung cancer

Tier II - Potential Clinical Significance

VARIANT	LEVEL	CLINICAL IMPACT
PDGFRA p.D842V c.2525A>T	C	May benefit from - Dasatinib *in gastrointestinal stromal tumor* Not likely to benefit from - Sunitinib, Imatinib *in gastrointestinal stromal tumor*

Other Biomarkers

VARIANT	LEVEL	VALUE	CLINICAL IMPACT
TMB	high	24 muts/Mb	May benefit from - Nivolumab, Nivolumab + Ipilimumab *in non-small cell lung cancer*
MSI	stable	5% Unstable Sites	

Clinically Critical Front Page

CGP REPORT

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PATIENT	DOB	DISEASE	MEDICAL RECORD #	REPORT DATE	REPORT STATUS
John Doe	02/04/1981	Non-small cell Lung Cancer	6563465346	02/18/2019	Final

PATIENT AND ORDER DETAILS

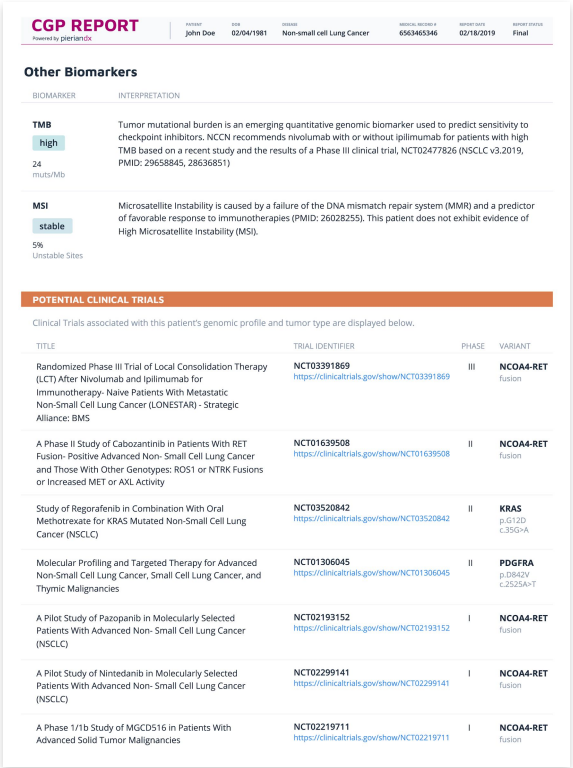
PATIENT	PHYSICIAN	SPECIMEN	CASE
DATE OF BIRTH 02/04/1981	ORDERING PHYSICIAN Bruce Banner	SPECIMEN TYPE Specimen from lung	REVIEW STATUS Final
SEX Male	FACILITY Organization Name	EXT. SPECIMEN ID 48998243	DATE ACCESSIONED 02/15/2019
ETHNICITY Not Hispanic or Latino		DATE COLLECTED 02/05/2019 13:53	DATE REPORTED Not Available
RACE Caucasian		DATE RECEIVED 02/08/2019 12:44	ACCESSION # ILMN_447
		% TUMOR IN SELECTED AREA 25	

CLINICALLY RELEVANT RESULTS

Tier I - Strong Clinical Significance

VARIANT	INTERPRETATION
NCOA4-RET fusion A	RET encodes a receptor tyrosine kinase involved in cell growth and differentiation which is known to undergo oncogenic activation in vivo and in vitro by cytogenetic rearrangement (provided by RefSeq, Jul 2008). NCOA4 encodes an androgen receptor coactivator which interacts with the androgen receptor in a ligand-dependent manner to enhance its transcriptional activity. Chromosomal translocations between NCOA4 and RET, both located on chromosome 10, have been associated with papillary thyroid carcinoma (provided by RefSeq, Feb 2009). RET rearrangements resulting in fusion with partner genes including KIF5B, CCDC6 and NCOA4 have been reported in non-small cell lung cancer (NSCLC) patients(PMID- 29128428). A NCOA4-RET fusion is identified in this case. The N terminus of the NCOA4 gene fuses with the C terminus of the RET gene in this fusion (PMID- 28011461). In PCCL3 cells, expression of NCOA4-RET fusion was reported to simultaneously activate DNA synthesis and apoptosis apart from interfering with thyroid differentiation at steps distal to the TSH-R (PMID- 12690093, 2003). The NCOA4-RET fusion has been reported in patients with NSCLC specifically in lung adenocarcinoma patients (COSMIC, February 2019, PMID- 23150706). RET rearrangements are one of the emerging biomarkers to identify novel therapies for patients with metastatic NSCLC (NCCN, NSCLC v.3.2019). NCCN recommends cabozantinib and vandatinib (category 2A) as targeted agents for NSCLC patients harbouring RET rearrangements (NCCN, NSCLC v.3.2019).
KRAS p.G12D c.35G>A A NM_004985.3 VAF % 10.0 DEPTH 5663	The KRAS protein has intrinsic GTPase activity and is an important mediator of growth factor receptor signaling resulting in the activation of several downstream pathways such as PI3K-mTOR and RAS-RAF-MEK pathway (RefSeq, Jul 2008). A missense alteration in KRAS, G12D, is identified in this case. Codon 12 lies within a GTP binding region of the KRAS protein (UniProt.org). Mutations in KRAS at codon 12 (within the GTP binding region), including KRAS G12D, result in reduced GTPase activity, which in turn leads to constitutive activation of KRAS and its downstream PI3K-AKT and MAPK signaling pathways (PMID- 26902995; 25705018).

