Facilitating precision oncology through enterprise-level implementation of next generation sequencing tumor panels

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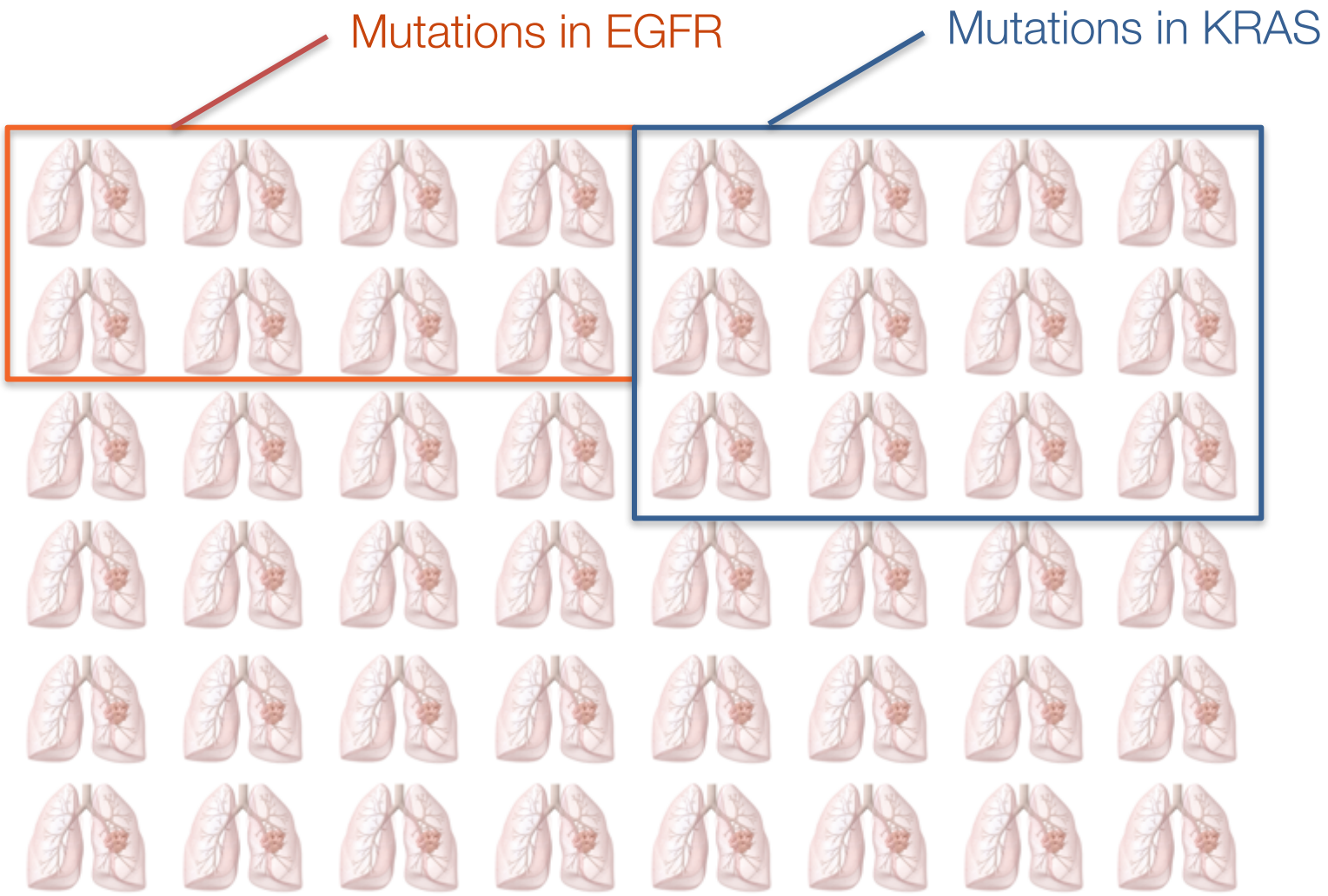
MIRACUM Symposium February 23rd

Memorial Sloan Kettering Cancer Center

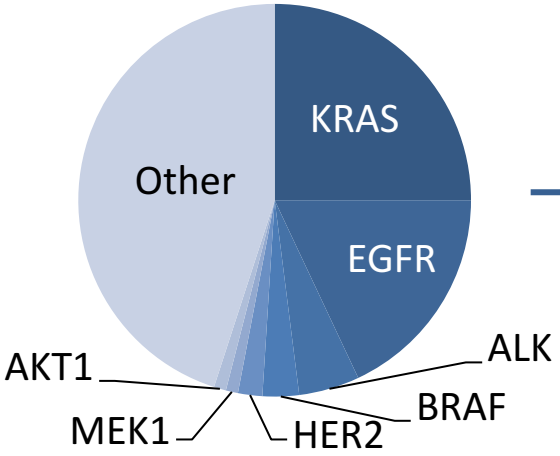
Precision Oncology



Cancer is a disease of the genome

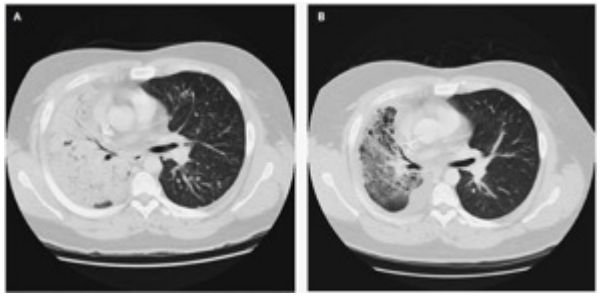
Precision Oncology

Lung Cancer



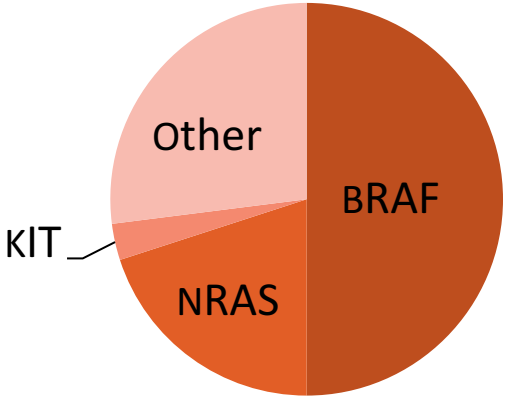
EGFR-mutant cancer

EGFR inhibitors:
erlotinib, gefinitib



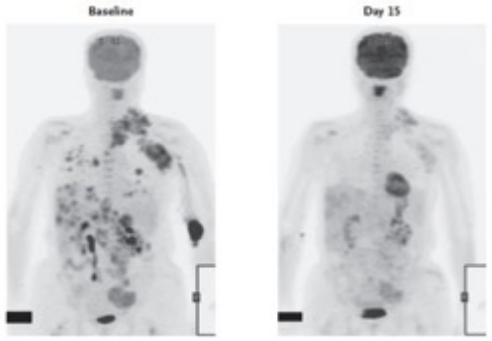
Lynch et al., NEJM, 2004
Paez et al., Science, 2004
Pao et al., PNAS, 2004

