BJ DOB 10/10/1967

Metastatic adenocarcinoma of the lung.

1

Presentation:

- He presented with a shortness of breath whilst playing touch football. He went on to have a chest x-ray and CT which showed evidence of quite widespread lymphadenopathy in the chest and upper abdomen as well as extensive pleuraly based disease on the left.

2

Histology

- The PDL1 staining was only 0% and the other tests such as EGFR activating mutation, ALK and ROS1 were all negative.
- He has gone on to have molecular testing at the Queensland University of Technology and initially testing showed no evidence of actionable targets and low TMB but recently we have shown evidence of RET-CCDC6 gene fusion event.

Fusion: RET_ENST00000355710.3:intron:11| 10:43611402 to CCDC6_ENST00000263102.6:intron:1|+10:61637528

3

Treatment so far

- BJ has been enrolled on the BR34 Study and he was allocated to treatment with a combination of Pemetrexed, Carboplatin, Durvalumab and Tremelimumab (PD-l1 and CTLA-4 antibodies).
- He has had the treatment since July 2018 and has done remarkably well.

4

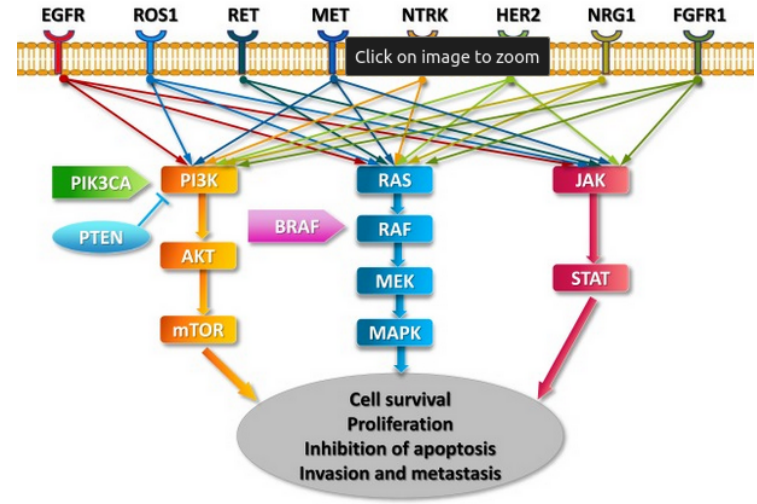
Disease Status

- His disease remains stable with evidence of a partial response.
- He is having ongoing maintenance treatment with Pemetrexed and Durvalumab.

- The purpose of presenting today is to check if there are any potential treatments or trials for the abnormality recently noted – RET CCDC6 gene fusion event. I understand high potency RET inhibitors are in development.

5

RET activates JAK/STAT , MAPK/ERK



Guo Y et al Onco Targets Ther. 2019 PMID:31819518

6

RET fusions in lung cancers

RET rearrangements in 1-2% of NSCLC and define a unique molecular subset.
(0.15% of all solid tumours). IHC not effective FISH is best

Correlate with

- adenocarcinoma histologic subtype,
- never-smoking status, (82%)
- younger age, 73% < 60yrs)
- more advanced disease stage, (but small primary lesions < 3cm but more N2 disease)
- radiological lymphangitic spread and psammoma bodies
- potentially higher chemosensitivity (high overall response rates (ORRs) (i.e., 40%) and progression-free survival (PFS) (i.e., 19 months) with pemetrexed-based chemotherapy)

A **RET-specific** agents are currently clinically available LOXO-292, BLU-667 but **several promiscuous kinase inhibitors** that target RET, among others, have been approved for MTC treatment

Ferrara R et al J Thorac Oncol. 2018 PMID: 29128428

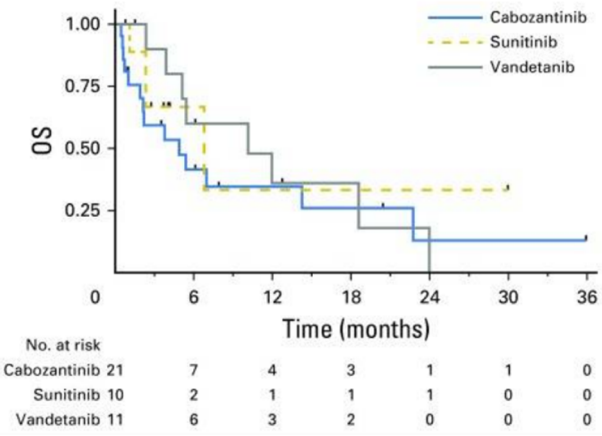
7

Pan- TKI inhibitors

- nintedanib-docetaxel case study 60yo F with no other mutation but RET-CCDC6 . Nintedanib alone with PFS for 33mths

Larger Studies: (n=165)

- E.g. Vandetanib (OS (11.6mths) PFS 4.5mths)
- Moderate to limited success.



Results from Multicenter RET registry J Clin Oncol 2017 PMC5559893

8

RET –specific inhibitors

- LOX0-292 (RET specific) 17/26 (65%) has a radio graphic response (well tolerated) compare to 79% for Medullay thyroid cancer. IN previously treated patients ORR of 68% and a CNS ORR of 91%.PFS median 18.4 mths
- BLU-667. ORR of 45% was observed among the 11 evaluable NSCLC patients, including heavily pre-treated patients, who had received prior RET-targeting agents (NCT03037385). clinical trails not in Australia

NCT03157128 https://meetinglibrary.asco.org/record/161573/abstract

9

Clinical trials in Australia associated with RET fusions identified using molecular match

- **NCT03157128** - Phase 1/2 Study of LOXO-292 in Patients With Advanced Solid Tumours, RET Fusion-Positive Solid Tumours, and Medullary Thyroid Cancer (Peter MacCallum VIC, Royal North Shore (NSW)
- **NCT03178552** - A Study to Evaluate Efficacy and Safety of Multiple Targeted Therapies as Treatments for Participants With Non-Small Cell Lung Cancer (NSCLC) (Pan-TKIs-Alectinib)
- **NCT04200404** - A Study of CS1001 in Subjects With Advanced or Refractory Solid Tumours (CS1001 PD-L1 inhibitor and Regorafenib – Pan TKI))
- **NCT03976375 – P3** Efficacy and Safety of Pembrolizumab (MK-3475) With Lenvatinib (VEGFR 1/2/3, FGFR, PDGFR, RET inhibitor) vs. Docetaxel in Participants With Metastatic Non-Small Cell Lung Cancer (NSCLC) and Progressive Disease (PD) After Platinum Doublet Chemotherapy and Immunotherapy (MK-7902-008/E7080-G000-316/LEAP-008**)**

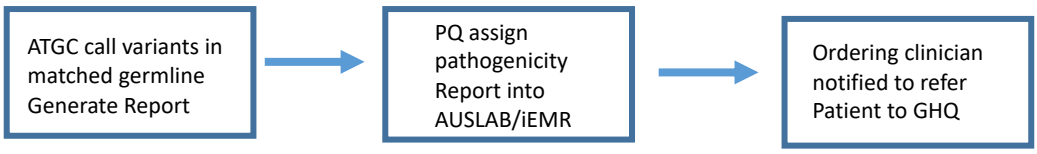
10

Incidental Germline Protocol

- NATA approved testing for BRCA1, BRCA2, PALB2, ATM, CHEK2*
- Why: Homologous recombination deficiency (PARP/ platinum therapy)

NCCN guidelines for Ovarian, Breast, prostate and Pancreatic cancers

- Workflow



Patients must be consented to our research study

11

Update

- NATA approval last week.
- 3 of 8 Breast Cancer Patients sent to PQ with positive reports
- Mutations under review

- BRCA2 p.Ser3366Ter (0/1 in ClinVar rs730881599 in last exon conflicting interpretations)
- BRCA2 p.Leu2327X (0/1 In ClinVar rs879255306 Reviewed ENIGMA)
- ATM p.Arg3008Cys (0/1 In ClinVar rs587782292 multiple submitters, no conflicts)

12

ATM is an interesting case

- Homozygous are reported as pathogenic Ataxia-telangiectasia syndrome
- Heterozygous are reported pathogenic for Cancer predisposition by Color and Ambry hover likely difficult to assign familiar risk
- Experimental studies have shown that this missense change results in inactivation of ATM kinase activity, increased radio-sensitivity, and cell cycle defects in vitro (PMID: 12552566, 15101044, 18573109)
- So important to HRR
- Clinical trails for HRR can us up to 15 genes (>1 in any gene)

NCT04123366, NCT03742895

13

Discussion

- Some the future other genes will be identified as biomarker for HRR

ARIEL 3 study:

BRCA1, *BRCA2*, *ATM*, *ATR*, *BARD1*, *BLM*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCI*, *FANCL*, *FANCM*, *MRE11*, *NBN*, *PALB2*, *RAD50*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD52*, *RAD54L*, and *RPA1*

BUT

May not confer quantifiable familiar risk

- Might be import to oncology but not referable to GHQ.
- How should these be described.