In-depth Interpretations



CGP REPORT

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PATIENT	DOB	DISEASE	MEDICAL RECORD #	REPORT DATE	REPORT STATUS
John Doe	02/04/1981	Non-small cell Lung Cancer	6563465346	02/18/2019	Final

Other Biomarkers

BIOMARKER	INTERPRETATION
TMB high 24 muts/Mb	Tumor mutational burden is an emerging quantitative genomic biomarker used to predict sensitivity to checkpoint inhibitors. NCCN recommends nivolumab with or without ipilimumab for patients with high TMB based on a recent study and the results of a Phase III clinical trial, NCT02477826 (NSCLC v3.2019, PMID: 29658845, 28636851)
MSI stable 5% Unstable Sites	Microsatellite Instability is caused by a failure of the DNA mismatch repair system (MMR) and a predictor of favorable response to immunotherapies (PMID: 26028255). This patient does not exhibit evidence of High Microsatellite Instability (MSI).

POTENTIAL CLINICAL TRIALS

Clinical Trials associated with this patient's genomic profile and tumor type are displayed below.

TITLE	TRIAL IDENTIFIER	PHASE	VARIANT
Randomized Phase III Trial of Local Consolidation Therapy (LCT) After Nivolumab and Ipilimumab for Immunotherapy- Naive Patients With Metastatic Non-Small Cell Lung Cancer (LONESTAR) - Strategic Alliance: BMS	NCT03391869 https://clinicaltrials.gov/show/NCT03391869	III	**NCOA4-RET** fusion
A Phase II Study of Cabozantinib in Patients With RET Fusion- Positive Advanced Non- Small Cell Lung Cancer and Those With Other Genotypes: ROS1 or NTRK Fusions or Increased MET or AXL Activity	NCT01639508 https://clinicaltrials.gov/show/NCT01639508	II	**NCOA4-RET** fusion
Study of Regorafenib in Combination With Oral Methotrexate for KRAS Mutated Non-Small Cell Lung Cancer (NSCLC)	NCT03520842 https://clinicaltrials.gov/show/NCT03520842	II	**KRAS** p.G12D c.35G>A
Molecular Profiling and Targeted Therapy for Advanced Non-Small Cell Lung Cancer, Small Cell Lung Cancer, and Thymic Malignancies	NCT01306045 https://clinicaltrials.gov/show/NCT01306045	II	**PDGFRA** p.D842V c.2525A>T
A Pilot Study of Pazopanib in Molecularly Selected Patients With Advanced Non- Small Cell Lung Cancer (NSCLC)	NCT02193152 https://clinicaltrials.gov/show/NCT02193152	I	**NCOA4-RET** fusion
A Pilot Study of Nintedanib in Molecularly Selected Patients With Advanced Non- Small Cell Lung Cancer (NSCLC)	NCT02299141 https://clinicaltrials.gov/show/NCT02299141	I	**NCOA4-RET** fusion
A Phase 1/1b Study of MGCD516 in Patients With Advanced Solid Tumor Malignancies	NCT02219711 https://clinicaltrials.gov/show/NCT02219711	I	**NCOA4-RET** fusion

Relevant Clinical Trials

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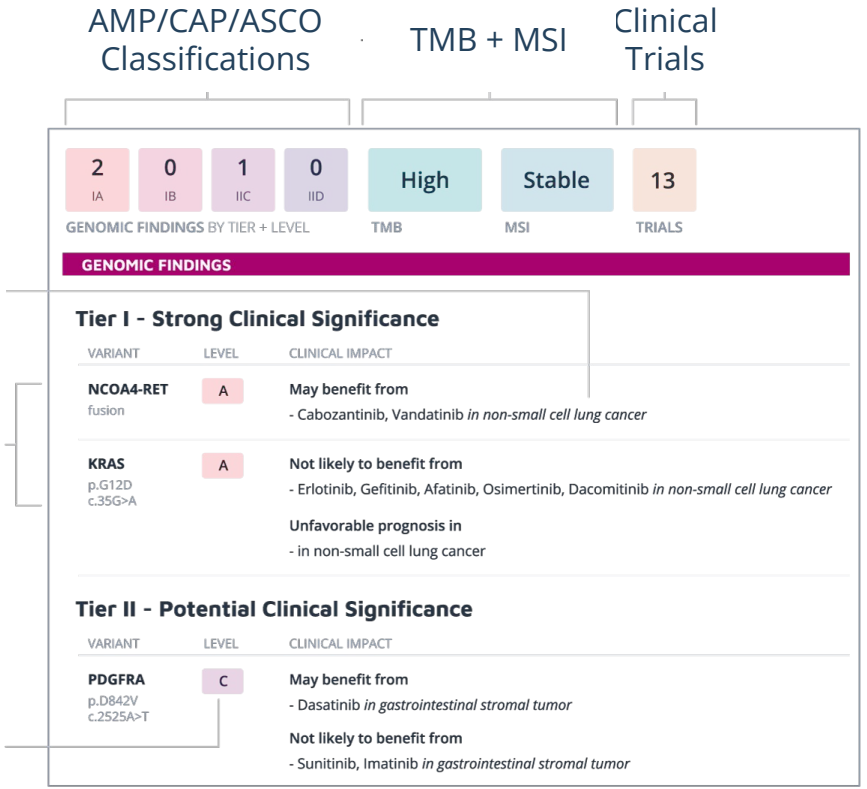
PierianDx Clinical Reporting and Sign-out

Editable interpretation and clinical impact statements

Supports all variant types and biomarkers across multiple tumor types: SNVs, indels, splice variants, CNVs, and fusions