Melanoma



BRAF-mutant cancer

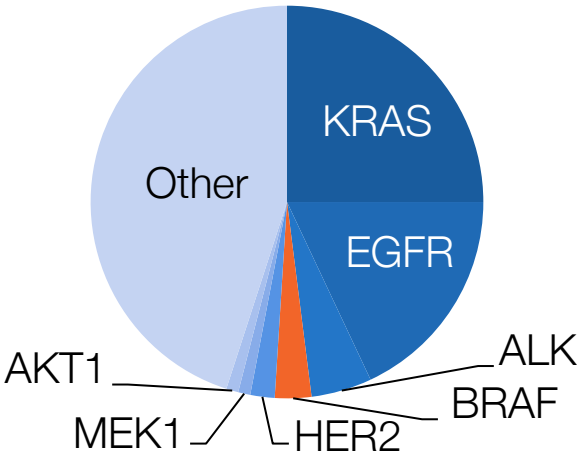
BRAF inhibitor:
vemurafenib, dabrafenib



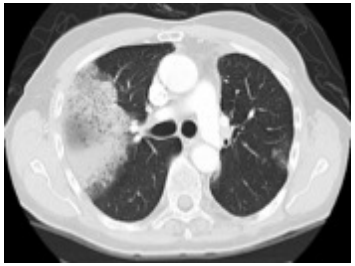
Flaherty et al., NEJM, 2010

Precision Oncology

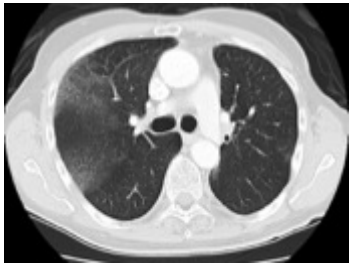
Lung Cancer



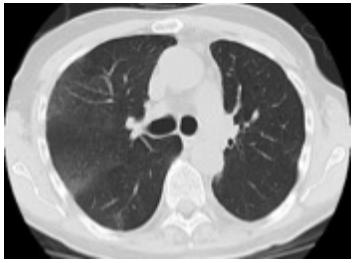
Response to Dabrafenib



Baseline



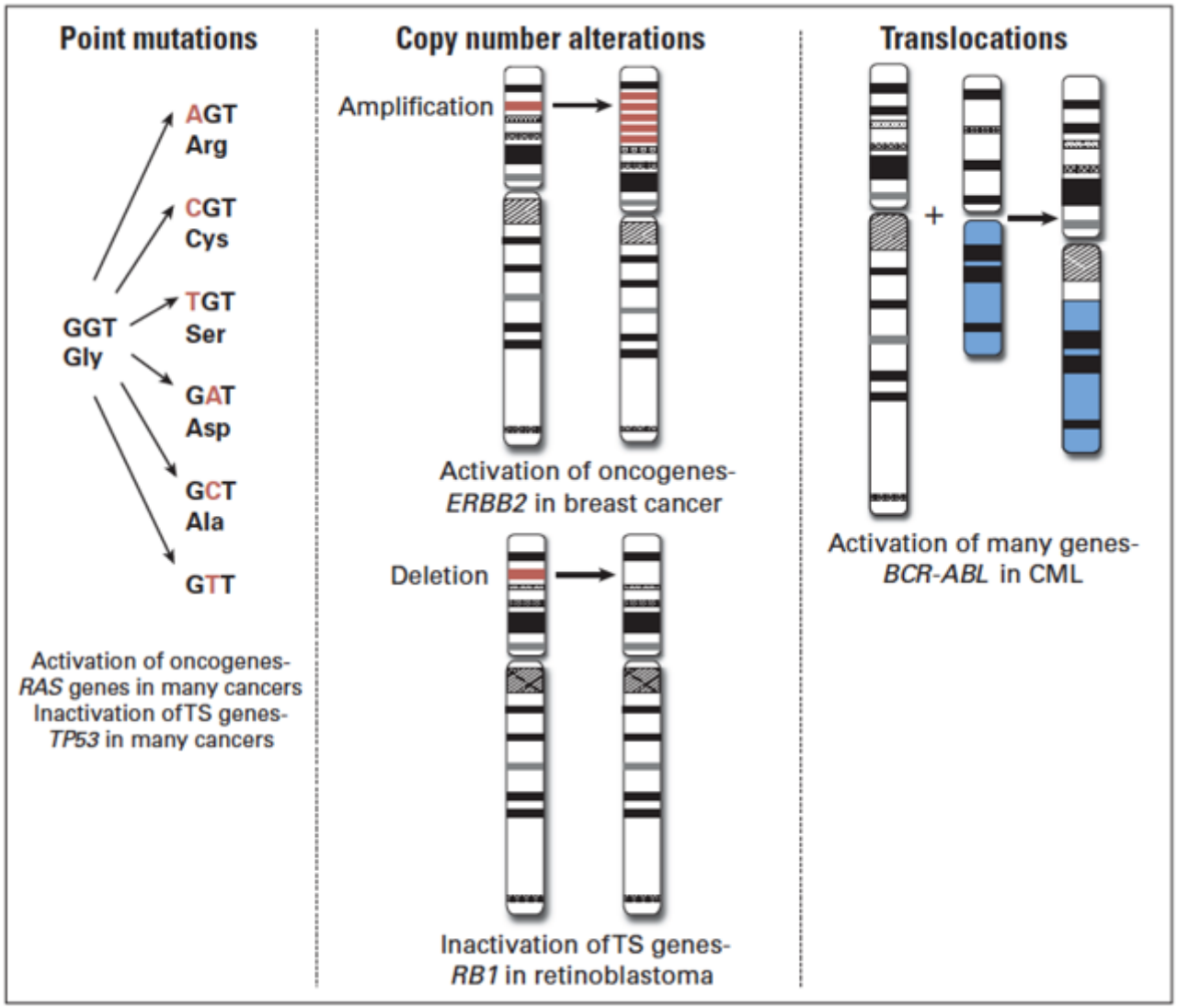
6 weeks on Dabrafenib



4 months on Dabrafenib

Courtesy Dr. Greg Reily

Challenge : Multiple Classes of Alterations

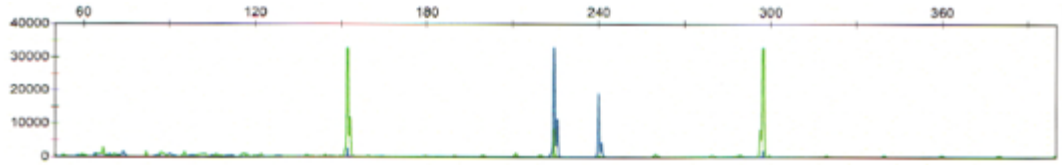


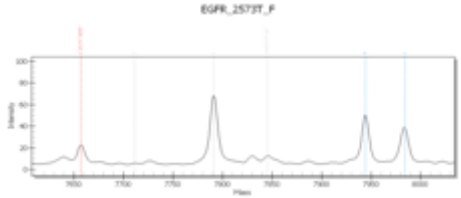
MacConaill and Garraway, *Journal of Clinical Oncology*, 2010

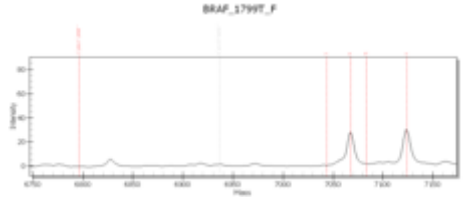
Challenge : Single Tumor, Many Tests

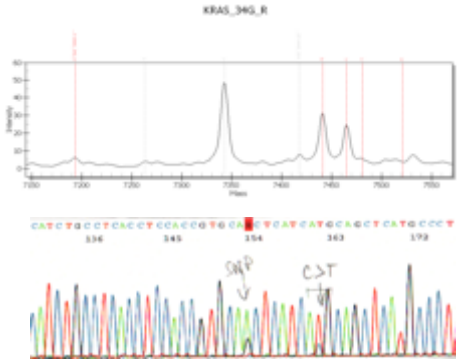
Lung adenocarcinoma

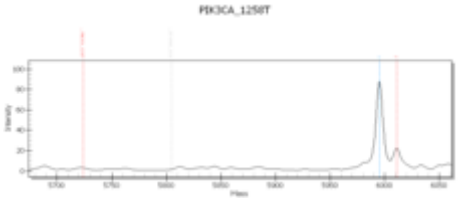
- EGFR exon 19
- EGFR exon 20
- ERBB2 exon 20
- EGFR exon 21
- PIK3CA
- BRAF
- KRAS
- EGFR T790M
- EML4-ALK
- MET

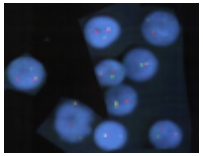


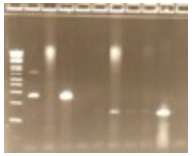






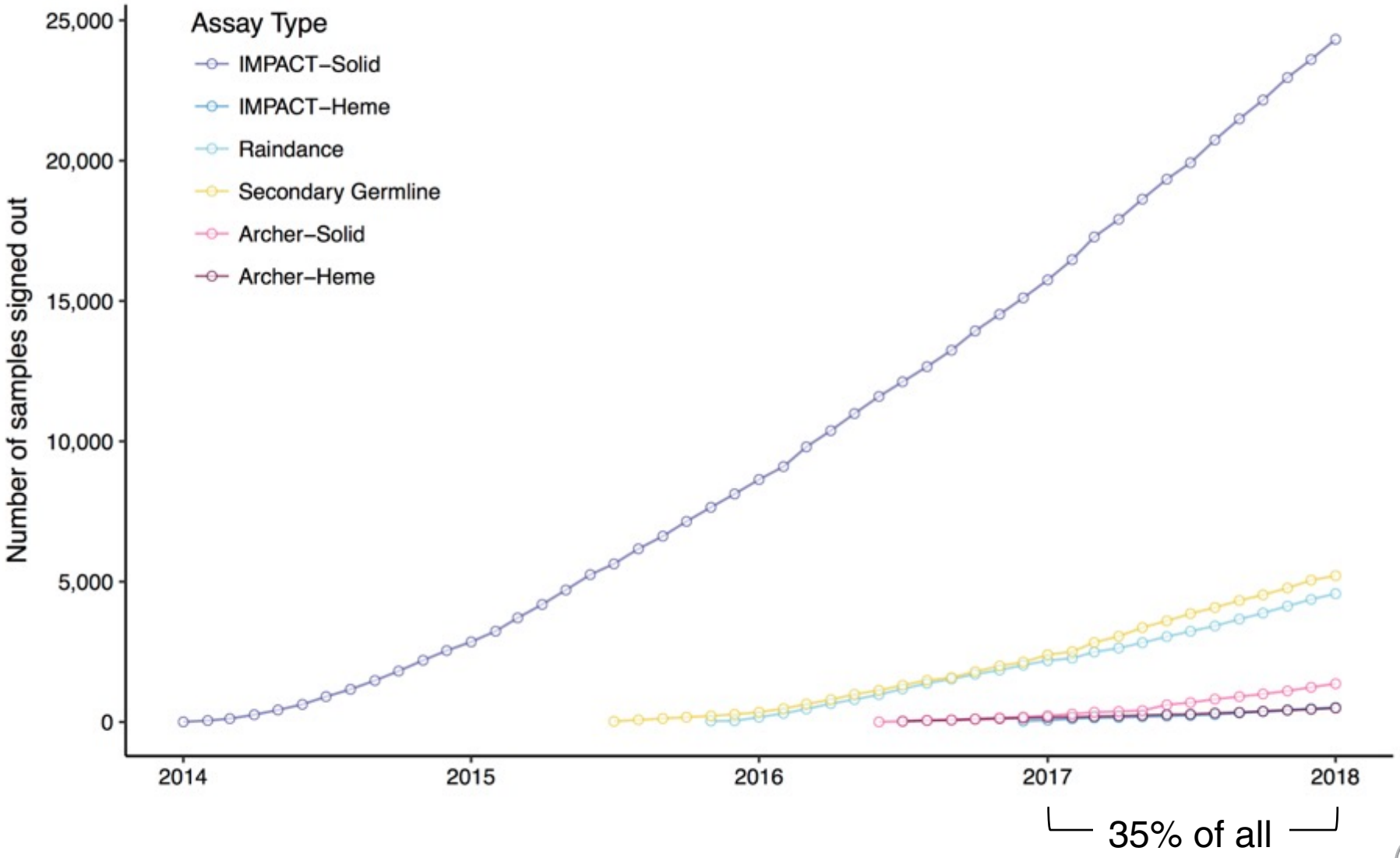






MSKCC – Molecular Diagnostics Service

Comprehensive set of next-generation sequencing (NGS) based assays

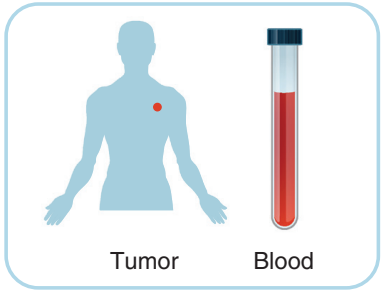


> 30,000 samples analyzed so far

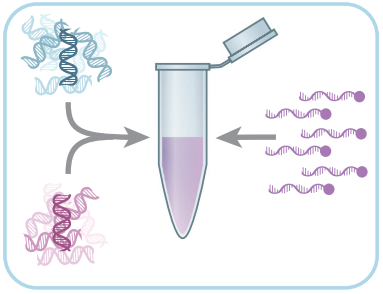
Precision Oncology with MSK-IMPACT

Identifies **mutations**, **copy number alterations**, **structural variants** in **468 genes** as well as biomarkers that could predict treatment response such as **MSI status** or **tumor mutation burden** (TMB)