14

Enabling Local Precision Medicine Programs

Joel Geoghegan
Manager, Oncology Specialists for Asia

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Overview

- Precision Medicine in Oncology
- Comprehensive Genomic Profiling (CGP)
- Illumina's TruSight Oncology 500 Product Portfolio for CGP and Liquid Biopsy
- Local Clinical Trial Programs

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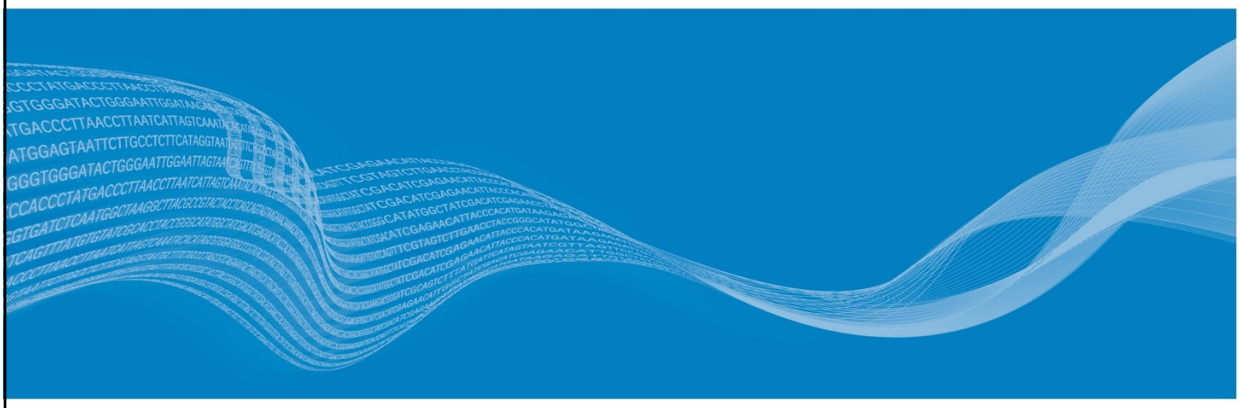
Precision Medicine



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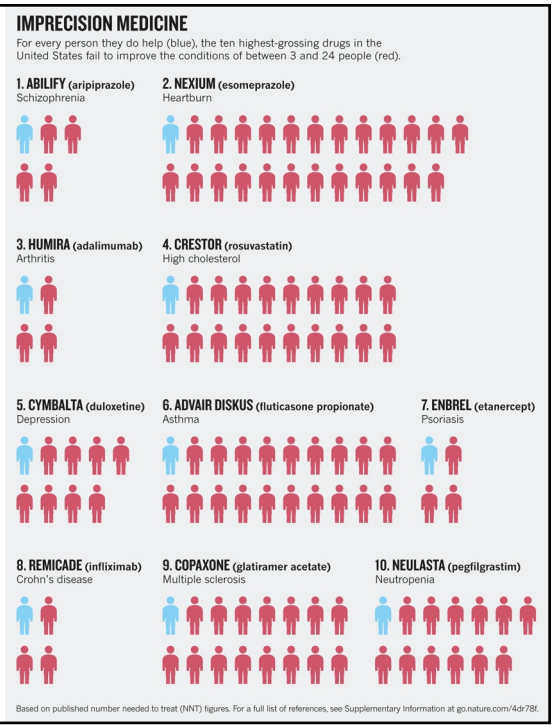
How do top selling drugs perform?

Imprecision Medicine

- **Top ten highest-grossing drugs in the United States (2013)**
  - At Worst benefit 1 in 25
  - At Best benefit 1 in 4
- **of the people who take them**



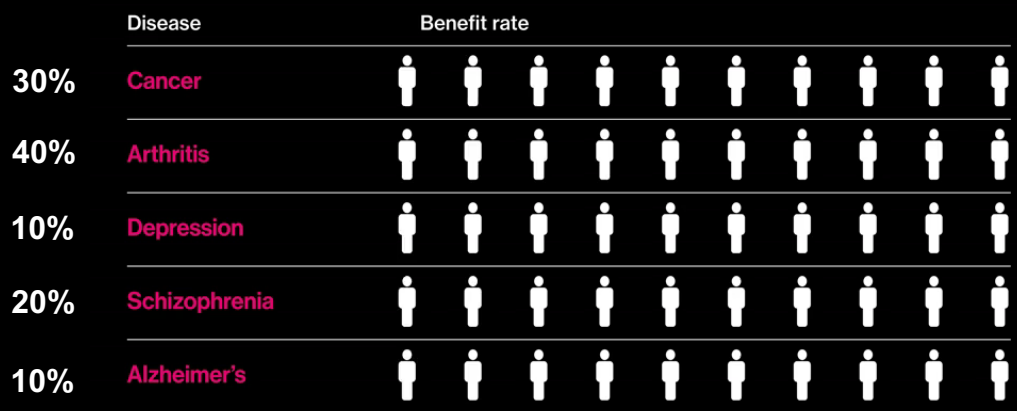
https://www.drugs.com/stats/top100/2013/sales

Schork NJ. Personalized Medicine. *Nature* 520, 609–611 (30 April 2015)

18

One Size Does Not Fit All

Percentage of patients who benefit from therapy



Antonio Regalado. Look how far precision medicine has come. MIT Technology Review. Oct 23, 2018
https://www.technologyreview.com/s/612281/look-how-far-precision-medicine-has-come/

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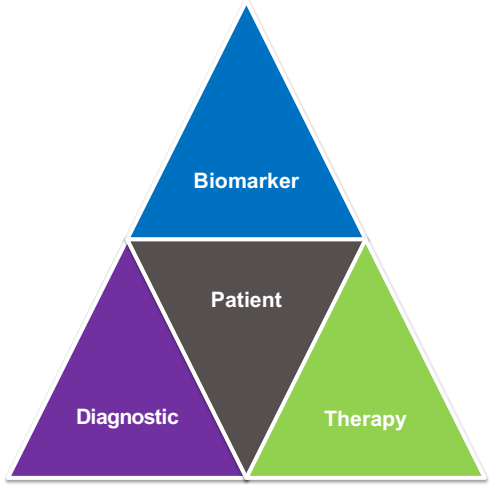
What is precision medicine?

- **Match the patient to the therapy that will provide the most benefit**
- **Increase efficacy through biomarker guided decision making**
- **Reduce unnecessary side effects by minimizing treatment less likely to work**
- **Cost savings and improved outcomes**



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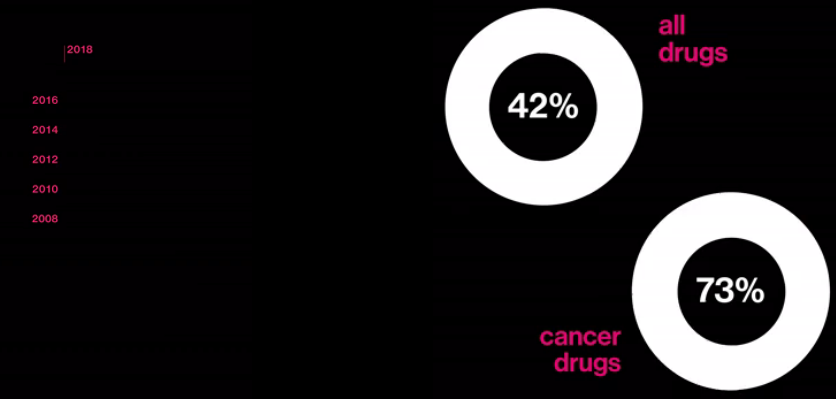
Biomarker driven medicine is growing

Pharma industry and regulatory bodies are key stakeholders



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Antonio Regalado. Look how far precision medicine has come. MIT Technology Review. Oct 23, 2018
https://www.technologyreview.com/s/612281/look-how-far-precision-medicine-has-come/

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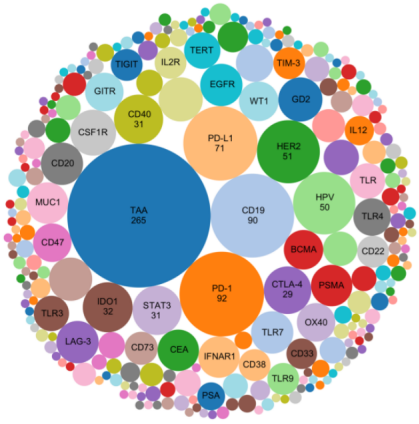
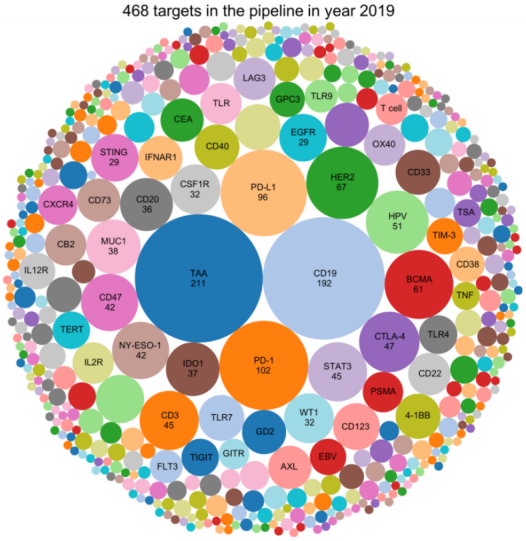
There are more cancer biomarkers and therapies in the pipeline