Evidence levels determined by expertly curated and up-to-date drug labels, practice guidelines, clinical trials, cancer research databases, millions of publications, shared medical interpretations, and more



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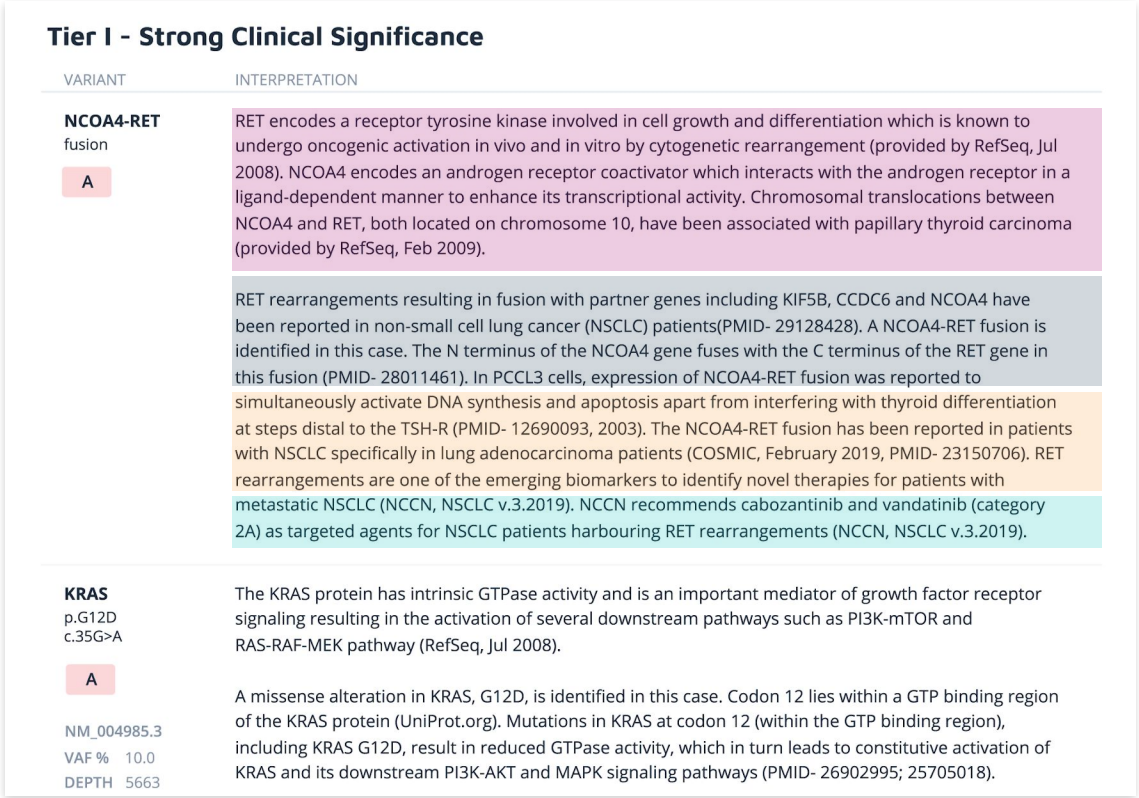
AMP/CAP/ASCO Guideline Recommended Elements

In-depth Variant Interpretations



Tier I - Strong Clinical Significance

VARIANT	INTERPRETATION
NCOA4-RET fusion A	RET encodes a receptor tyrosine kinase involved in cell growth and differentiation which is known to undergo oncogenic activation in vivo and in vitro by cytogenetic rearrangement (provided by RefSeq, Jul 2008). NCOA4 encodes an androgen receptor coactivator which interacts with the androgen receptor in a ligand-dependent manner to enhance its transcriptional activity. Chromosomal translocations between NCOA4 and RET, both located on chromosome 10, have been associated with papillary thyroid carcinoma (provided by RefSeq, Feb 2009). RET rearrangements resulting in fusion with partner genes including KIF5B, CCDC6 and NCOA4 have been reported in non-small cell lung cancer (NSCLC) patients(PMID- 29128428). A NCOA4-RET fusion is identified in this case. The N terminus of the NCOA4 gene fuses with the C terminus of the RET gene in this fusion (PMID- 28011461). In PCCL3 cells, expression of NCOA4-RET fusion was reported to simultaneously activate DNA synthesis and apoptosis apart from interfering with thyroid differentiation at steps distal to the TSH-R (PMID- 12690093, 2003). The NCOA4-RET fusion has been reported in patients with NSCLC specifically in lung adenocarcinoma patients (COSMIC, February 2019, PMID- 23150706). RET rearrangements are one of the emerging biomarkers to identify novel therapies for patients with metastatic NSCLC (NCCN, NSCLC v.3.2019). NCCN recommends cabozantinib and vandatinib (category 2A) as targeted agents for NSCLC patients harbouring RET rearrangements (NCCN, NSCLC v.3.2019).
KRAS p.G12D c.35G>A A NM_004985.3 VAF % 10.0 DEPTH 5663	The KRAS protein has intrinsic GTPase activity and is an important mediator of growth factor receptor signaling resulting in the activation of several downstream pathways such as PI3K-mTOR and RAS-RAF-MEK pathway (RefSeq, Jul 2008). A missense alteration in KRAS, G12D, is identified in this case. Codon 12 lies within a GTP binding region of the KRAS protein (UniProt.org). Mutations in KRAS at codon 12 (within the GTP binding region), including KRAS G12D, result in reduced GTPase activity, which in turn leads to constitutive activation of KRAS and its downstream PI3K-AKT and MAPK signaling pathways (PMID- 26902995; 25705018).