1. Patient consent

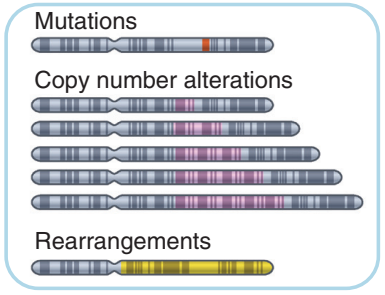


2. Sample accessioning



3. Sample preparation

4. Sequencing



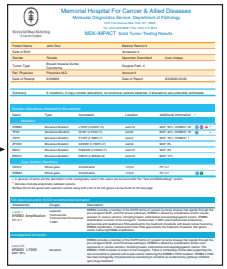
5. Bioinformatics analysis

6. Case review and sign out

Genomic variants database

MPath

Precision oncology knowledge base



Clinical report

Clinical trial matching

Data mining & interpretation

cBioPortal for Cancer Genomics

Zehir, Benayed *et al.*, Nature Med 2017

MSK-IMPACT current panel design

Panel includes exons, introns, and other noncoding regions

24,350 cases

341 genes: 2,861 cases

410 genes: 10,004 cases

468 genes: 11,525 cases

Cancer Gene Exons:
6,614 protein-coding exons of 468 genes
- actionable mutations
- targets of investigational agents
- frequently mutated in cancer
- cancer susceptibility genes

Cancer Gene Introns:
70 introns of 20 recurrently rearranged genes

Cancer Gene Promoters: TERT promoter

SNP Probes: >1,000 non-coding SNPs
- copy number analysis, various QC checks

Target Territory = 1.52 Mb

Transitioning into NGS assays

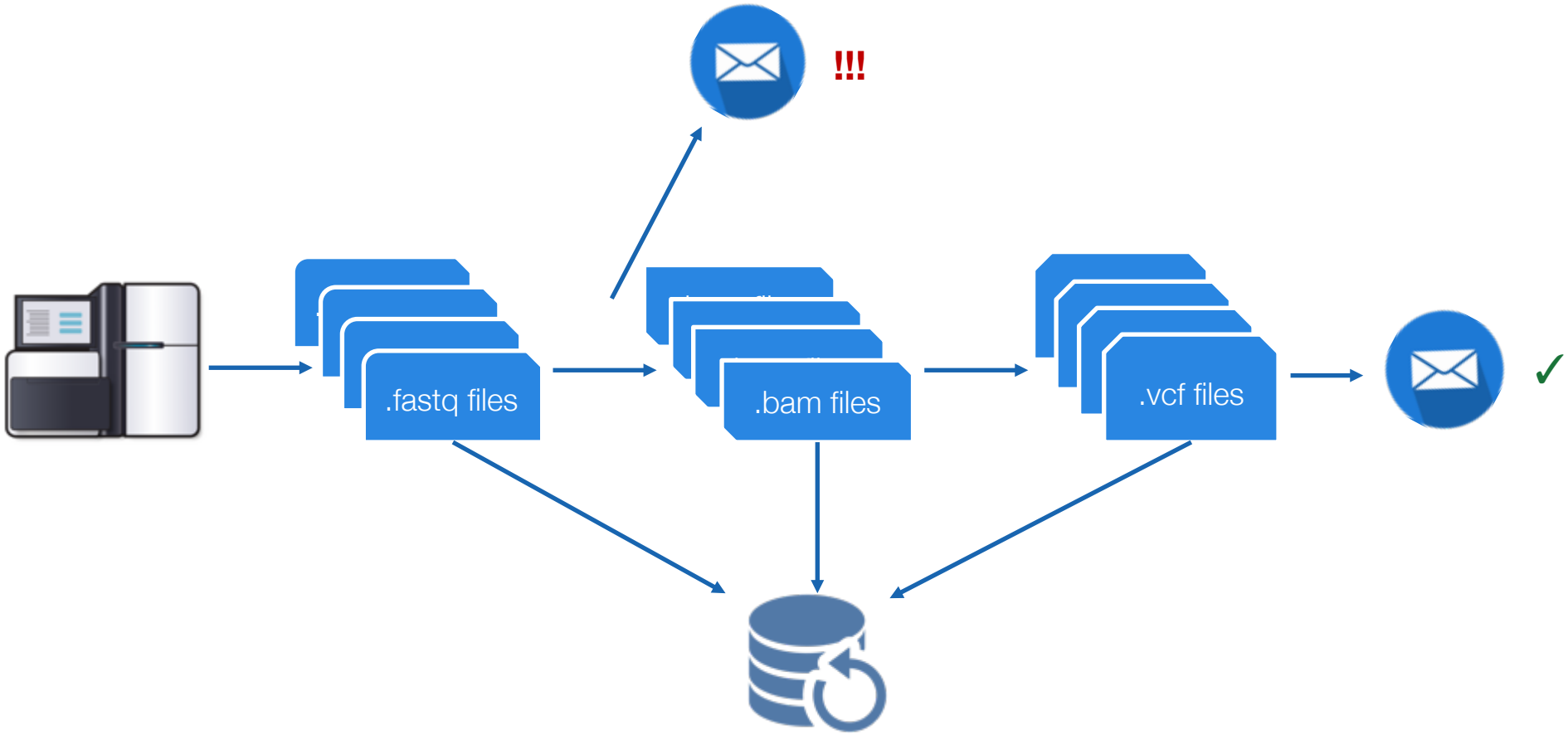
Technical needs for a comprehensive clinical sequencing program

- **Track certain meta-data** elements for the sample
- Review **quality control** data points and confirm technical success
- Track **sample failures**, **repeats**, **multiple samples** from the **same patient**

- **Review & track mutations** at the raw data level (.bam files)
- **Review & track copy number alterations** and **structural variants**

- **Share data** with the institute (**clinicians + researchers**)
- **Create clinical reports** easily
- **Ease of accessibility** and **accountability**

MPath – Data Management System (DMS)



- Kicks-off whenever a run is completed on the sequencer
- Automatically **starts analysis pipeline** and **monitors progress**
- Monitors file storage and automatically **backs up the data** files
- **Tracks sample meta data, sample QC**
- **De-identifies samples** based on patient identifier and the assay type
- **Alerts** users of **successful completion** of pipeline as well as **errors** regarding the analysis

Memorial Sloan Kettering Cancer Center

MPath DMS

JIRA

Select one: All | Impact | Impact Heme | Raindance | Year | Status

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MPath DMS

JIRA

Demultiplex Stats | QC Metrics | CNV-Results | CNV-Loess | CNV-NormalVsNormal | UnrepBC Analysis | AnalysisQC Dir | LIMs Dashboard | Krona

Switch HTML file

QC Results for:

IMPACTv6-RES-20170001R

Run: IMPACTv6-RES-20170001R QC results

Memorial Sloan Kettering Cancer Center-Diagnostics Molecular Pathology Lab

Memorial Sloan Kettering Cancer Center

MPath DMS

JIRA

Demultiplex Stats | QC Metrics | CNV-Results | CNV-Loess | CNV-NormalVsNormal | UnrepBC Analysis | AnalysisQC Dir | LIMs Dashboard | Krona

Switch HTML file

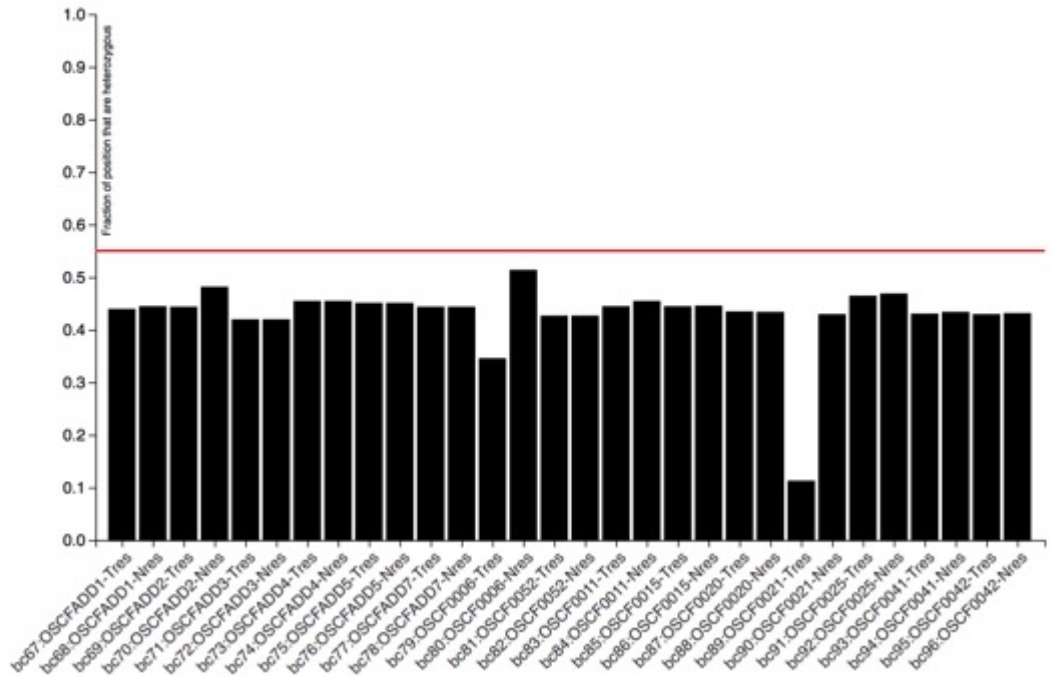
QC Results for:

IMPACTv6-RES-20170001R

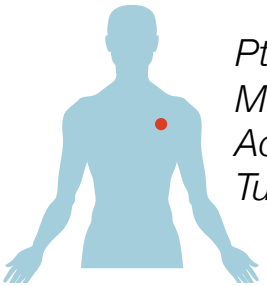
Sample QC Results

Navigation

Major Contamination



MPath - Results Management System (RMS)