Nearly 80% growth in just the past 2 years

263 targets in the pipeline in year 2017



468 targets in the pipeline in year 2019



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Xin Yu et al. Imuno-oncology drug development goes global. Nature Reviews Drug Discovery 27 September 2019.
Supplemental Figure 2 https://www.nature.com/articles/d41573-019-00167-9

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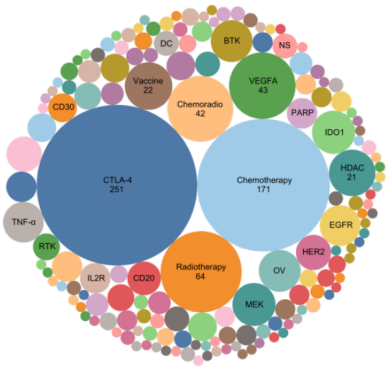
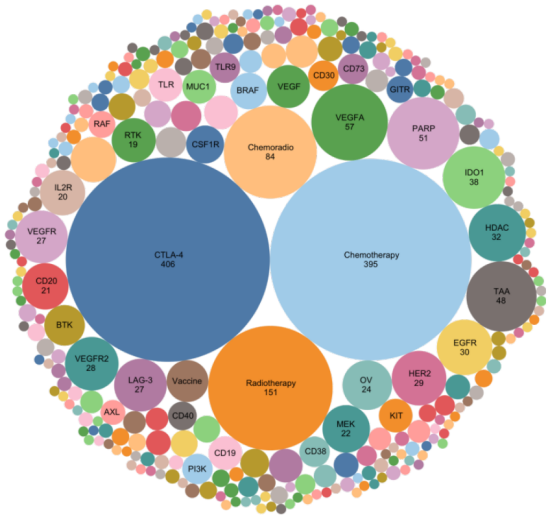
Immuno-Oncology is growing

Clinical trials with immune checkpoint inhibitors have doubled in the past 2 years

1,103 active trials testing 159 targets in 2017



2,251 active trials testing 295 targets in 2019



23

Xin Yu et al. Trends in clinical development for PD-1/PD-L1 inhibitors. Nature Reviews Drug Discovery 04 November 2019. Supplemental Figure 1 https://www.nature.com/articles/d41573-019-00182-w

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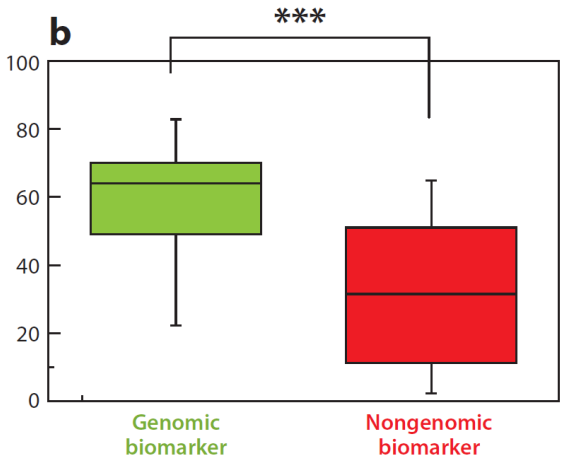
Precision medicine is showing progress

FDA-approved drugs or combinations (between January 1, 2006, and June 1, 2018)

- **Dependent on the detection of a specific genomic alteration in tumors (Mean: 60% ORR)**
- **Not dependent on the detection of a specific genomic alteration in tumors (Mean: 31% ORR)**



24

Doherty et al. Cancer Treatment in the Genomic Era. *Annu. Rev. Biochem.* 2019. 88:247–80

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This huge number of
biomarkers,
targeted therapies,
immunotherapies and
clinical trials
requires a new paradigm.

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Comprehensive Genomic Profiling

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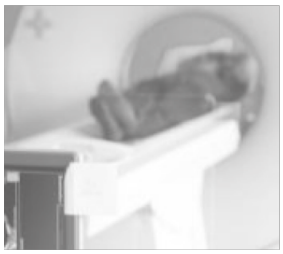
NGS and the Cancer Patient Journey

Hereditary Factors

Germline

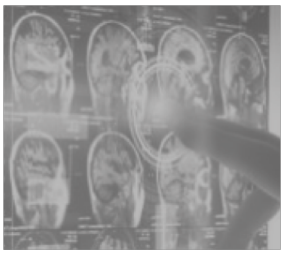


Screening

ctDNA

Therapy Selection

Tissue ctDNA



Monitoring

ctDNA

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Same location. Different mutations.

Lack of tissue and time to result favor implementation of NGS based tests.

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Many Questions, But Little or no Tissue

NSCLC

- 31% | **Unknown Oncogenic Driver Detected**
- 25% | **KRAS**
- 17% | **EGFR Sensitizing** • Gefitinib[4] • Erlotinib[4] • Afatinib[4] • Osimertinib[4] • Necitumumab[4] • Rociletinib[3]
- 7% | **ALK** • Crizotinib[4] • Alectinib[4] • Ceritinib[4] • Lorlatinib[4] • Brigatinib[2]
- 4% | **EGFR** • Other
- 3% | **MET** • Crizotinib[2] • Cabozantinib[2]
- 3% | **>1 Mutation**
- 2% | **HER2** • Trastuzumab emtansine[2] • Afatinib[2] • Dacomitinib[2]
- 2% | **ROS1** • Crizotinib[4] • Cabozantinib[2] • Ceritinib[2] • Lorlatinib[2] • DS-6051b[1]
- 2% | **BRAF** • Vemurafenib[2] • Dabrafenib[2]
- 2% | **RET** • Cabozantinib[2] • Alectinib[2] • Apatinib[2] • Vandetanib[2] • Ponatinib[2] • Lenvatinib[2]
- 1% | **NTRK1** • Entrectinib[2] • LOXO-101[2] • Cabozantinib[2] • DS-6051b[1]
- 1% | **PIK3CA** • LY3023414[2] • PQR 309[1]
- <1% | **MEK1** • Trametinib[2] • Selumetinib[3] • Cobimetinib[1]

KEY
1 – Phase I 3 – Phase III
2 – Phase II 4 – Approved

FFPE Sample
Tiny to Small

▼

Limited number of slides available

29 • Tsao AS, Scagliotti GV, Bunn PA Jr et al. Scientific Advances in Lung Cancer 2015. *J Thorac* Oncol. 11:613-638, 2016
• Roy-Chowdhuri S, Stewart J. Preanalytic Variables in Cytology: Lessons Learned From Next-Generation Sequencing—The MD Anderson Experience. Arch Pathol Lab Med 140:1191-1199, 2016

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Different locations. Same mutation. Pan-Cancer Indications.

Growing evidence that tumors in different locations may present the same mutation.

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The Number of Pan-Cancer Biomarkers is Growing

1. Le et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science.* 2017 Jul 28;357(6349):409–413.
2. https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm560167.htm
3. Drilon et al. Efficacy of Larotrectinib in TRK Fusion–Positive Cancers in Adults and Children *N Engl J Med* 2018; 378:731–739
4. https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm626720.htm
5. Samstein, R. M. et al Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nature Genet.* https://doi.org/10.1038/s41588-018-0312-8 (2019).
6. Zehir et al., Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients *Nat Med.* 2017 Jun;23(6):703-713

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A change in paradigm:

Growing number of biomarkers + Pan-Cancer Biomarkers + Limited biopsy tissue available

= need for a single multiplex assay comprehensive genomic profiling (CGP) assay

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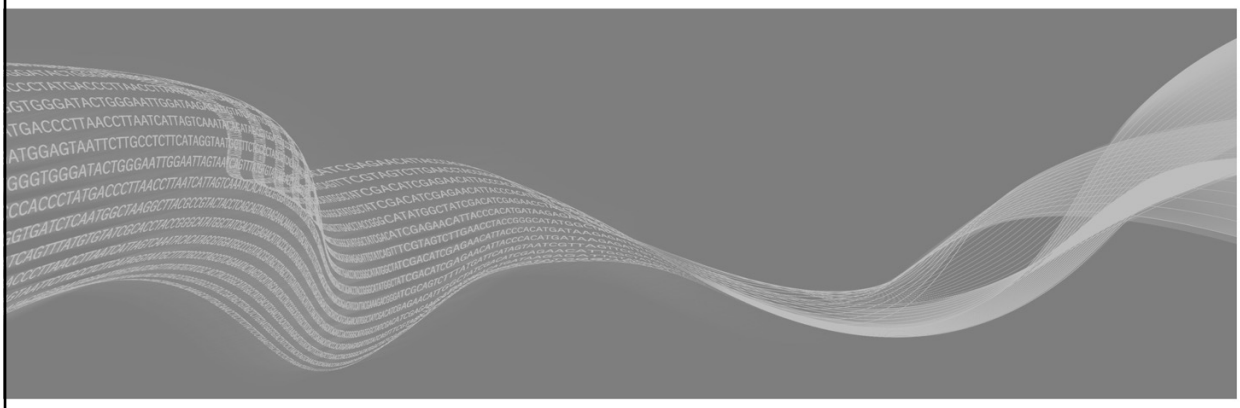
TruSight ™ Oncology 500 Product Portfolio