Gene Level Info

Variant Level Info

Supporting Evidence

Therapy, Guideline Info

HGVS nomenclature: p. and c. notation

Transcript, variant allele fraction, depth

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Evidence-based Reporting

PierianDx Clinical Reporting and Sign-out

CGP REPORT

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PATIENT	DOB	DISEASE	MEDICAL RECORD #	REPORT DATE	REPORT STATUS
John Doe	02/04/1981	Non-small cell Lung Cancer	6563465346	02/18/2019	Final

A Phase 1b Study of Abemaciclib in Combination With Pembrolizumab for Patients With Stage IV Non- Small Cell Lung Cancer or Hormone Receptor Positive, HER2 Negative Breast Cancer	NCT02779751 https://clinicaltrials.gov/show/NCT02779751	I	KRAS p.G12D c.35G>A
A Phase Ib, Open-label, Multicenter Study of Oral LXH254 in Combination With Oral LTT462 in Adult Patients With Advanced or Metastatic KRAS or BRAF Mutant Non- Small Cell Lung Cancer	NCT02974725 https://clinicaltrials.gov/show/NCT02974725	I	KRAS p.G12D c.35G>A
A Phase I Dose Finding Study of Oral LXH254 in Adult Patients With Advanced Solid Tumors Harboring MAPK Pathway Alterations	NCT02607813 https://clinicaltrials.gov/show/NCT02607813	I	KRAS p.G12D c.35G>A
A Phase Ib Trial of Pembrolizumab (MK-3475) and Trametinib Focused on Advanced KRAS Mutant Non-small Cell Lung Cancer	NCT03299088 https://clinicaltrials.gov/show/NCT03299088	I	KRAS p.G12D c.35G>A
Phase 1/1b Study of MGCD516 in Patients With Advanced Cancer	NCT02219711 https://clinicaltrials.gov/show/NCT02219711	I	PDGFRA p.D842V c.2525A>T
Nintedanib in Molecularly Selected Patients With Advanced Non-Small Cell Lung Cancer	NCT02299141 https://clinicaltrials.gov/show/NCT02299141	I	PDGFRA p.D842V c.2525A>T

TIER III - VARIANTS OF UNCERTAIN SIGNIFICANCE

AKT3 p.P449S NM_001206729.1 c.1345C>T	ATM p.N1240Kfs*4 NM_000051.3 c.3720_3736del17	AKT3 p.E450K NM_001206729.1 c.1348G>A	APC p.V2194I NM_000038.5 c.6580G>A	APC p.D1794V NM_000038.5 c.5381A>T	APC p.A1793E NM_000038.5 c.5378C>A	APC p.N1792K NM_000038.5 c.5376T>A
APC p.L148H NM_000038.5 c.443T>A	APC p.L148I NM_000038.5 c.442C>A	BRCA2 p.P2283H NM_000059.3 c.6848C>A	ATM p.G3011Vfs*19 NM_000051.3 c.9006delA	BRCA2 p.S2984* NM_000059.3 c.8951C>A	BRCA2 p.S2984T NM_000059.3 c.8950T>A	BRCA2 p.E2301K NM_000059.3 c.6901G>A
BRCA2 p.I2296M NM_000059.3 c.6888A>G	BRCA2 p.D2294E NM_000059.3 c.6882C>G	BRCA2 p.N2291D NM_000059.3 c.6871A>G	BRCA2 p.H1731N NM_000059.3 c.5191C>A	BRCA2 p.P2283T NM_000059.3 c.6847C>A	BRCA2 p.G1761E NM_000059.3 c.5282G>A	BRCA2 p.D1737V NM_000059.3 c.5210A>T
BRCA2 p.D1737Y NM_000059.3 c.5209G>T	BRCA2 p.E1734* NM_000059.3 c.5200G>T	BRCA2 p.E1734K NM_000059.3 c.5200G>A	BRCA2 p.L1732P NM_000059.3 c.5195T>C	BRCA2 p.Y1313* NM_000059.3 c.3939C>A	BRCA2 p.Y1313C NM_000059.3 c.3938A>G	BRCA2 p.T1310I NM_000059.3 c.3929C>T

Variants of Unknown Significance

CGP REPORT

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PATIENT	DOB	DISEASE	MEDICAL RECORD #	REPORT DATE	REPORT STATUS
John Doe	02/04/1981	Non-small cell Lung Cancer	6563465346	02/18/2019	Final

BRCA2 p.T1310Mfs*25 NM_000059.3 c.3929delC	BRCA2 p.N1297K NM_000059.3 c.3891T>A	BRCA2 p.N1287fs*6 NM_000059.3 c.3860delA	BRCA2 p.S1284R NM_000059.3 c.3852T>G	BRCA2 p.S1284R NM_000059.3 c.3852T>A	BRCA2 p.V1283I NM_000059.3 c.3847G>A	BRCA2 p.V1283* NM_000059.3 c.3847delG
BRCA2 p.E866K NM_000059.3 c.2596G>A	BRCA2 p.P606Q NM_000059.3 c.1817C>A	BRCA2 p.Q347K NM_000059.3 c.1039C>A	CCND3 p.S178A NM_001136017.2 c.532T>G	CTNNB1 p.N287S NM_001098209.1 c.860A>G	KRAS p.R164Q NM_004985.3 c.491G>A	KRAS p.G174S NM_004985.3 c.520G>A
KRAS p.M188L NM_004985.3 c.562A>C	MSH2 p.S566K NM_000251.2 c.1698T>A	MSH2 p.T564N NM_000251.2 c.1691C>A	MSH2 p.Y563S NM_000251.2 c.1688A>C	MSH2 p.Y563N NM_000251.2 c.1687T>A	MSH2 p.E562Q NM_000251.2 c.1686G>C	MSH2 p.E562V NM_000251.2 c.1685A>T
MSH2 p.E562* NM_000251.2 c.1684G>T	MSH2 p.E562Q NM_000251.2 c.1684G>C	MSH2 p.E561* NM_000251.2 c.1681G>T	MSH2 p.E561K NM_000251.2 c.1681G>A	MSH2 p.N560I NM_000251.2 c.1679A>T	MSH2 p.S558F NM_000251.2 c.1673C>T	MSH2 p.S558Y NM_000251.2 c.1673C>A
PIK3R1 p.Q92K NM_001242466.1 c.274C>A	RB1 p.S302Y NM_000321.2 c.905C>A	RB1 p.W195C NM_00321.2 c.585G>T				