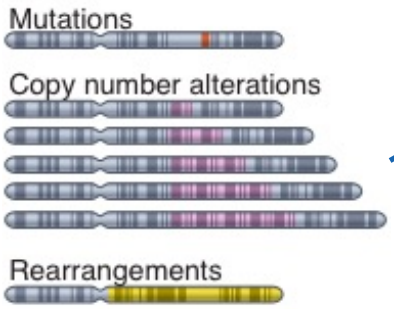


Pt. Name
MRN
Accession #
Tumor Type

Meta-data

+

MPath NGS



Analysis results

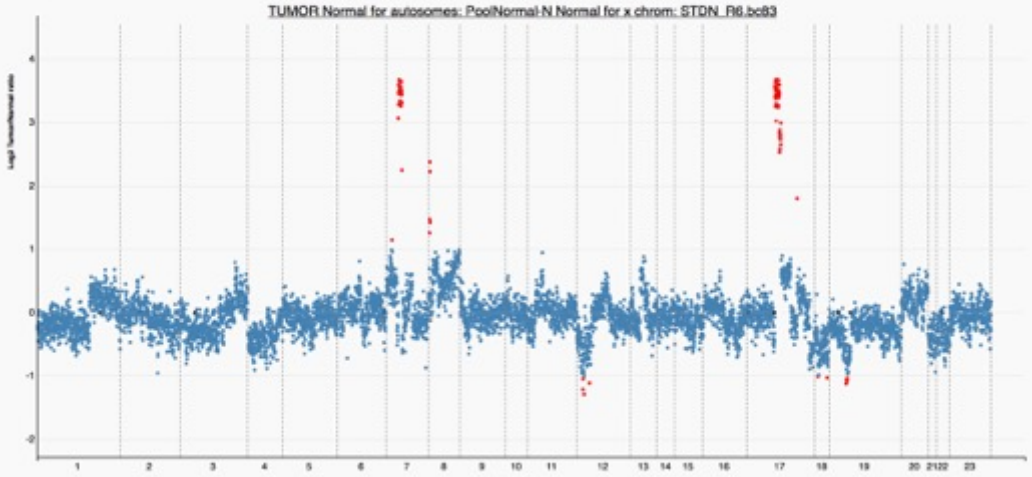
MPath - NGS

Memorial Sloan Kettering Cancer Center **MSK-IMPACT®**

MSK-IMPACT® All | Search | JIRA | zehira

Patient Info | Mutations | Copy Number Variants | Structural Variants | DMS | LIMS QC | IMPACTv6-CLIN-20170488

SCNA Results



Mutation Calls

Show / hide columns | Search:

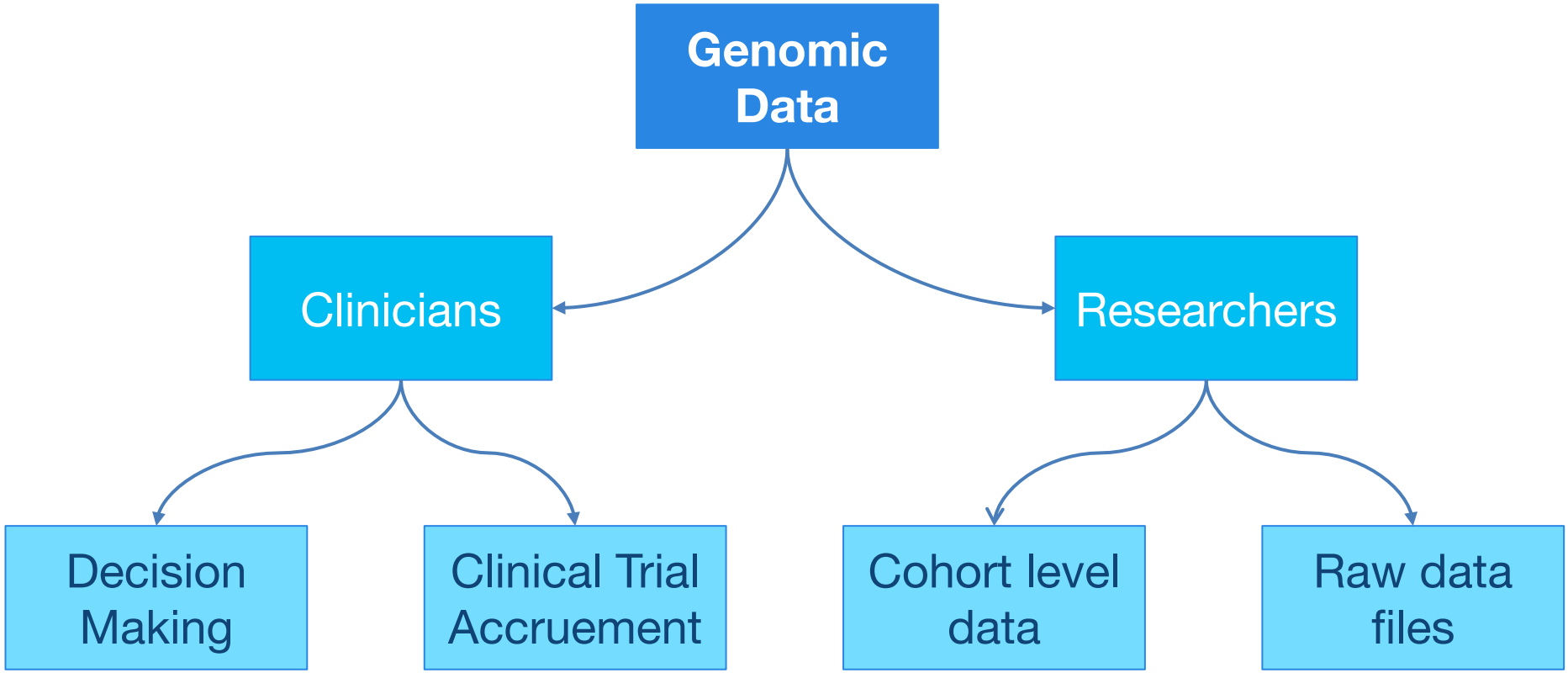
Chr:Pos	Ref	DNA	AA
7:140453136	A	799T>A	p.V600E
12:25398285	C	34G>A	p.G12S
12:25378562	C	36G>A	p.A146T
2:209113112	C	95G>A	p.R132H
19:3118942	A	26A>T	p.Q209L
3:178952085	A	40A>G	p.H1047R
7:55241707	G	55G>A	p.G719S
15:66727451	A	67A>C	p.Q56P
3:178936082	G	24G>A	p.E542K
7:140453137	C	98G>A	p.V600M
1:115256528	T	83A>T	p.Q61H
12:25398281	C	8G>A	p.G13D
3:41266101	C	8C>A	p.S33Y

Whole gene level SCNA calls

Show / hide columns | Search: Significant

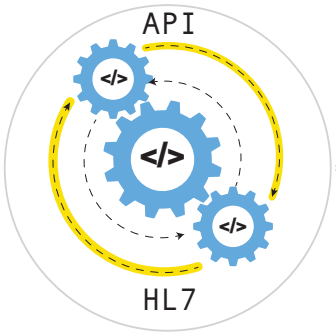
Cytoband	Gene Name	Fold Change	p_value	Significance	Comments	Annotat
17q12	CDK12	7.5083	0.0	Significant	None	None
17q12	ERBB2	11.0219	0.0	Significant	None	None
17q21	RARA	6.71832	0.0	Significant	None	None
7p11	EGFR	10.7302	0.0	Significant	None	None
8p11	WHSC1L1	1.62315	0.397694	Significant	ab	Note:
8q24	AGO2	1.76455	0.154817	Significant	ab	Note:
8q24	RECQL4	1.68892	0.27688	Significant	ab	Note:

Institutional data sharing



MPath - Reporter

MPath NGS



MPath Reporter

HL-7

RESTful APIs

Memorial Hospital For Cancer & Allied Diseases
Molecular Diagnostics Service, Department of Pathology
1275 York Avenue New York, NY, 10065
Tel: (212) 639-8360 | Fax: (212) 717-3515
MSK-IMPACT Testing OncoKB Annotation Amended Report

Patient Name	Redacted	Medical Record #	Redacted
Date of Birth	Redacted	Accession #	Redacted
Gender	Redacted	Specimen Submitted	Sigmoid colon
Tumor Type	Colon Adenocarcinoma	Surgical Path. #	Redacted
Ref. Physician	Redacted	Account #	Redacted
Date of Receipt	Redacted	Date of Report	Redacted
Date of Procedure	Redacted		

Summary: 4 mutations, no copy number alterations, no structural variants detected, 1 alteration has OncoKB interpretation

Somatic alterations detected in this sample:

Gene	Type	Annotation	Location	Additional. Info.
Mutations				
BRAF	Missense Mutation	V600E (c.1799T>A)	exon15	MAF: 7.3%
PTEN	Missense Mutation	D92N (c.274G>A)	exon5	MAF: 7.5%
TP53	In-frame Del	N131del (c.393_395del)	exon5	MAF: 10.4%
NBGR1	Nonsense Mutation	R173* (c.517C>T)	exon3	MAF: 5.3%

+: A glossary of terms and icons used in this report can be found after the "Test and Methodology" section
^: Denotes clinically/analytically validated variants.
RefSeq IDs for the genes with reported variants along with a list of all 410 genes can be found on the last page

Investigational biomarkers

Alteration(s)	Drugs(s)	Description
Level 2B BRAF V600E MAF: 7.3%	Trametinib + Dabrafenib (2B), Vemurafenib + Cobimetinib (2B), Dabrafenib (2B), Vemurafenib (2B), Vemurafenib + Panitumumab (3A), Trametinib (2B)	BRAF, an intracellular kinase, is frequently mutated in melanoma, thyroid and lung cancers. The BRAF V600E mutation is known to be oncogenic. The FDA-approved selective RAF-inhibitors dabrafenib and vemurafenib have minimal activity as single agents in BRAF V600-mutant colorectal cancer. There is promising clinical data supporting regimens that combine a RAF-inhibitor together with an anti-EGFR antibody with or without a MEK-inhibitor, such as the doublet vemurafenib plus panitumumab or the triplet dabrafenib, trametinib, plus panitumumab in patients with these tumors.

Technical Assessments

Coverage	875X	Test Version	410 genes
Status	Matched Sample	Run Number	2015-224

Coverage assessment: Unless specified, all exons tested had minimum depth of coverage of 100X.
Mutation assessment: Mutation assessment: Mutations are called against the patient's matched normal sample. This assay reports somatic variants confirmed to be absent in the matched normal.