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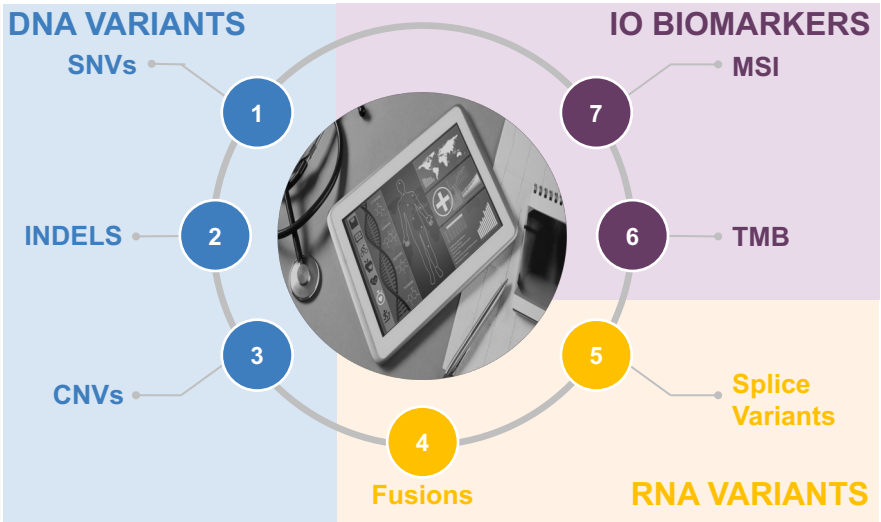
Content of a CGP Assay

Cover all biomarkers present in current **guidelines**

Cover biomarkers in **clinical trials**

Cover biomarkers for both **targeted therapies** and **immuno-oncology**



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TruSight™ Oncology 500 Menu

	Specimen	Library Prep and Enrichment	Sequence	Secondary Analysis	Interpretation and Reporting
TSO500 ctDNA 30 ng cfDNA	Plasma	TruSight™ Oncology 500 ctDNA kit	NovaSeq™ 6000 System: Samples 8 / 24, Flowcell S2 / S4	DRAGEN™ Bio-IT platform	Under Development
TSO500 HT* 40 ng DNA, 40 – 80ng RNA	FFPE	TruSight™ Oncology 500 HT kit	NovaSeq™ 6000 System: Samples 16 / 32 / 72 / 192, Flowcell SP / S1 / S2 / S4	Local Docker	pieriandx
TSO500 40 ng DNA, 40 – 80ng RNA	FFPE	TruSight™ Oncology 500	NextSeq™ 550 / NextSeq™ 550 Dx (in research mode): Samples 8, Flowcell HO	Local Docker	pieriandx

*Under Development, launch in April

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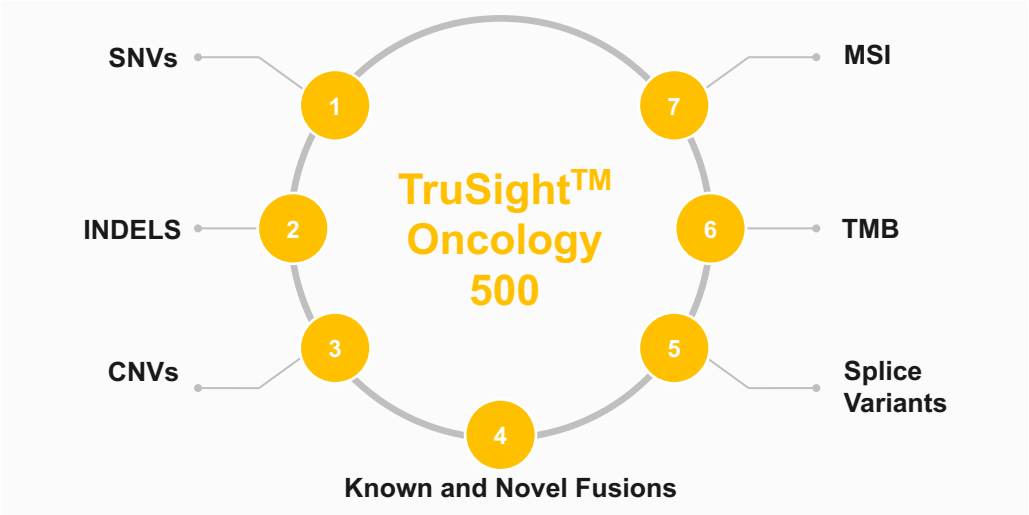
TruSight™ Oncology 500 DNA/RNA Bundle

Pan-Cancer DNA & RNA Analysis: All Main variant types + TMB & MSI



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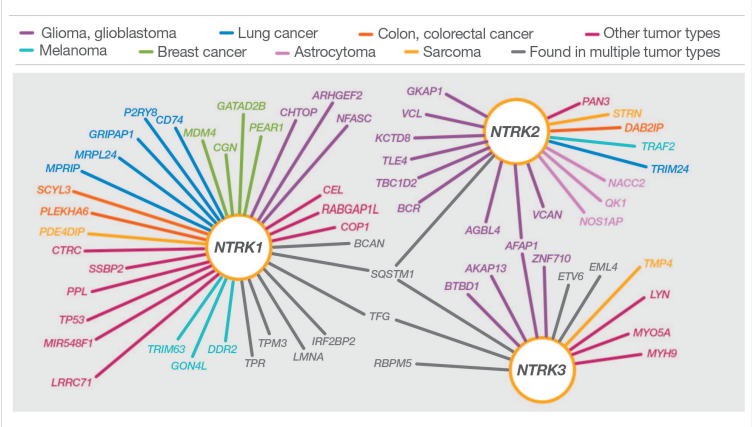
Fusions Are Complex

Multiple actionable fusion genes have numerous fusion partners, many still unknown



NTRK1, NTRK2, NTRK3…

…have 61 known fusion partners today, across multiple tumor types. This number is continuously growing as new partners are detected. NTRK fusions can be targeted with TRK inhibitor drugs such as Vitrakvi.

Learn more: www.trkcancer.com

Figure adapted from: Kummar S. TRK Inhibition: A New Tumor-Agnostic Treatment Strategy. *Target Oncol*. 2018;13(5):545-556

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TruSight Oncology 500™ - DNA and RNA Sequencing

A single assay for targeted therapies

Targeted Therapies			Pan-Cancer Markers	
Germline SNVs	BRCA1/2	PARP inhibitors	Fusions + FDA approved biomarkers * Emerging biomarkers	NTRKs+
Activating SNVs	BRAFV600E	BRAF inhibitors		NRG1*
Resistance SNVs	EGFRT790M	osimertinib		FGFR*
Deletions	EGFR ex19 Del	1st, 2nd or 3rd gen EGFR TKI		ALK*
Insertions	ERBB2 ex20 Ins	poziotinib		ROS1*
CNVs	MET Amp	EGFR and MET TKIs	Activation Mutations	FGFR*
Fusions	ELM4-ALK	ALK TKIs		ERBB2*
Splice Variants	MET ex14 skip	MET inhibitors	Amplifications	ERBB2*

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TruSight Oncology 500™ - DNA and RNA Sequencing

A single assay for immune checkpoint inhibitors

Immune Checkpoint Inhibitors	
MSI+	Intermediate, High
MMR+	Deficient
TMB*1	>10, >20 mut/MB
Hypermutation*2	POLD1, POLE mut
Fusions*3	Neoantigens
Splice Variants*4	Neojunctions
HLA-I*5	Het, HLA-B44

1. Yarchoan M et al. Tumor Mutational Burden and Response Rate to PD-1 Inhibition *N Engl J Med.* 2017;377:2500-2501
2. Campbell B et al. Comprehensive Analysis of Hypermutation in Cancers *Cell* 171, 1042–1056, November 16, 2017
3. Yang W et al. Immunogenic Antigens Derived from Gene Fusions *Nature Medicine* Epub ahead of print 22 April 2019
4. Kahles et al. Comprehensive Analysis of Alternative Splicing Across Tumors from 8,705 Patients *Cancer Cell* 34, 211–224 August 13, 2018
5. Chowell D et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science* 359, 6375, 582-587

+ FDA approved biomarkers
* Emerging biomarkers

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Current Pathology Lab Pain Point