CLASSIFICATION AND LEVELS OF EVIDENCE

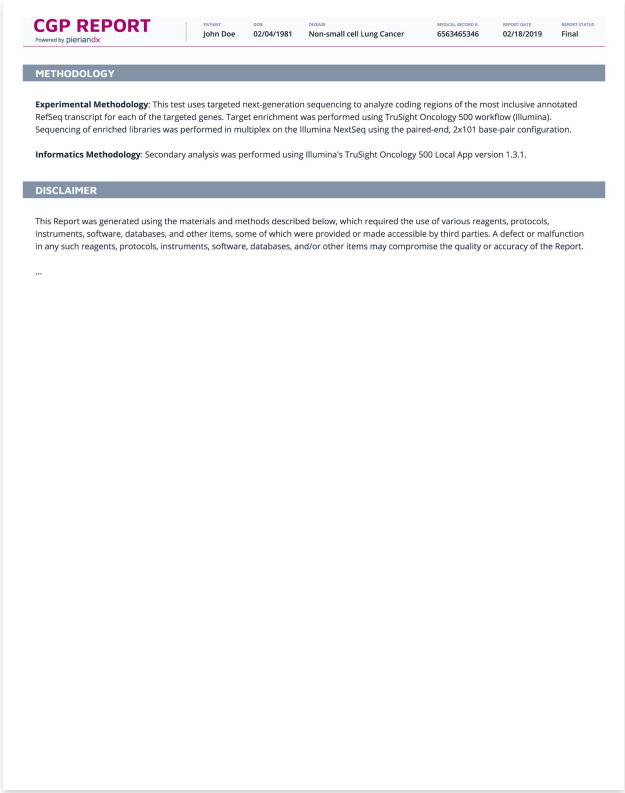
The variant classification system used in this report is based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and the College of American Pathologists (J Mol Diagn 2017, 19:4-23). Tiers IA, IB, IIC, IID, III and IV describe variant categories of descending clinical significance in the patient. Variants in Tier IV are not reported in accordance with the consensus recommendations.

IA	IB	IIC	IID
Variant of strong clinical significance, Level A evidence (*FDA approved therapy or practice guideline in patient's tumor type*)	Variant of strong clinical significance, Level B Evidence (*consensus in the field based on well-powered studies in patient's tumor type*)	Variant of potential clinical significance, Level C evidence (*FDA approved therapy or practice guideline in other tumor type(s), evidence from multiple small published studies, or based on availability of investigational therapies*)	Variant of potential clinical significance, Level D evidence (*case reports or preclinical studies*)

III	Variant of uncertain clinical significance
IV	Benign or likely benign variant

FOOTER AREA

Classification and Evidence Legend



CGP REPORT

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PATIENT	DOB	DISEASE	MEDICAL RECORD #	REPORT DATE	REPORT STATUS
John Doe	02/04/1981	Non-small cell Lung Cancer	6563465346	02/18/2019	Final

METHODOLOGY

Experimental Methodology: This test uses targeted next-generation sequencing to analyze coding regions of the most inclusive annotated RefSeq transcript for each of the targeted genes. Target enrichment was performed using TruSight Oncology 500 workflow (Illumina). Sequencing of enriched libraries was performed in multiplex on the Illumina NextSeq using the paired-end, 2x101 base-pair configuration.

Informatics Methodology: Secondary analysis was performed using Illumina's TruSight Oncology 500 Local App version 1.3.1.

DISCLAIMER

This Report was generated using the materials and methods described below, which required the use of various reagents, protocols, instruments, software, databases, and other items, some of which were provided or made accessible by third parties. A defect or malfunction in any such reagents, protocols, instruments, software, databases, and/or other items may compromise the quality or accuracy of the Report.

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Methodology, Disclaimer

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Enabling Rapid Validation and Deployment

LDT Support of RUO Assays

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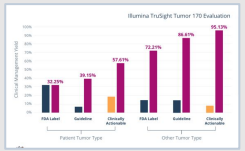
A Seamless Experience

Enabling TruSight™ Oncology 500

Enabling Sample-to-Answer

The combination of **TruSight™ Oncology 500** with **PierianDx** variant interpretation and reporting solutions will provide customers with a **seamless, high-quality experience**.