Page 1 of 4

Electronic Medical Record

cBio Portal

Traditional clinical reports

Plain text based reporting

POSITIVE FOR THE FOLLOWING SOMATIC ALTERATIONS IN THE CLINICALLY VALIDATED PANEL:

1. TP53 (NM_000546) exon10 p.R342Pfs*5 (c.1024dupC)
MICROSATELLITE STABLE (MSS). See MSI note below.

POSITIVE FOR THE FOLLOWING SOMATIC ALTERATIONS IN

THE INVESTIGATIONAL PANEL:
2. TERT (NM_198253 - 5p15.33) Amplification (Fold Change: 3.7)
3. FGFR4 (NM_213647 - 5q35.2) Amplification (Fold Change: 2.6)
4. NSD1 (NM_022455 - 5q35.3) Amplification (Fold Change: 2.5)
5. SDHA (NM_004168 - 5p15.33) Amplification (Fold Change: 2.0)
6. CDKN2B (NM_004936 - 9p21.3) Deletion (Fold Change: -2.0)
7. CDKN2Ap16INK4A (NM_000077 - 9p21.3) Deletion (Fold Change: -2.0)
8. CDKN2Ap14ARF (NM_058195 - 9p21.3) Deletion (Fold Change: -2.0)
9. IGF2 (NM_001127598) exon3 p.G58E (c.173G>A)
10. KDM5C (NM_004187) exon15 p.E698K (c.2092G>A)
11. SOS1 (NM_005633) exon19 p.R989S (c.2967G>C)
12. TERT (NM_198253) exon1 p.R41C (c.121C>T)
13. ZFHX3 (NM_006885) exon2 p.V190Afs*17 (c.569_576delTGTACCCG)
14. NSD1 (NM_022455) rearrangement: chr5:g.8599249_c.6258+66:NSD1inv

Note: The NSD1 rearrangement is predicted to result in the inversion of exons 1-21. The functional significance is undetermined.
MSI Note: The MSIsensor score is 0.23.

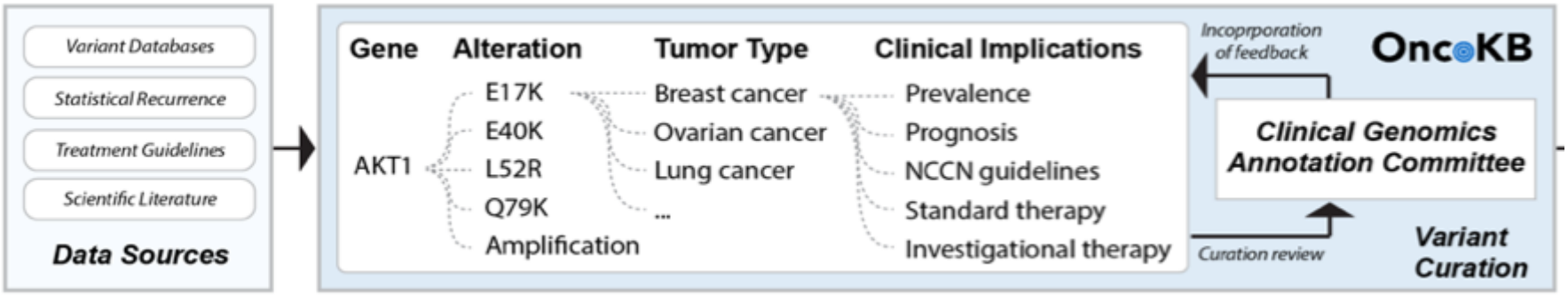
MEAN OVERALL COVERAGE (SEQUENCING DEPTH) IN THIS SAMPLE: 748XUnless specified, all exons tested had minimum depth of coverage of 100X.Mutation assessment: Mutations are called against the patient's matched normal sample.

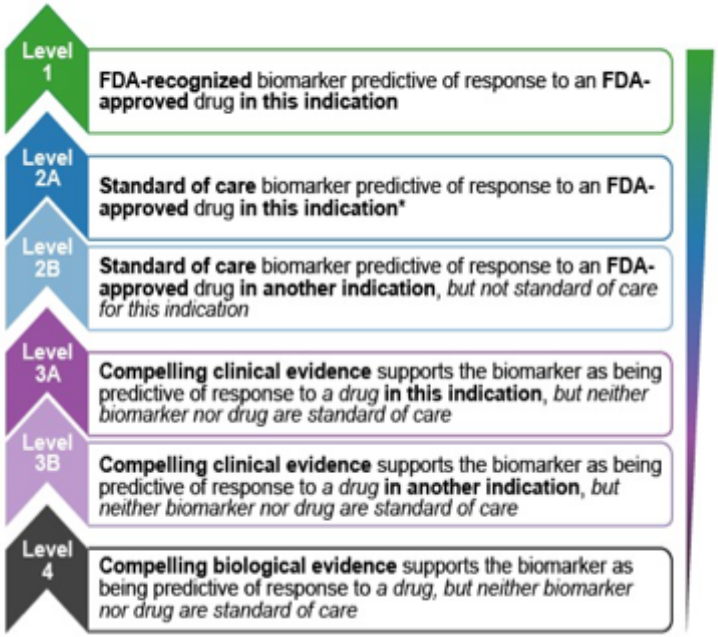
This assay reports somatic variants confirmed to be absent in the matched normal.

Microsatellite instability (MSI) assessment by MSIsensor: MSIsensor Methodology: Microsatellite instability (MSI) status is assessed using the MSIsensor program (Niu B et al, 2014) that interrogates the length distribution of all genomic microsatellite loci included in the

OncoKB: Precision oncology knowledge base

Clinical and biological effects of oncogenic variants

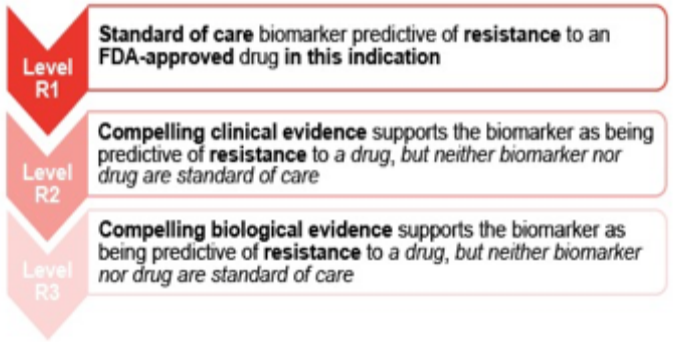




Standard Therapeutic Implications
*Includes biomarkers that are recommended as standard of care by the NCCN or other expert panels but not necessarily FDA-recognized for a particular indication

Investigational Therapeutic Implications
possibly directed to clinical trials

Hypothetical Therapeutic Implications
based on preliminary, non-clincial data



Standard Therapeutic Implications

Hypothetical Therapeutic Implications
based on preliminary, non-clincial data

Chakravarty et al, JCO Precision Oncology (2017)

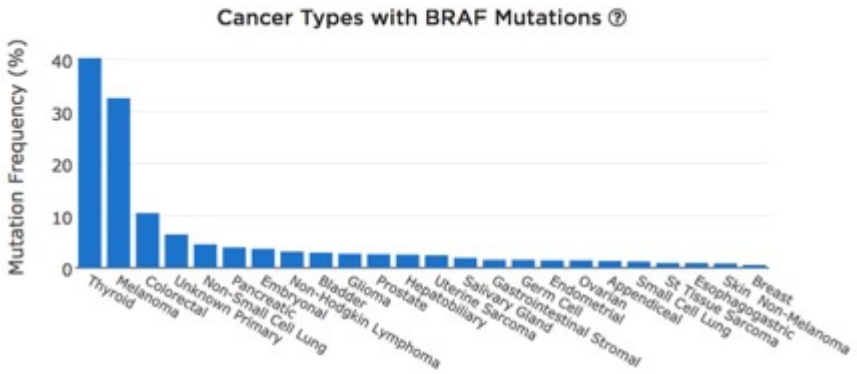
https://oncokb.org

BRAF 117 annotated variants

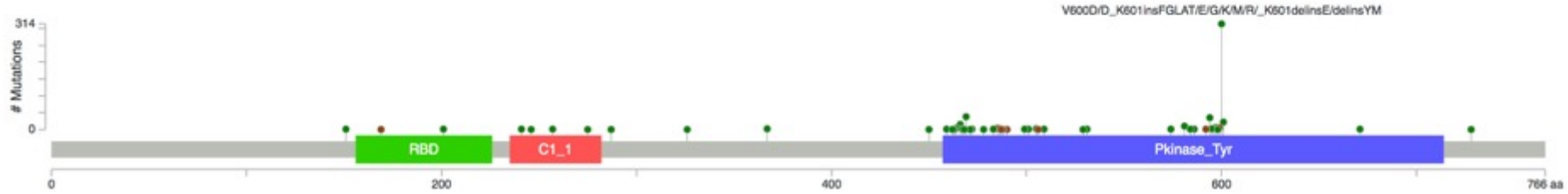
Oncogene
Highest level of evidence: Level 1
Also known as NS7, B-raf, BRAF1, RAFB1, B-RAF1
Isoform: ENST00000288602 RefSeq: NM_004333.4

BRAF, an intracellular kinase, is frequently mutated in melanoma, thyroid and lung cancers.

See BRAF background



Annotated Mutation Distribution in MSK-IMPACT Clinical Sequencing Cohort (Zehir et al., Nature Medicine, 2017)



Clinically Relevant Variants (35) | All Annotated Variants (117)

If you notice any mistakes or missing variants / citations, please send an email to feedback@oncokb.org.