Multiple individual assays lead to sample, time and cost implications

NSCLC FFPE Single Gene Assays | Tissue Slide Requirements

EGFR by PCR Highest incidence	ALK, ROS1, RET & MET by FISH	MET Exon 14 skipping by qPCR	BRAF, KRAS & ERBB2 by PCR	ALGORITHM reflex approach
5	+ 4	+ 5	+ 15	= 29 slides

Reflex analysis leads to time implications

https://www.mayocliniclabs.com/test-catalog/Specimen/35404
https://ltd.aruplab.com/Tests/Pub/2002440
https://www.molecular.abbottrealdx.ie/sites/default/files/staticAssets/ALK-US-CE-Clinical-PI_R3_mw001_3060.pdf
https://neogenomics.com/test-menu/met-exon-14-deletion-analysis
https://www.geisingermedicallabs.com/catalog/details.cfm?tid=1740
https://www.geisingermedicallabs.com/catalog/details.cfm?tid=1638

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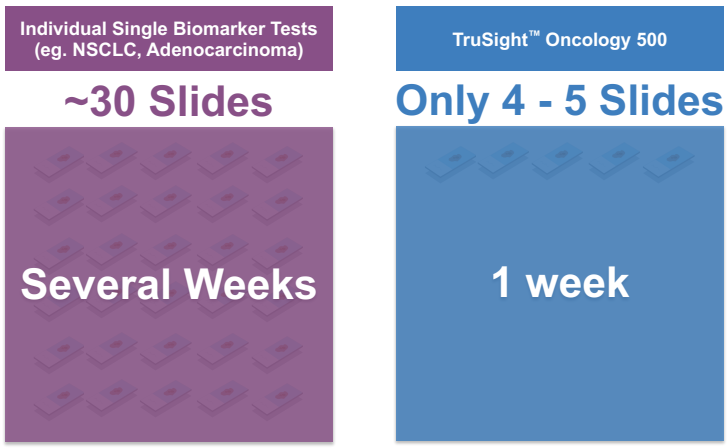
TruSight™ Oncology 500

Allow labs to consolidate assays, providing sample, cost and time benefits



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The Value of Providing the Assay In-house

Stay relevant within your institution and keep the sample data

Category	In-House Assay	Send-out
Sample	✓ Simpler logistic	⊘ More Complex logistic
	✓ More control over sample	⊘ Less control over sample
Relationship within your Institution	✓ More involvement in Molecular Tumor Boards	⊘ Less involvement in Molecular Tumor Boards
Data	✓ Keep the sample data, build a database, generate your own reports	⊘ Limited or little data is shared, only final report available
	✓ Better ability to interpret data	⊘ Less ability to interpret data
TAT	✓ ~ 1 – 2 weeks	⊘ Typically 2 weeks or more

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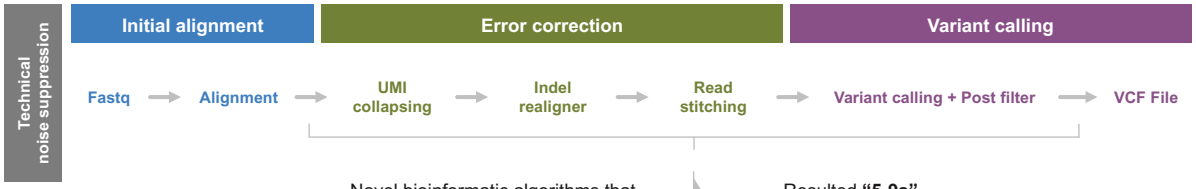
TruSight™ Oncology 500 | 99.9998% Specificity

Sophisticated error correction for minimizing false positives



Novel bioinformatic algorithms that required **~6** bioinformaticians for 1 year

Resulted **"5-9s"** specificity **(99.9998%)**

Why do you need **"5-9s"** specificity?

Specificity	Max. # of false positives (523 gene panel)
95%	65,000
99%	13,000
99.9%	1,300
99.99%	130
99.999%	13
99.9998%	< 2

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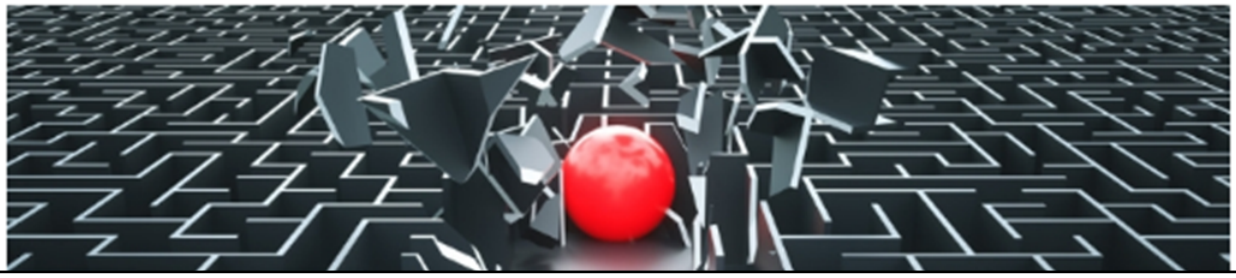
The FDA Grants Breakthrough Device Designation for Illumina's TruSight Assay

Based on the content of TruSight Oncology 500, the proposed in vitro diagnostic will receive prioritized review and resources



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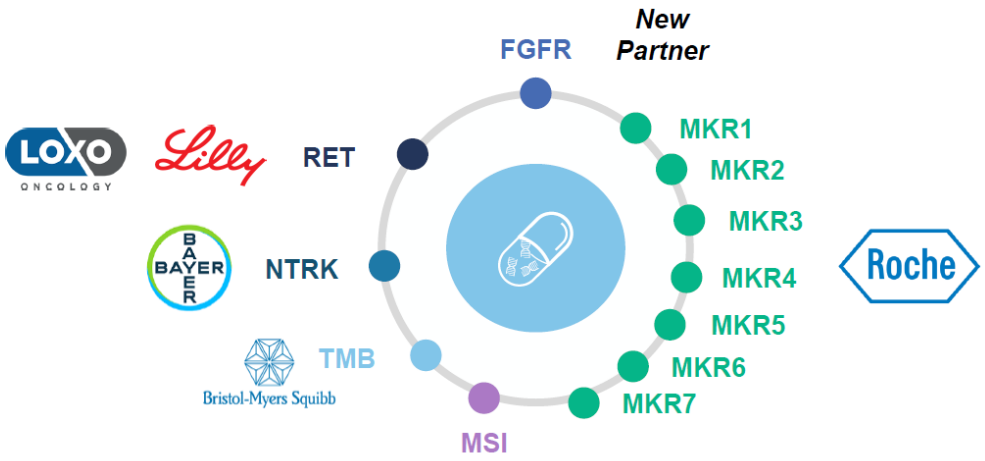
Enabling CDx Pharm Partnerships



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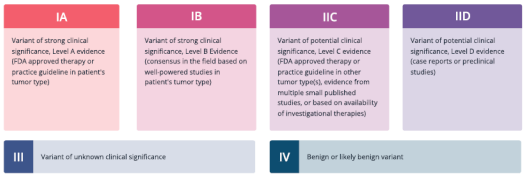
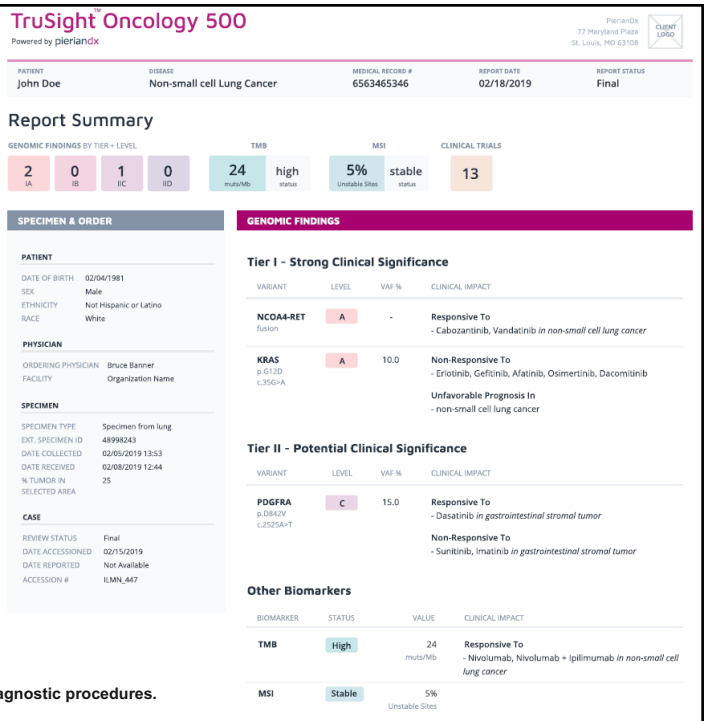
The Report – Summary