					Powered by PierianDx	
FFPE specimens	Sample	Library prep and enrichment	Sequence	Analysis	Annotation, Classification	Interpretation, Reporting
						
Supports multiple tissue types	Commercial DNA/RNA extraction kits	TruSight Oncology UMI kit DNA Probes (523 genes) RNA Probes (55 genes) Automatable workflow	NextSeq™ System	TruSight Oncology 500 Local App		

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Software Specifications

PierianDx Clinical Genomics Workspace

Complete Molecular Testing Solution

- Complete HL7 integration: LIMS EHR
- Fully HIPAA-compliant cloud-based Application
- Secure site-to-site Virtual Private Network (VPN)
- API (Application Programming Interface) for workflow automation and data retrieval
- Support for next generation sequencing and clinical microarray

Clinical Sharing Network

- Access to ~20K prior medical interpretations of genomic variants
- Aggregation of de-identified patients data across disease populations

Variant Analysis and Classification

- FASTQ to VCF
- Variant annotation
- Intuitive variant classification
- Phenolyzer scoring
- Inheritance model filtering

Quality Assurance/Control

- Run-to-run variability and reproducibility versus validated reference averages
- Complete provenance tracking
- Role-based access privileges

Clinical Reporting

- Somatic cancer and constitutional disorders
- Branded and customized clinical report creation
- Clinical trial matching
- Intuitive genome browser

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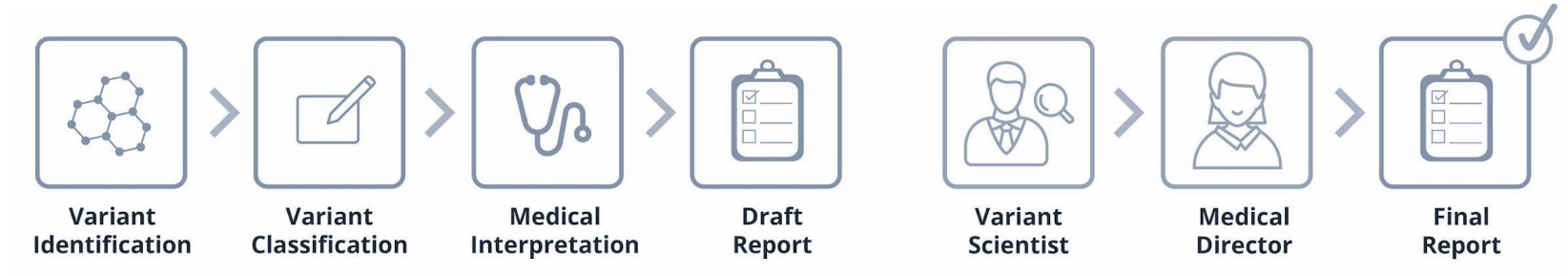
Optimal Mix of Technology + Human Expertise



	Software Automated	Variant Scientist & Medical Review
Classifications	Auto classification and interpretation of variants based on sophisticated rules engine	Assess existing variant classification/interpretation and update based on newest literature
Interpretations	Wide range of suggested therapeutic options and risk information	Evaluate pathways, variant co-occurrence, and patient data to create cohesive interpretations
Clinical Trials	Clinical trial matching based on molecular findings	Refine trial matching based on pathway information and client-specific SOP

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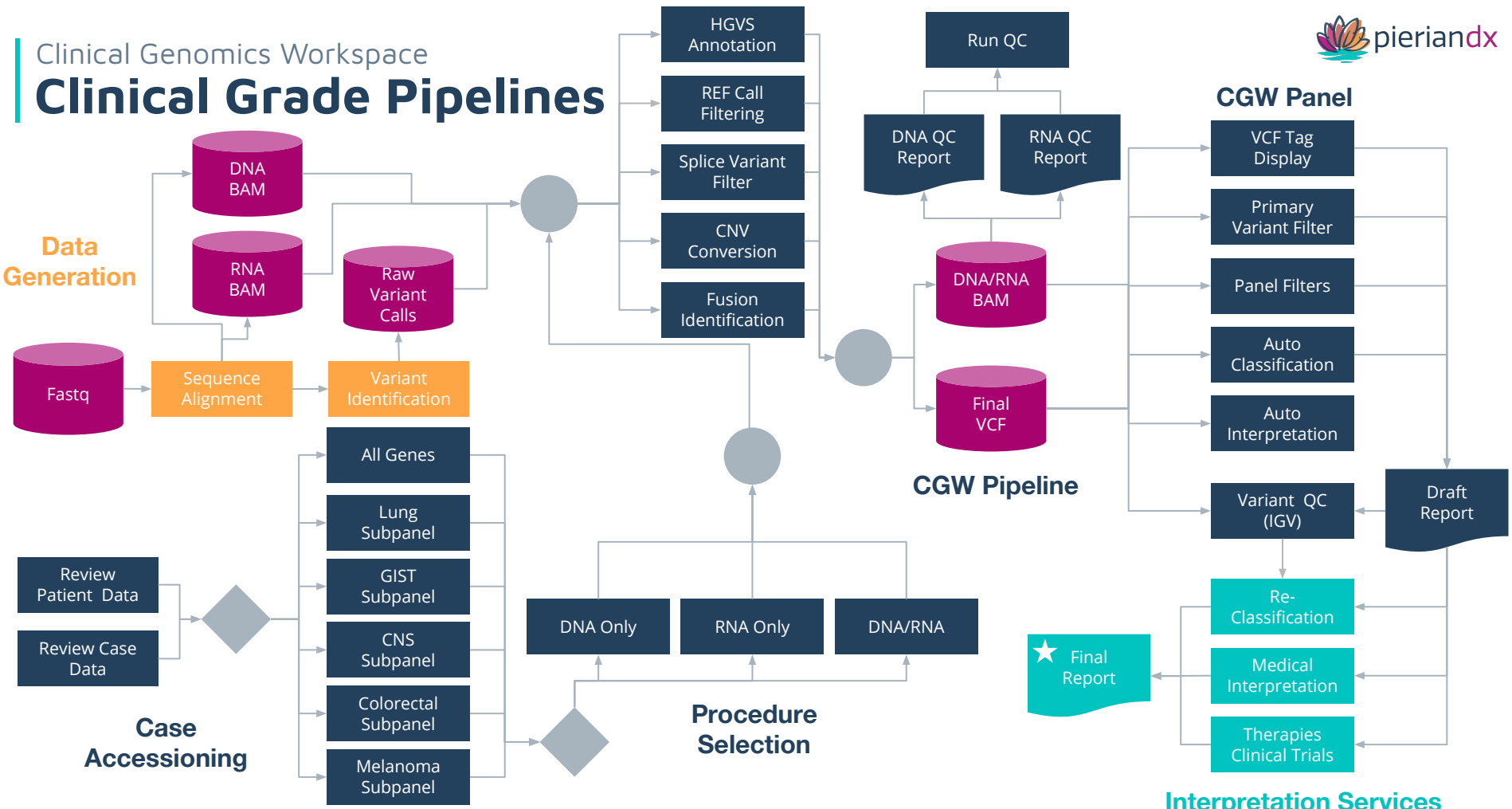
Clinical Genomics Workspace