Search:

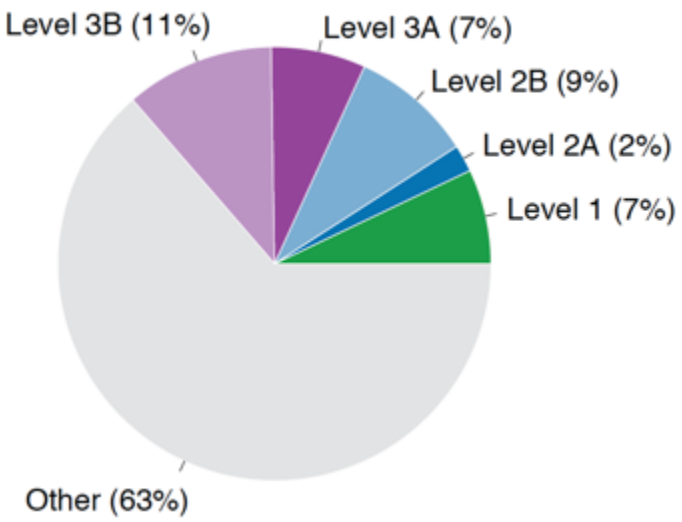
Variant	Cancer Type	Drug(s)	Level	Citations
V600G	Melanoma	Vemurafenib Dabrafenib Dabrafenib + Trametinib Vemurafenib + Cobimetinib	1	14 references

Chakravarty et al, JCO Precision Oncology (2017)

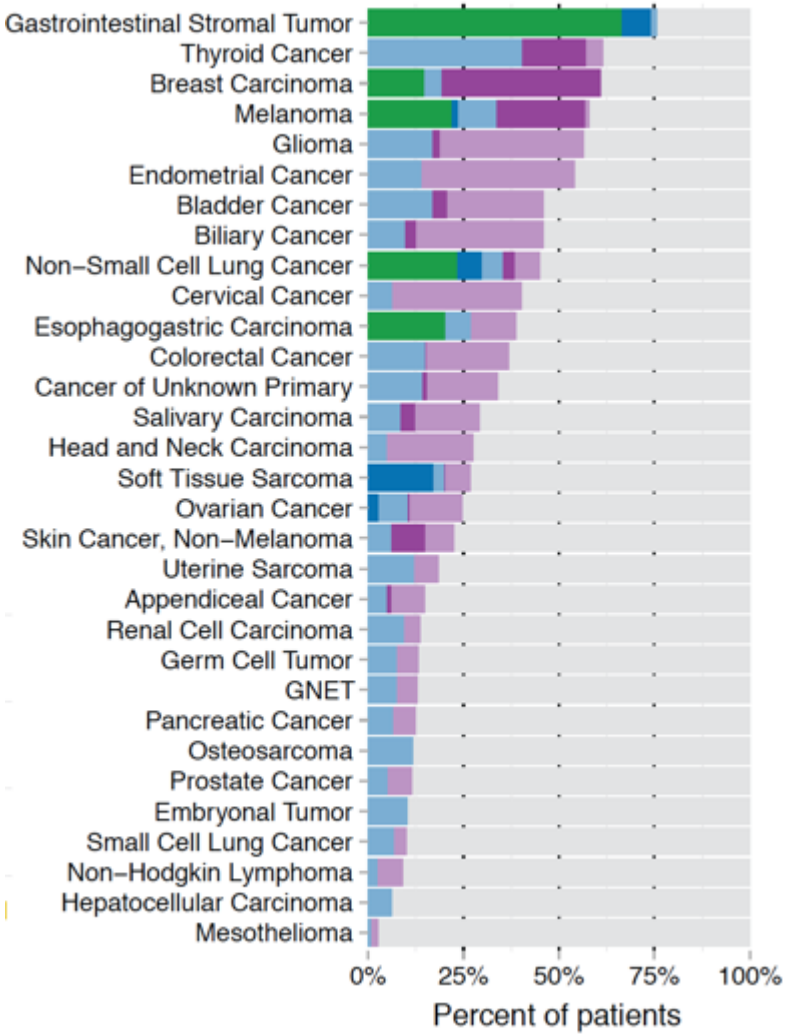
https://oncokb.org

Prevalence of Actionable Mutations in MSK-IMPACT Cohort

37% of patients harbored ≥1 OncoKB-annotated actionable mutation



Level 1	FDA-recognized biomarker for an FDA-approved drug in the same indication
Level 2A	Standard of care biomarker for an FDA-approved drug in the same indication
Level 2B	Standard of care biomarker for an FDA-approved drug in another indication
Level 3A	Compelling clinical evidence supporting the biomarker as being predictive of drug response in the same indication
Level 3B	Compelling clinical evidence supporting the biomarker as being predictive of drug response in another indication



Debyani Chakravarty, Sarah Phillips, JJ Gao, Hongxin Zhang,
Ritika Kundra, Jioajioa Wang, Julia Rudolph, Niki Schultz

Enhanced reports with clinical annotations

OncoKB Level 1 to 3 annotations included in molecular diagnostics reports

Memorial Sloan Kettering Cancer Center

Memorial Hospital For Cancer & Allied Diseases
Molecular Diagnostics Service, Department of Pathology
1275 York Avenue New York, NY, 10065
Tel: (212) 639-8280 | Fax: (212) 717-3515
MSK-IMPACT Testing Report

Patient Na
Date of Bi
Gender
Tumor Ty
Ref. Phys
Date of Re

Summary
MSI Statu
Tumor Mu Burden
Comment

FDA Approved and/or NCCN recommended biomarker:

Alteration(s)	Drugs(s)	Annotation
Level 1 **EGFR** L747_P753delinsS MAF: 42.3%	Erlotinib, Afatinib, Gefitinib	EGFR, a receptor tyrosine kinase, is altered by amplification, mutation and/or overexpression in various cancers, most frequently in lung and brain cancers. The EGFR L747_P753delinsS alteration is known to be oncogenic. The EGFR tyrosine kinase inhibitors erlotinib, afatinib and gefitinib are FDA-approved for the treatment of patients with non-small cell lung cancer harboring an EGFR exon 19 deletion such as L747_P753delinsS. OncoKB version: v1.12.

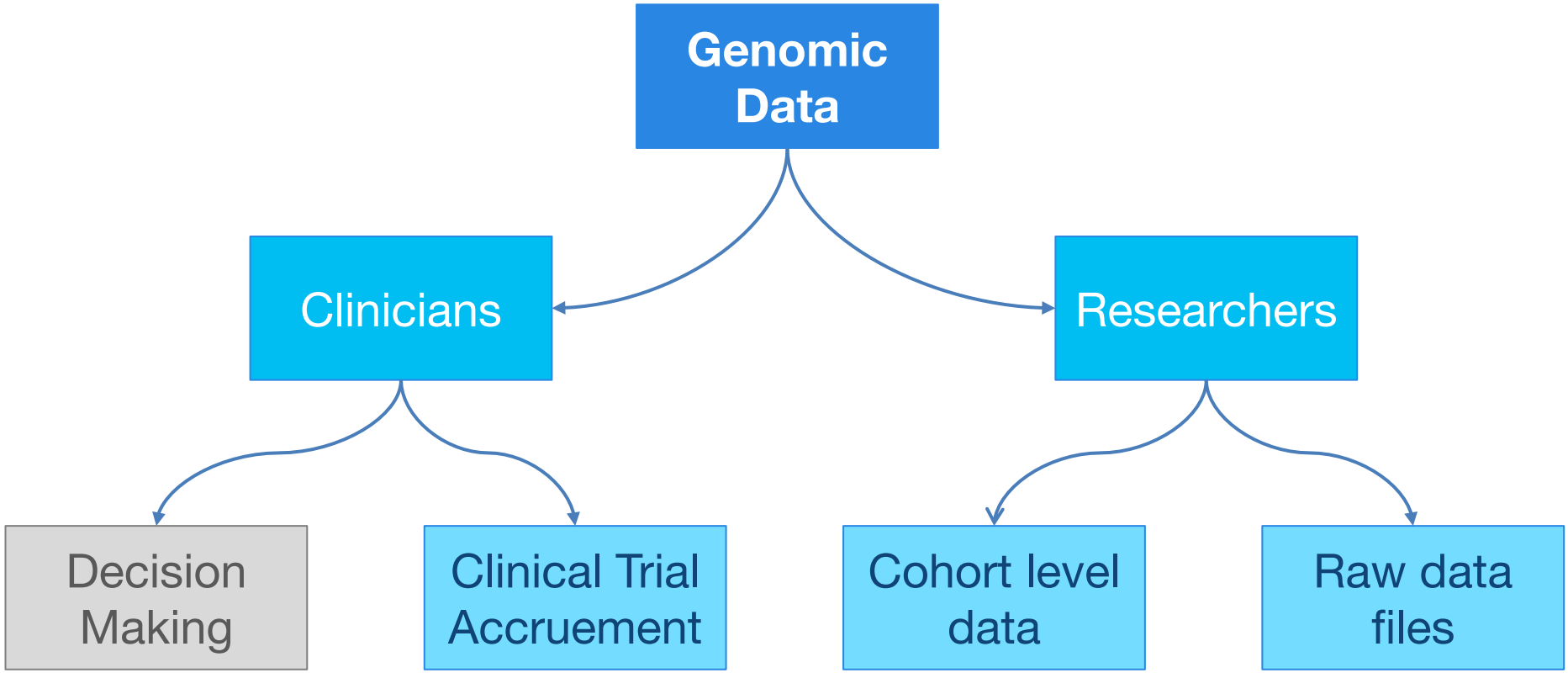
Investigational biomarker:

Level 2B **EML4-ALK** Fusion	Crizotinib, Ceritinib, Alectinib, Brigatinib	ALK, a receptor tyrosine kinase, is recurrently altered by chromosomal rearrangements in various cancers including anaplastic large cell lymphoma, non-small cell lung cancer and inflammatory myofibroblastic tumor. The EML4-ALK fusion is known to be oncogenic. While crizotinib, ceritinib, alectinib and brigatinib are FDA-approved for the treatment of patients with ALK-fusion positive lung cancer, their clinical utility in patients with ALK-fusion positive adenocarcinoma, NOS is unknown. OncoKB version: v1.12.
Level 3B **MDM2** Amplification FC: 13.5	RG7112, DS-3032b	MDM2, a ubiquitin ligase and p53 inhibitor, is amplified in a diverse range of cancers including well-differentiated liposarcomas. MDM2 amplification is known to be oncogenic. While there is promising clinical data supporting the use of MDM2-inhibitors such as RG7112 and DS-3032b in patients with MDM2-amplified liposarcomas, their clinical utility in patients with MDM2-amplified lung adenocarcinoma is unknown. OncoKB version: v1.12.

Somatic

Gene						
Mu						
EGFR						
CTNNB1						
Co						
MDM2						
MYC	Whole gene	Amplification	8q24.21	FC: 2.0		
AGO2	Whole gene	Amplification	8q24.3	FC: 2.0		
Structural Variants						
EML4-ALK	Fusion	c.667+4775:EML4_c.3068-389:ALKinv	EML4 exons 1-5 with ALK exons 19-29		2B	

Institutional data sharing



Data sharing with Clinicians

Clinical Trial Accruement

How do you tell clinicians that there is a patient whose tumor harbors genomic alterations making them eligible for the clinical trial they are the PI of?