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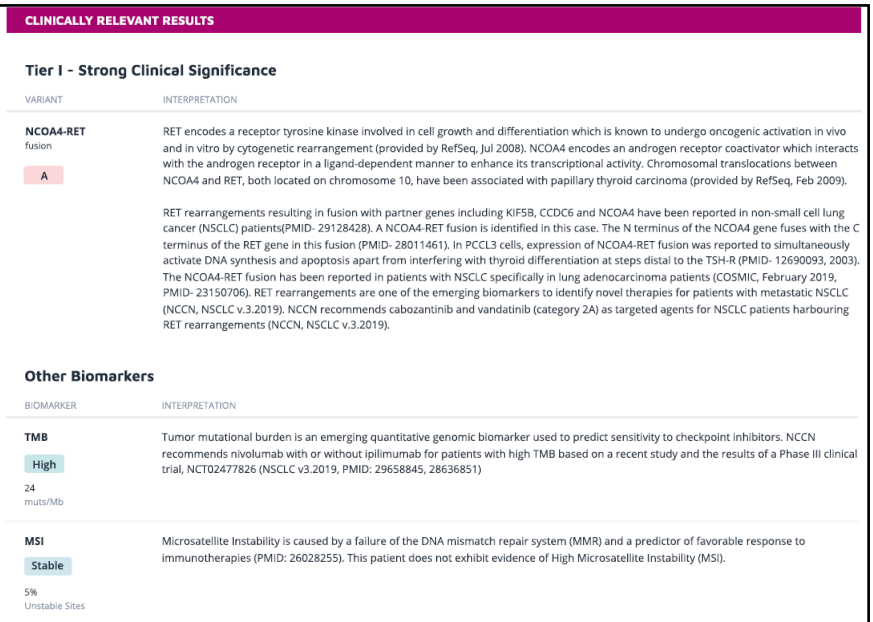
The Report – More Detail



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).

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).

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The Report – Clinical Trials

CLINICAL TRIALS

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
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

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Liquid Biopsy

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Key Drivers for Liquid Biopsy Based Tests

Tissue Biopsies Limitation[1]

Inclusion in Guidelines[2]

Increased yield with faster TAT[1]

Complement to tissue results[3]
Tumor heterogeneity

TMB emerging as predictive biomarker[4]

Liquid Biopsy Based Assays

1. Rolfo et al. Tissue biopsy limitations... *Journal Thoracic Onc.* 2018
2. Inclusion in NCCN Guidelines... NSCLC *NCCN Guidelines v3.* 2019 – Jan 2019
3. Leighl et al. Compliment and Concordance with Tissue Results"... *Clin Cancer Res.* 2019
4. Kim ES, Velcheti V, et al. Emergence of liquid biomarkers such as bTMB... LBA55 –*ESMO Abstract.* 2018

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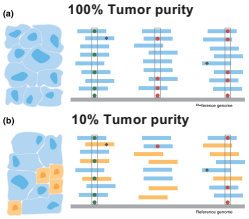
Common Challenges with Tissue Biopsies

QNS

Quantity not Sufficient

Invasive

Not an Option



Low Tumor Purity

$$$$$

Side Effects

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TruSight™ Oncology 500ctDNA

Enabling Comprehensive Genomic Profiling in plasma

NTRK, MSI, TMB

Small Variants – 523 Genes

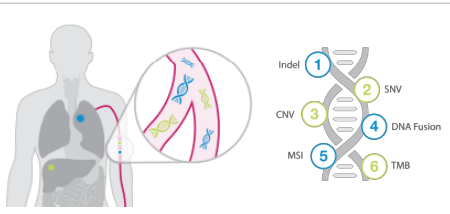
ABL1	BRCA2	CUX1	FANCC	GNA13	INHBA	MDM4	PAK5	PTPN11	SMAD2	TP53
ABL2	BRD4	CXCR4	FANCD2	GNAQ	INPP4A	MED12	PALB2	PTPRD	SMAD3	TP63
ABRAXAS1	BRIP1	CYLD	FANCE	GNAS	INPP4B	MEF2B	PARP1	PTPRS	SMAD4	TRAF2
ACVR1	BTG1	DAXX	FANCF	GPS2	INSR	MEN1	PAX3	PTPRT	SMARCA4	TRAF7
ACVR1B	BTK	DCUN1D1	FANCG	GREM1	IRF2	MET	PAX5	QKI	SMARCB1	TSC1
ADGRA2	CALR	DDR2	FANCI	GRIN2A	IRF4	MGA	PAX7	RAB35	SMARCD1	TSC2
AKT1	CARD11	DDX41	FANCL	GRM3	IRS1	MITF	PAX8	RAC1	SMC1A	TSHR
AKT2	CASP8	DHX15	FAS	GSK3B	IRS2	MLH1	PBRM1	RAD21	SMC3	U2AF1
AKT3	CBFB	DICER1	FAT1	H3F3A	JAK1	MLLT3	PDCD1	RAD50	SMO	VEGFA
ALK	CBL	DIS3	FBXW7	H3F3B	JAK2	MPL	PDCD1LG2	RAD51	SNCAIP	VHL
ALOX12B	CCN6	DNAJB1	FGF1	H3F3C	JAK3	MRE11	PDGFRA	RAD51B	SOCS1	VTCN1
AMER1	CCND1	DNMT1	FGF10	HGF	JUN	MSH2	PDGFRB	RAD51C	SOX10	WT1
ANKRD11	CCND2	DNMT3A	FGF14	HIST1H1C	KAT6A	MSH3	PDK1	RAD51D	SOX17	XIAP
ANKRD26	CCND3	DNMT3B	FGF19	HIST1H2BD	KDM5A	MSH6	PDPK1	RAD52	SOX2	XPO1
APC	CCNE1	DOT1L	FGF2	HIST1H3A	KDM5C	MST1	PGR	RAD54L	SOX9	XRCC2
AR	CD274	E2F3	FGF23	HIST1H3B	KDM6A	MST1R	PHF6	RAF1	SPEN	YAP1
ARAF	CD276	EED	FGF3	HIST1H3C	KDR	MTOR	PHOX2B	RANBP2	SPOP	YES1
ARFRP1	CD74	EGFL7	FGF4	HIST1H3D	KEAP1	MUTYH	PIK3C2B	RARA	SPTA1	ZBTB2
ARID1A	CD79A	EGFR	FGF5	HIST1H3E	KEL	MYB	PIK3C2G	RASA1	SRC	ZBTB7A
ARID1B	CD79B	EIF1AX	FGF6	HIST1H3F	KIF5B	MYC	PIK3C3	RB1	SRSF2	ZFHX3
ARID2	CDC73	EIF4A2	FGF7	HIST1H3G	KIT	MYCL	PIK3CA	RBM10	STAG1	ZNF217
ARID5B	CDH1	EIF4E	FGF8	HIST1H3H	KLF4	MYCN	PIK3CB	RECQL4	STAG2	ZNF703
ASXL1	CDK12	ELOC	FGF9	HIST1H3I	KLHL6	MYD88	PIK3CD	REL	STAT3	2RSR2
ASXL2	CDK4	EML4	FGFR1	HIST1H3J	KMT2A	MYOD1	PIK3CG	RET	STAT4	
ATM	CDK6	EMSY	FGFR2	HIST2H3A	KMT2B	NAB2	PIK3R1	RHEB	STAT5A	
ATR	CDK8	EP300	FGFR3	HIST2H3C	KMT2C	NBN	PIK3R2	RHOA	STAT5B	
ATRX	CDKN1A	EPCAM	FGFR4	HIST2H3D	KMT2D	NCOA3	PIK3R3	RICTOR	STK11	
AURKA	CDKN1B	EPHA3	FH	HIST3H3	KRAS	NCOR1	PIM1	RIT1	STK40	
AURKB	CDKN2A	EPHA5	FLCN	HLA-A	LAMP1	NEGR1	PLCG2	RNF43	SUFU	
AXIN1	CDKN2B	EPHA7	FLI1	HLA-B	LATS1	NF1	PLK2	ROS1	SUZ12	
AXIN2	CDKN2C	EPHB1	FLT1	HLA-C	LATS2	NF2	PMAIP1	RPS6KA4	SYK	
AXL	CEBPA	ERBB2	FLT3	HNF1A	LMO1	NFE2L2	PMS1	RPS6KB1	TAF1	
B2M	CENPA	ERBB3	FLT4	HNRNPK	LRP1B	NFKBIA	PMS2	RPS6KB2	TBX3	
BAP1	CHD2	ERBB4	FOXA1	HOXB13	LYN	NKX2-1	PNRC1	RPTOR	TCF3	
BARD1	CHD4	ERCC1	FOXL2	HRAS	LZTR1	NKX3-1	POLD1	RUNX1	TCF7L2	
BBC3	CHEK1	ERCC2	FOXO1	HSD3B1	MAGI2	NOTCH1	POLE	RUNX1T1	TENT5C	
BCL10	CHEK2	ERCC3	FOXP1	HSP90AA1	MALT1	NOTCH2	PPARG	RYBP	TERC	
BCL2	CIC	ERCC4	FRS2	ICOSLG	MAP2K1	NOTCH3	PPM1D	SDHA	TERT	
BCL2L1	COP1	ERCC5	FUBP1	ID3	MAP2K2	NOTCH4	PPP2R1A	SDHAF2	TET1	
BCL2L11	CREBBP	ERG	FYN	IDH1	MAP2K4	NPM1	PPP2R2A	SDHB	TET2	
BCL2L2	CRKL	ERRFI1	GABRA6	IDH2	MAP3K1	NRAS	PPP6C	SDHC	TFE3	
BCL6	CRLF2	ESR1	GATA1	IFNGR1	MAP3K13	NRG1	PRDM1	SDHD	TFRC	
BCOR	CSF1R	ETS1	GATA2	IGF1	MAP3K14	NSD1	PREX2	SETBP1	TGFBR1	
BCORL1	CSF3R	ETV1	GATA3	IGF1R	MAP3K4	NTRK1	PRKAR1A	SETD2	TGFBR2	
BCR	CSNK1A1	ETV4	GATA4	IGF2	MAPK1	NTRK2	PRKCI	SF3B1	TMEM127	
BIRC3	CTCF	ETV5	GATA6	IKBKE	MAPK3	NTRK3	PRKDC	SH2B3	TMPRSS2	
BLM	CTLA4	ETV6	GEN1	IKZF1	MAX	NUP93	PRKN	SH2D1A	TNFAIP3	
BMPR1A	CTNNA1	EWSR1	GID4	IL10	MCL1	NUTM1	PRSS8	SHQ1	TNFRSF14	
BRAF	CTNNB1	EZH2	GLI1	IL7R	MDC1	PAK1	PTCH1	SLIT2	TOP1	
BRCA1	CUL3	FANCA	GNA11	INHA	MDM2	PAK3	PTEN	SLX4	TOP2A	