Clinical Grade Pipelines



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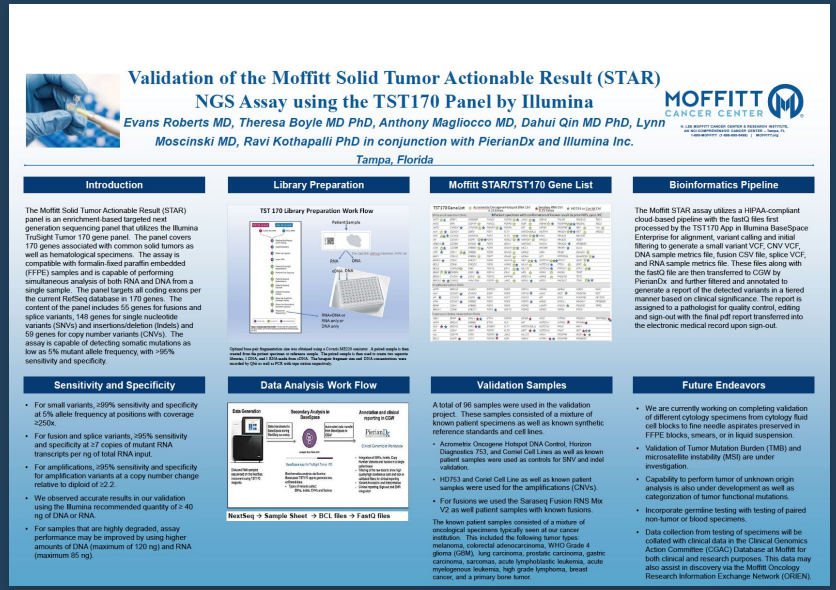
Trusted Assay Validation Services

Accelerate Your Go-Live

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

Clinical Laboratory Improvement Amendments

NGS Assay Validation Support
Development of draft validation plan ✓
Assay familiarization and optimization ✓
Validation analyses and data review ✓
Analytical sensitivity and specificity ✓
Precision concordance, reproducibility ✓
QC references, exon/hotspot coverage ✓
Sample procurement ✓
Report, requisition & consent forms ✓
CAP/CLIA compliant documentation ✓



Case Study: Moffit STAR

1st Clinically Validated & Live TST 170 Assay

PierianDx was instrumental in Moffitt's validation and implementation of Illumina's TruSight Tumor 170.