MPath Reporter

DARWIN
Institutional database

Clinician

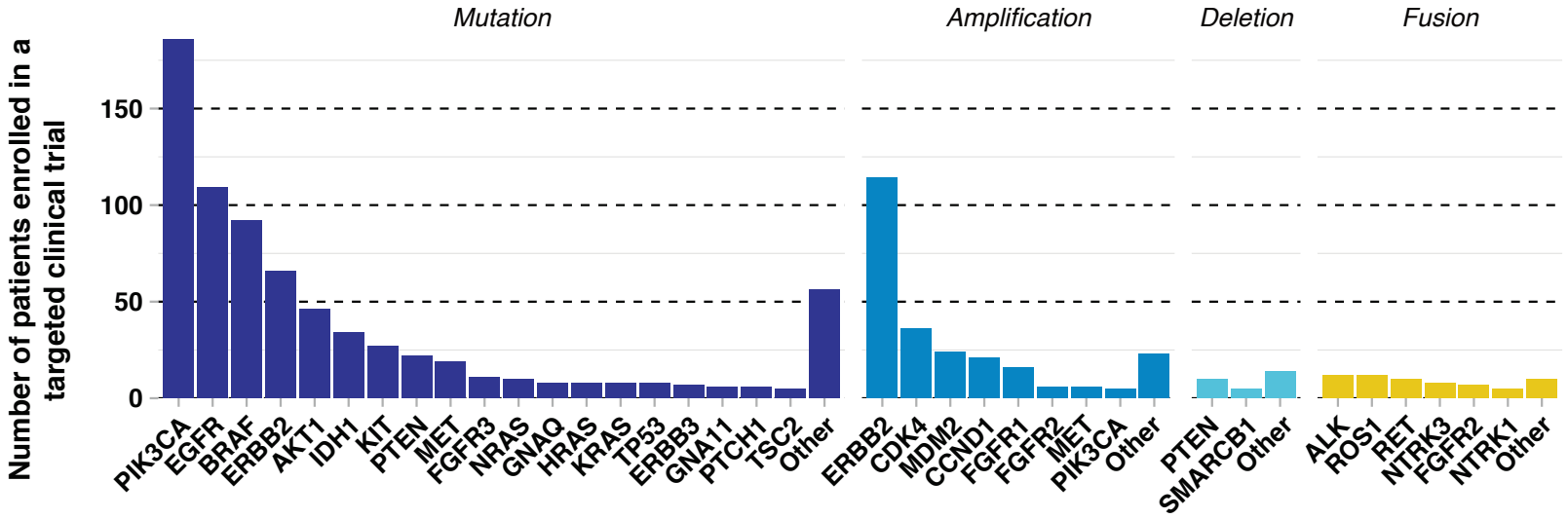
Able to connect with hospital wide information systems and identify patients based on predetermined criteria

Stuart Gardos, Mike Eubank

Enrollment on Genomically Matched Trials

11% of patients with MSK-IMPACT were enrolled on an MSK trial based on a target aberration*

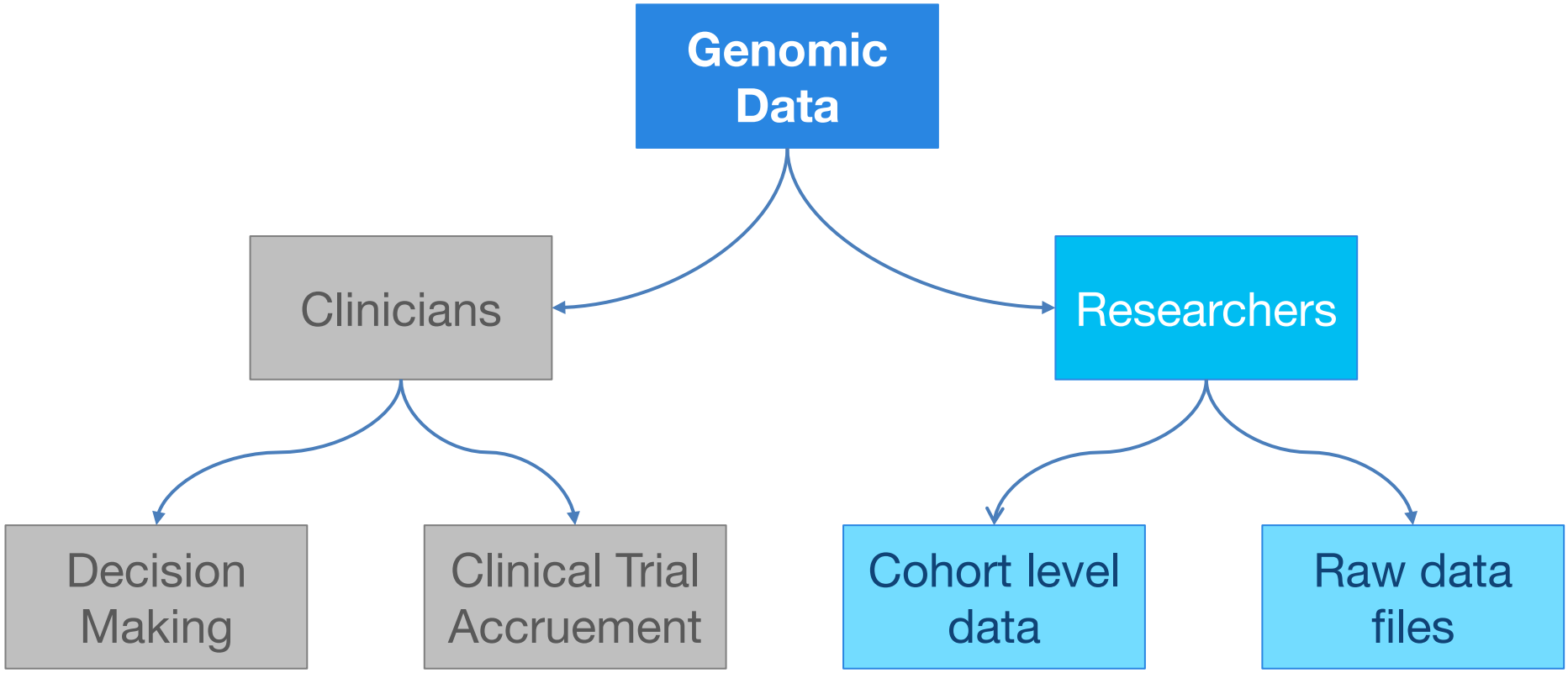
Total patients	100%
…on any MSK trial	38%
…on a targeted trial	16%
…with a target aberration	11%