CNV 59 genes

AKT2	PDGFRB
ALK	PIK3CA
AR	PIK3CB
ATM	PTEN
BRAF	RAF1
BRCA1	RET
BRCA2	RICTOR
CCND1	RPS6KB1
CCND3	TFRC
CCNE1	
CDK4	
CDK6	
CHEK1	
CHEK2	
EGFR	
ERBB2	
ERBB3	
ERCC1	
ERCC2	
ESR1	
FGF1	
FGF10	
FGF14	
FGF19	
FGF2	
FGF23	
FGF3	
FGF4	
FGF5	
FGF6	
FGF7	
FGF8	
FGF9	
FGFR1	
FGFR2	
FGFR3	
FGFR4	
JAK2	
KIT	
KRAS	
LAMP1	
MDM2	
MDM4	
MET	
MYC	
MYCL	
MYCN	
NRAS	
NRG1	
PDGFRA	

DNA Fusions 23 genes

ABL1
ALK
BCR
BRAF
CD74
EGFR
ETV1
ETV4
ETV6
EWSR1
FGFR2
FGFR3
NAB2
NTRK1
NTRK2
NUTM1
PAX3
PAX8
PPARG
RET
ROS1
TFE3
TMPRSS2



TruSight Oncology 500 ctDNA

- An alternative assay when quantity of sample is not sufficient for enabling CGP with TSO500 from FFPE samples.
- To address tumor heterogeneity or to complement genomic profiling in tissue
- Enabling CGP including immuno-oncology biomarkers from Liquid Biopsy

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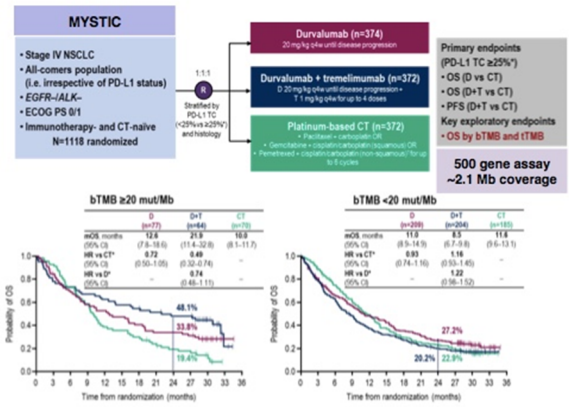
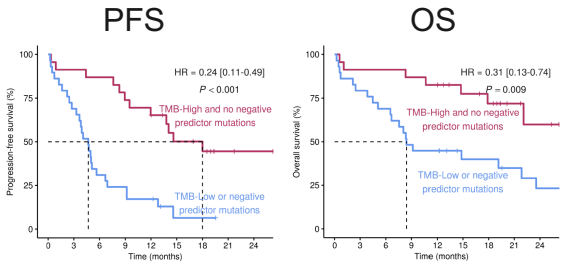
Blood based TMB emerging as biomarker





bTMB + Loss of function mutations in STK11, KEAP1, and PTEN, and an activating ERBB2 exon 20 insertion in NSCLC with pembrolizumab

Aggarwal C et al. Baseline plasma tumor mutation burden predicts response to pembrolizumab-based therapy in patients with metastatic non-small cell lung cancer. Clinical Cancer Research. February 26, 2020 doi: 10.1158/1078-0432.CCR-19-3663

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TruSight Oncology 500 ctDNA

~35,000x coverage and 99.9995 % Specificity

	Sensitivity	Specificity
Small Variants 0.5% VAF	≥ 95%	≥ 99.9995%
Gene Amplifications ≥ 1.4 Fold Change	≥ 95%	≥ 95%
Gene Deletions ≤ 0.6 Fold Change	≥ 95%	≥ 95%
MSI High Detection at 2% Tumor Fraction	≥ 95%	≥ 95%
Gene rearrangements	≥ 95%	≥ 95%

Small variants – SNV and Del

VAF	Sensitivity
0.2% to 0.5%	95.04%
0.5% to 1.0%	99.70%

Sensitivity by LoD in HotSpot Variants

Input ng	0.20%	0.40%	0.50%	0.70%	0.80%	1.00%
10	38.46	74.54	84.57	94.71	96.97	99.04
30	90.83	99.70	99.95	100.00	100.00	100.00
50	99.02	100.00	100.00	100.00	100.00	100.00
70	99.91	100.00	100.00	100.00	100.00	100.00
100	100.00	100.00	100.00	100.00	100.00	100.00

Sensitivity

<75%

75–95%

>95%

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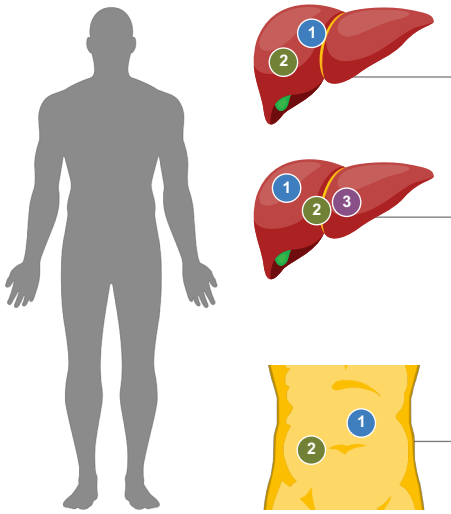
Will tissue and plasma results match?

Tumor Heterogeneity Revealed by ctDNA Analysis



	Plasma	Biopsy 1	Biopsy 2	Biopsy 3
TPS001				
TP53 p.E171	✓	✓	✓	
MAP2K1 p.K57T	✓	✓		
KRAS p.Q61H	✓		✓	
TPS007				
PBRM1 p.R710	✓	✓	✓	✓
FGFR2 p.K641R	✓	✓		✓
FGFR2 p.N549H	✓		✓	
FGFR2 p.E565A	✓			✓
FGFR2 p.V654F	✓			
FGFR2 p.L617V	✓			
TPS011				
APC p.K1250fs	✓	✓	✓	
EGFR p.V411G	✓	✓		
KRAS p.G12D	✓		✓	

Corcoran et al, Liquid vs tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers, *Nature Medicine*, Vol 25, September 2019

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NCI-MATCH Chooses TruSight™ Oncology 500 ctDNA for Liquid Biopsy


CANCER RESEARCH, ONCOLOGY

TruSight Oncology 500 Selected to Power Liquid Biopsy Studies

Illumina to collaborate with the Frederick National Laboratory for Cancer Research

Collaboration with Frederick National Laboratory

- 7000 samples
- Concordance between tissue and circulating tumor DNA
- Largest tissue/ctDNA concordance data sets ever analyzed
- "TSO 500 ctDNA offers a breadth of coverage"
  – Mickey Williams Director of MoCha Lab-

"Many more genes can be interrogated compared to other platforms"

https://www.illumina.com/company/news-center/feature-articles/tso-500-selected-to-power-liquid-biopsy-studies.html
https://www.biospace.com/article/frederick-national-laboratory-partners-with-illumina-for-clinical-validation-of-liquid-biopsies/

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Local Trials

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Australian Genomic Cancer Medicine Centre

- **Group of 8 Hospitals:**
  - ACT – Canberra Hospital
  - NSW – Garvan Institute of Medical Research
  - NT – Royal Darwin Hospital
  - QLD – Princess Alexandra Hospital
  - SA – Royal Adelaide Hospital
  - TAS – Royal Hobart Hospital
  - VIC – Peter MacCallum Cancer Centre
  - WA – Sir Charles Gairdner Hospital

- **Testing Performed at:**
  - NSW – Garvan
  - SA – SA Path
  - VIC – PeterMac
  - WA – PathWest

  - QLD – PAH/ATGC ?