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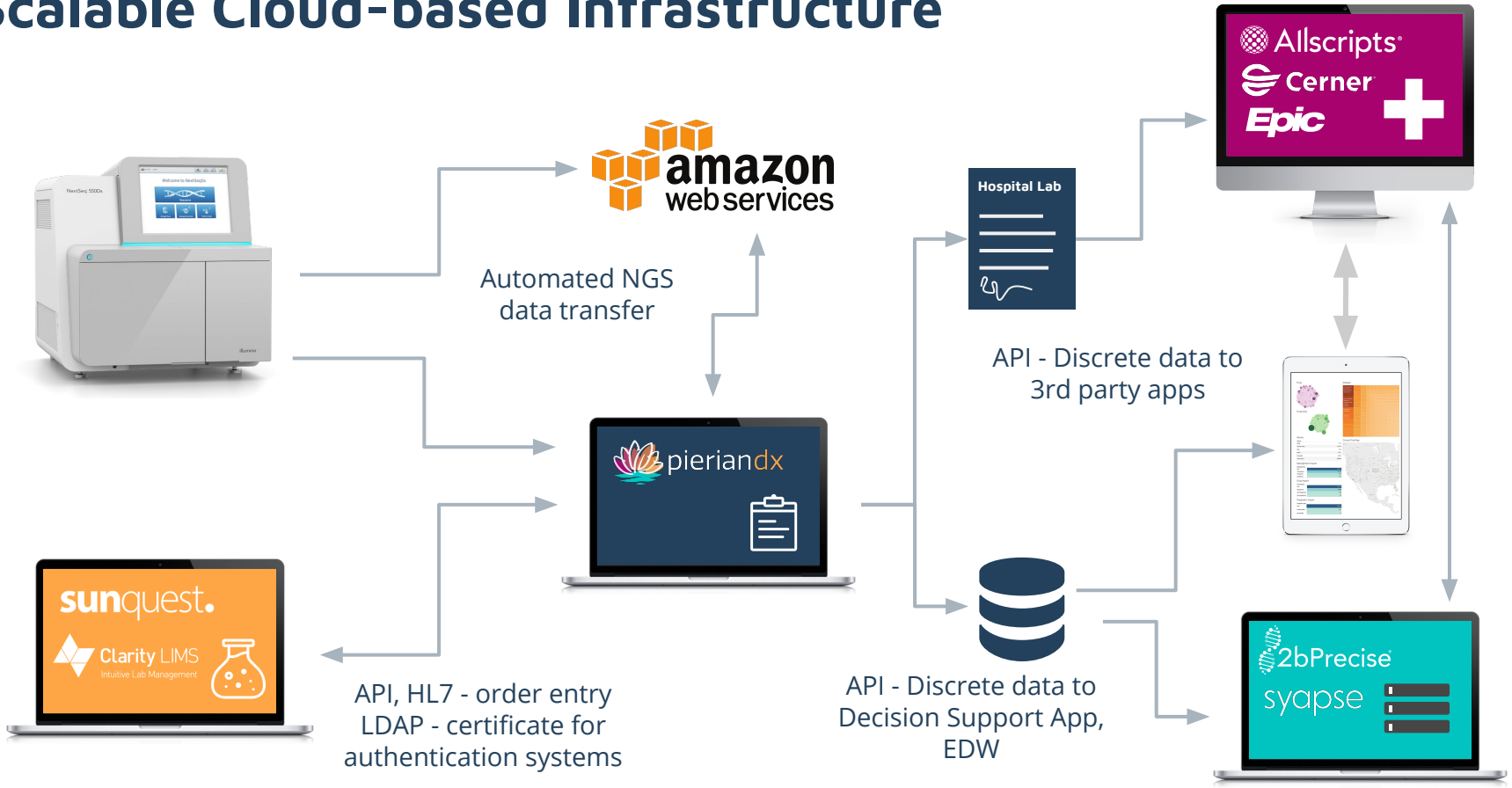
Scalable Cloud-based Infrastructure



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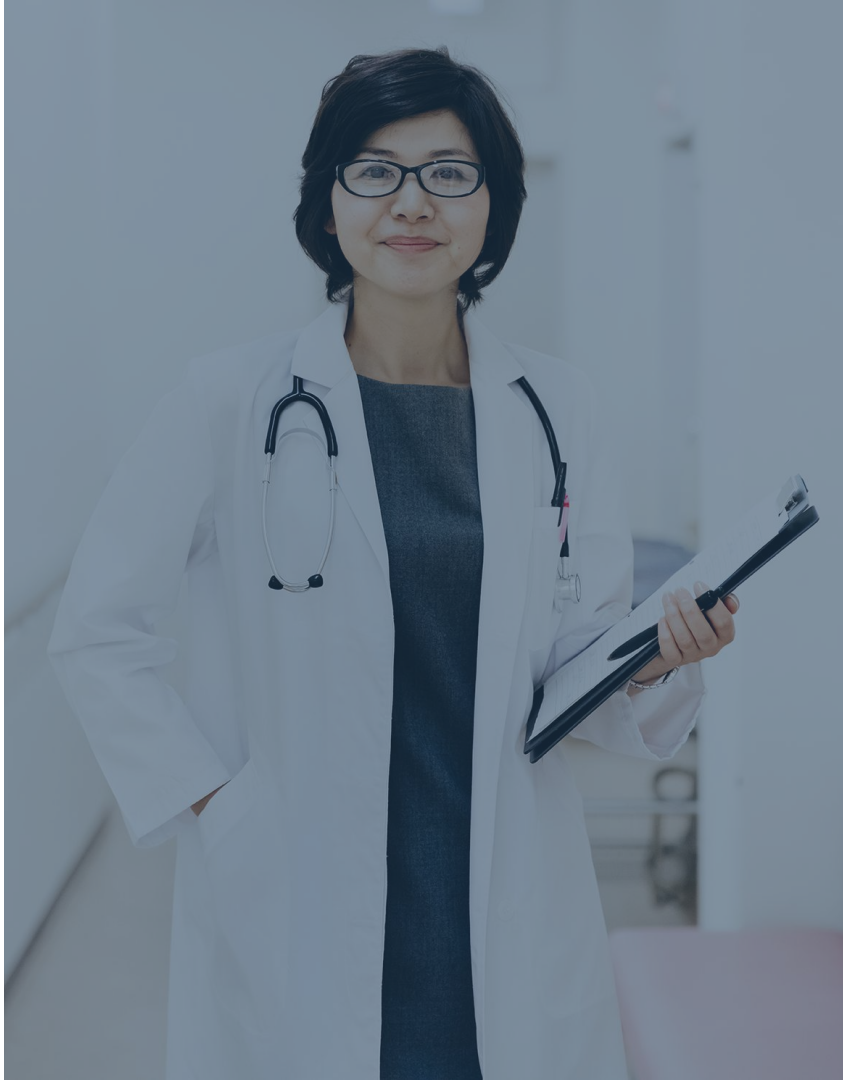
That's a Wrap

Summary

- Comprehensive genomic profiling provides **high value for patients and laboratories**
- TruSight™ Oncology 500 enables **in-house comprehensive genomic profiling** with excellent content and performance
- PierianDx interpretation and evidence-based reporting solutions enable a **streamlined sample-to-answer experience**
- PierianDx software and expert services support **rapid clinical validation and deployment** of research use only assays as laboratory developed tests



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