Alison Schram, David Hyman, Stu Gardos, Jonathan Wills

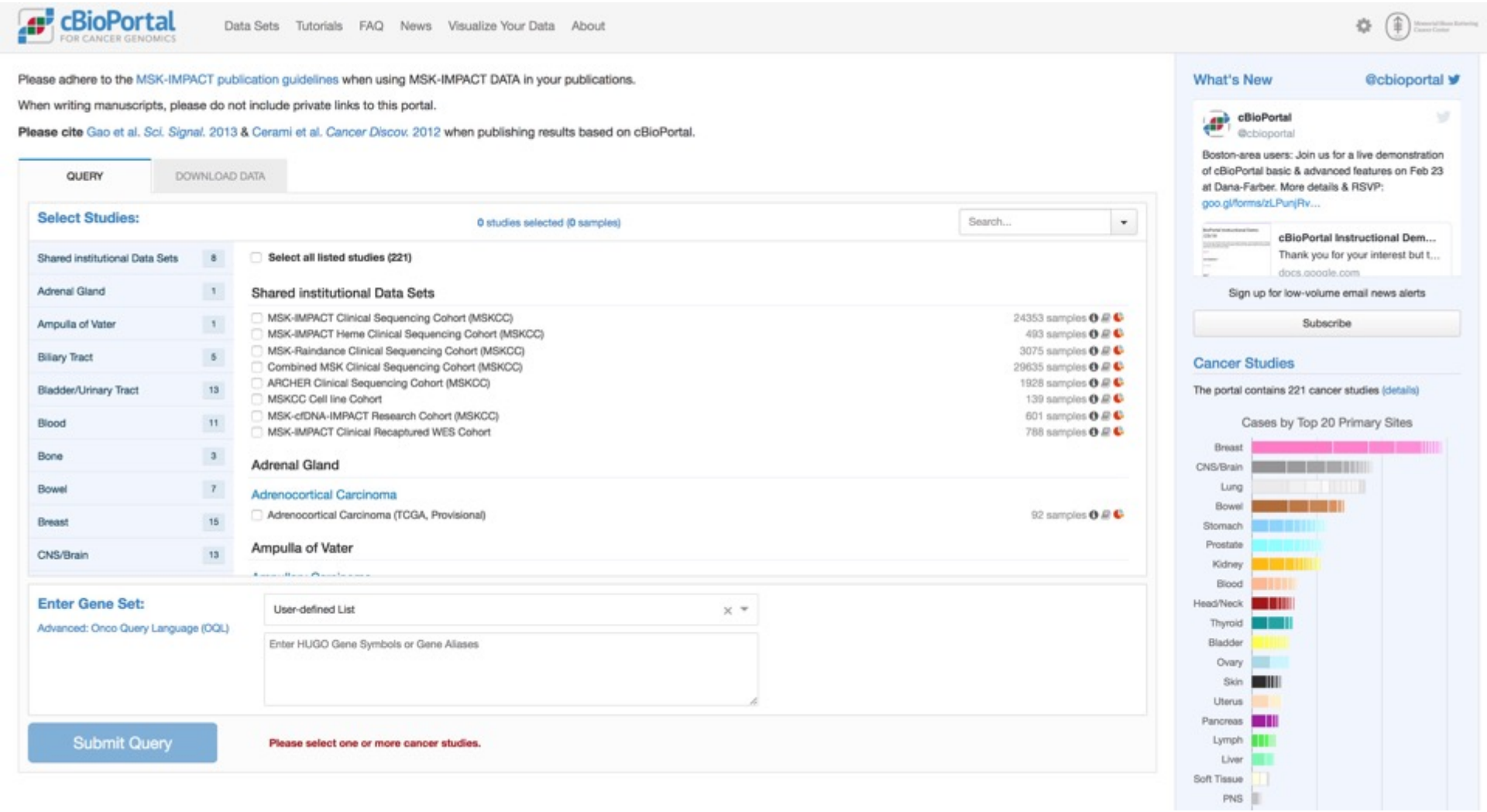
* as of Sept 2016, first 5,000 patients

Institutional data sharing



Data sharing with Researchers

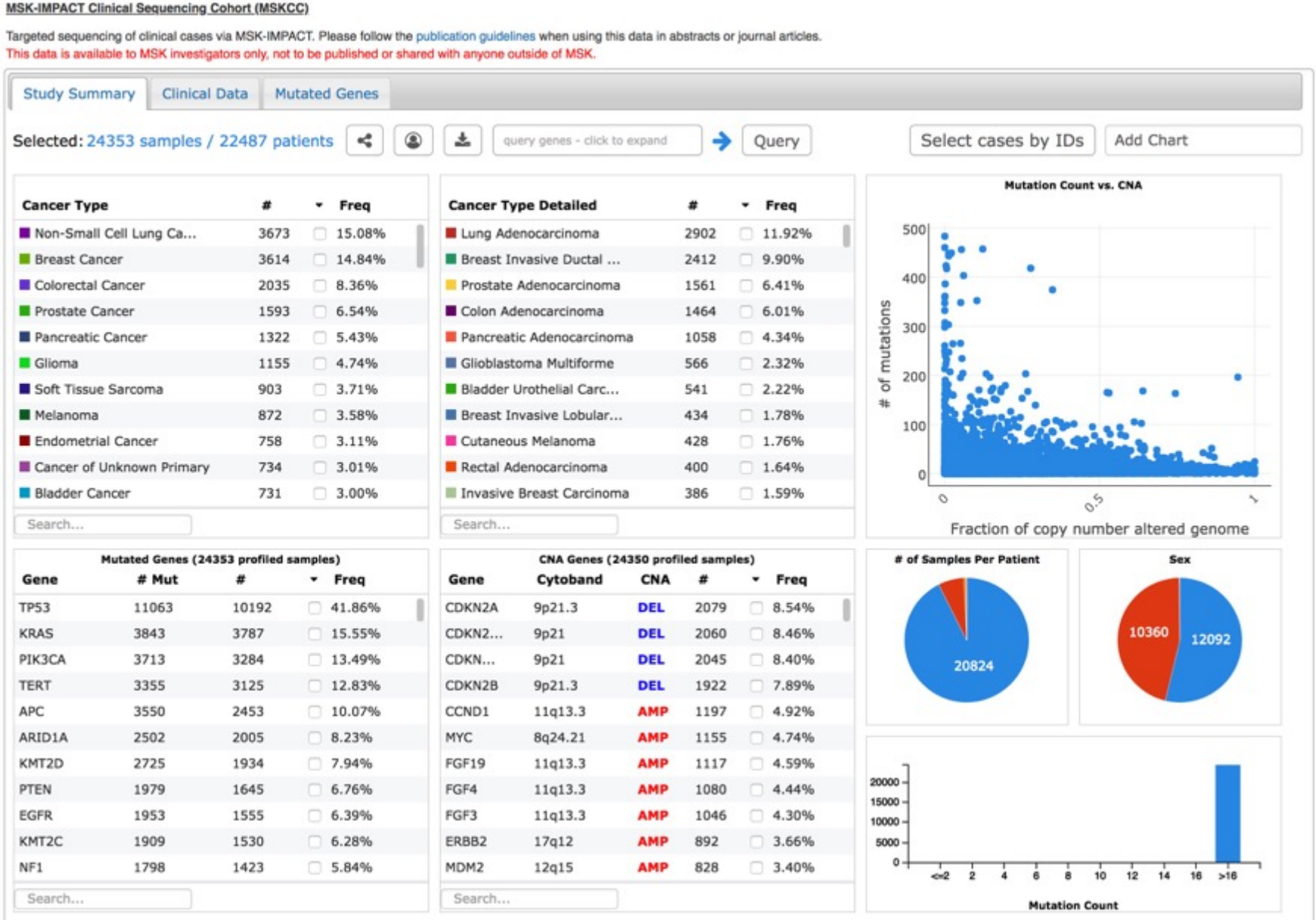
cBio Portal for cancer genomics



www.cbioportal.org

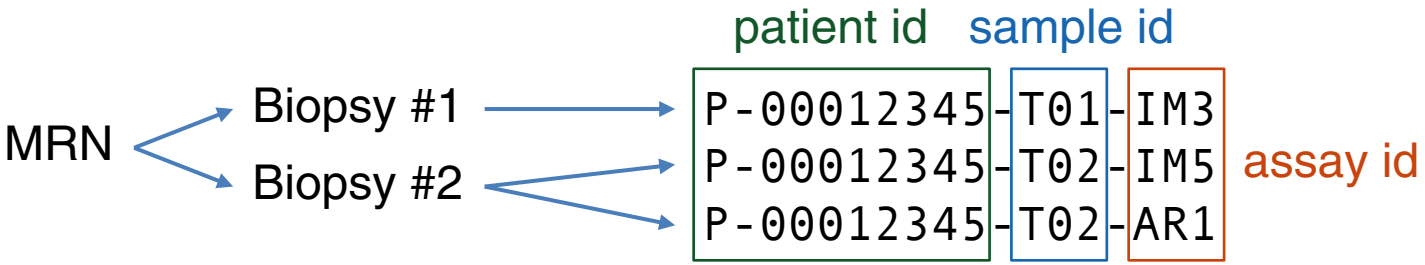
Data sharing with Researchers

Cohort level data



Data sharing with Researchers

De-identifying patients



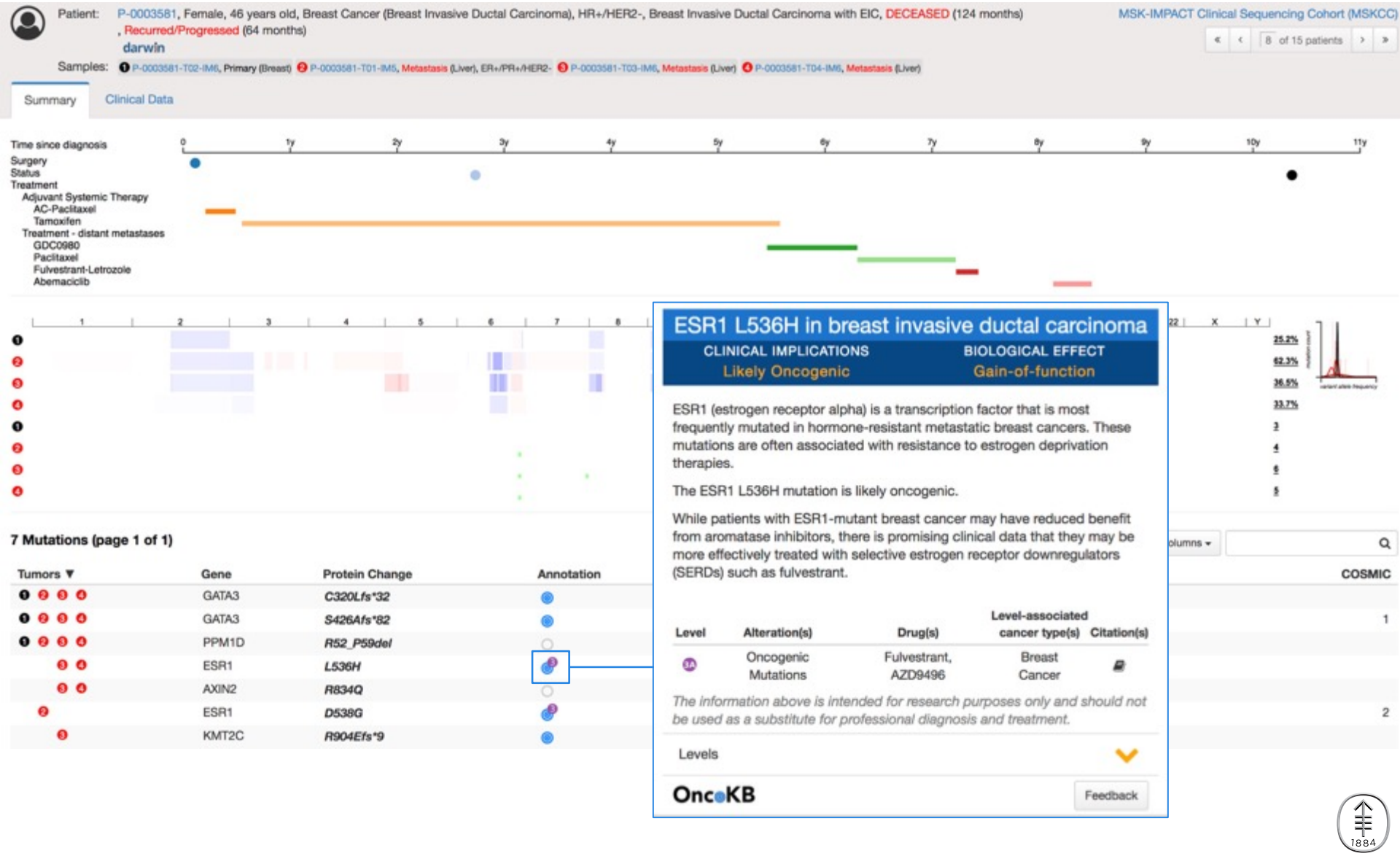
MPath generates IDs for new samples as they are processed through the pipeline and keeps track of the look up table

Clinical staff gets both IDs and researchers get only the de-identified ID

Unfortunately, clinical data collection is still mostly manual, done by medical fellows, and is not standardized.

Data sharing with Researchers

Patient level data: integrated clinical and genomic data



Data sharing with Researchers

Raw data sharing

Challenges:

- Allow researchers to run their own algorithms, analyses on the **data without PHI**
- Allow researchers to combine clinical data with data generated in the research setting
- Make sure different teams are not working on the same project without realizing it

Solution:

- Create a **de-identified .bam file repository**, updated nightly
- Require researchers to register their projects with the **Data Usage Committee**
- Require researchers to register their projects with **IRB via data protocols**

MSKCC Ecosystem

Patient

Pathology

Physicians

Researchers


Memorial Hospital For Cancer & Allied Diseases
Molecular Diagnostics Service, Department of Pathology
MSK-IMPACT Testing OncoKB Annotation Amended Report

identified data

MPath

de-identified data

trial eligibility

clinical data

DARWIN

Acknowledgements

Clinical Bioinformatics

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Diana Mandelker
Lulu Wang
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Talia Mitchell
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Keith Killian
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Knowledge Systems

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Paul Sabbatini
Melissa Pessin
John Petrini
Chris Sander
Mark Robson
Mike Eubank
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