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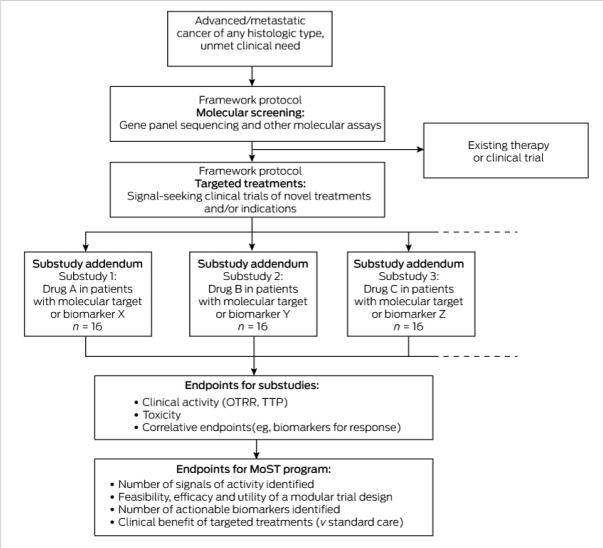
Molecular Screening & Therapeutics (MoST) study

- **Funding to enroll 3,000 patients with rare and hard to treat cancers**
- **Perform molecular screening with TSO500**
- **Upon actionable finding enroll in substudy arm or find other eligible trial**



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MoST Study Arms

- CDK4/6 inhibitor palbociclib in patients with tumours with amplified D-type cyclins or CDK4 or inactivation of CDKN2A.
- Durvalumab (MEDI4736) in combination with Tremelimumab in patients with advanced rare or neglected cancers.
- Olaparib in combination with Durvalumab in patients with tumours with homologous recombination repair defects
- Vismodegib in patients with tumours harbouring PTCH1 or SMO mutations
- Eribulin in patients with advanced CD31 positive angiosarcoma and selected CD31 positive sarcomas.
- Larotrectinib in patients with advanced NTRK1-3 positive tumours.
- Trastuzumab emtansine (T-DM1) in patients with tumours harbouring HER2 amplifications or mutations

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ASPiRATION Lung Cancer Trial

- **Multi-centre prospective study**
- **Benchmark CGP vs SoC testing (EGFR, ALK, ROS1)**
- **1,000 patients with mNSCLC**
  - 500 tested with Foundation Medicine
  - 500 tested through the AGCMC with TSO500
- **Federal Government Funding + Roche Funding**
- **Leverage AGCMC infrastructure**

- **Primary Endpoints:**
  - Percentage of patients with actionable findings by CGP and SoC
  - Time take for CGP and SoC
  - Percentage of patients requiring repeat biopsy by CGP and SoC
- **Secondary Endpoints:**
  - Percentage of patients with a change in treatment recommendation based on CGP
  - Clinical outcomes of enrolled patients (ORR, PFS, OS)
  - Evaluation of clinician preferences for CGP vs SoC testing

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ASPiRATION Lung Cancer Trial

- **Demonstrate the feasibility, efficiency and utility of CGP in Australia**
- **Numerous arms including:**
  - ALK, ROS1, EGFR, BRAF, METex14, HER2, NTRK, PDL1/CTLA4 inhibitors
- **17 enrolling sites + 5 screening labs + Foundation Medicine**
-

- **The Numbers:**
  - 1,000 patients
  - SoC testing – 212 patients with diagnosis
  - 788 remaining
  - ~54% with genomic alteration by CGP (424/788)
  - Estimate this with 95% CI ± 3.5%

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Summary

- Through biomarker based patient selection precision medicine has the potential to significantly improve therapy efficacy and outcomes
- Illumina is enabling broad adoption of comprehensive genomic profiling with the TSO500 product portfolio through a distributed model allowing labs to provide Foundation Medicine or Guardant Health like local services
- Liquid Biopsy has tremendous potential to enhance therapy decision making, monitoring treatment response and for relapse detection.
- The Australian Federal Government is funding new initiatives to evaluate the impact of precision medicine in Australia

TruSight™ Oncology 500 Products

Feature	TSO500	TSO500 HT	TSO500 ctDNA
Sample Type	FFPE Tissue	FFPE Tissue	cfDNA
Input Amount	40 ng DNA, 40 - 80 ng RNA	40 ng DNA, 40 - 80 ng RNA	30 ngs (no RNA)
Variants Detected	• Small DNA variants (SNVs, MNVs, indels) • Copy number variants • RNA fusions • RNA splice variants • MSI • TMB	• Small DNA variants (SNVs, MNVs, indels) • Copy number variants • RNA fusions • RNA splice variants • MSI • TMB	• Small DNA variants (SNVs, MNVs, indels) • Copy number variants • Gene rearrangements • MSI • TMB
Sequencer	NextSeq 500/550	NovaSeq 6000	NovaSeq 6000
Throughput	8 Samples (8 DNA + 8 RNA)	SP:16 Samples, S1: 32, S2: 72, S4: 192	S2: 8 Samples S4: 24 Samples
Bioinformatics	Local Docker Application Local Run Manager	Local Docker Application	DRAGEN
Coverage (Reads)	3000 - 3500X (40 - 50 Million)	3000 - 3500X (40 - 50 Million)	30,000 - 35,000X (400 – 500 Million)
Reporting	Pierian Dx Software	Pierian Dx Software	Pierian Dx Software

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TruSight™ Oncology 500 Tissue or HT DNA/RNA Bundle

Enabling quality Comprehensive Genomic Profiling

SINGLE ASSAY – 523 GENES

Detection of currently relevant DNA & RNA variants for multiple types of cancer.

Some key biomarkers included:

Pan-cancer biomarkers: NTRK1, NTRK2, NTRK3, MSI | TMB (emerging)

LUNG	MELANOMA	COLON	OVARY	BREAST	GASTRIC	BLADDER	MYELOID	Sarcoma
AKT1	*BRAF*	*AKT1*	*BRAF*	*AKT1*	*BRAF*	*MSH6*	*ALBL1*	ALK
ALK	*CTNNB1*	*BRAF*	*BRCA1*	*AR*	*KIT*	*PMS2*	*ASXL1*	APC
BRAF	*GNA11*	*HRAS*	*BRCA2*	*BRCA1*	*KRAS*	*TSC1*	*CALR*	BRAF
DDR2	*GNAQ*	*KRAS*	*KRAS*	*BRCA2*	*MET*		*CEBPA*	CDK4
EGFR	*KIT*	*MET*	*PDGFRA*	*ERBB2*	*MLH1*		*ETV6*	CTNNB1
ERBB2	*MAP2K1*	*MLH1*	*FOXL2*	*FGFR1*	*PDGFRA*		*EZH2*	ETV6
FGFR1	*NF1*	*MSH2*	*TP53*	*FGFR2*	*TP53*		*FLT3*	EWSR1
FGFR3	*NRAS*	*MSH6*		*PIK3CA*			*GATA2*	FOXO1
KRAS	*PDGFRA*	*NRAS*		*PTEN*			*IDH1*	GLI1
MAP2K1	*PIK3CA*	*PIK3CA*					*IDH2*	KIT
MET	*PTEN*	*PMS2*					*JAK2*	MDM2
NRAS	*TP53*	*PTEN*					*KIT*	MYOD1
PIK3CA		*SMAD4*					*MPL*	NAB2
PTEN		*TP53*					*NPM1*	NF1
RET							*RUNX1*	PAX3
TP53							*SF3B1*	PAX7
TMB							*SRSF2*	PDGFRA
							TP53	PDGFRB
								SDHB
								SDHC
								SMARCB1
								TFE3
								WT1

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For Research Use Only. Not for use in diagnostic procedures.

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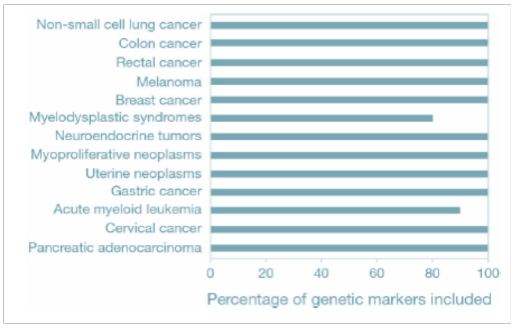
TruSight™ Oncology 500 DNA/RNA Bundle

Content aligned to guidelines and more than 1,200 clinical trials

Content Alignment to Guidelines



100% coverage of guidelines for 11 tumor types

Coverage of genes and signatures included in 1,233 clinical trials in the USA

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