Secuenciación masiva en investigación de vanguardia. Aplicación en oncología como modelo

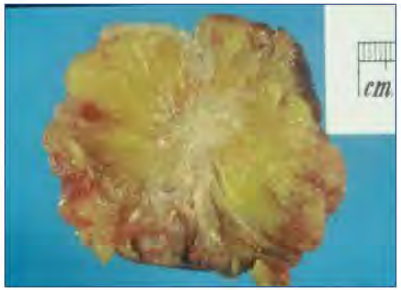
Federico Rojo
Fundación Jiménez Díaz

Sesión Paralela 1. La investigación clínica en España, preparándose para 2018

Evolution from Clinical to Precision Oncology

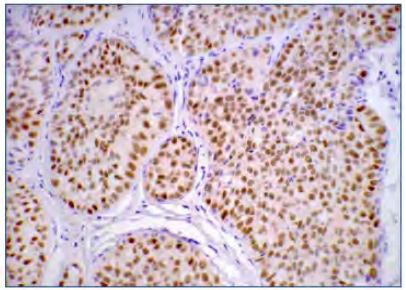
Today

Clinical Oncology → Pathological Oncology → Molecular Oncology → **Precision Oncology**



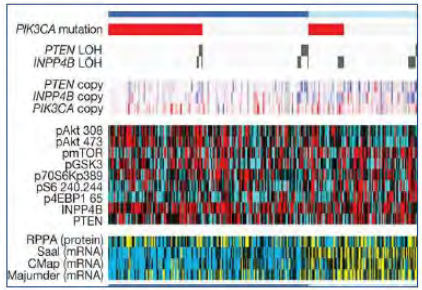
Few therapeutic options to treat tumors:

- Surgery
- Radiotherapy
- Few chemotherapies



Increase on therapeutic options allowed specific treatments for different tumor types:

-Combined chemo-radiation
-Specific protocols



Targeted agents that work in specific molecular alterations:

-Broad knowledge of molecular tumor biology

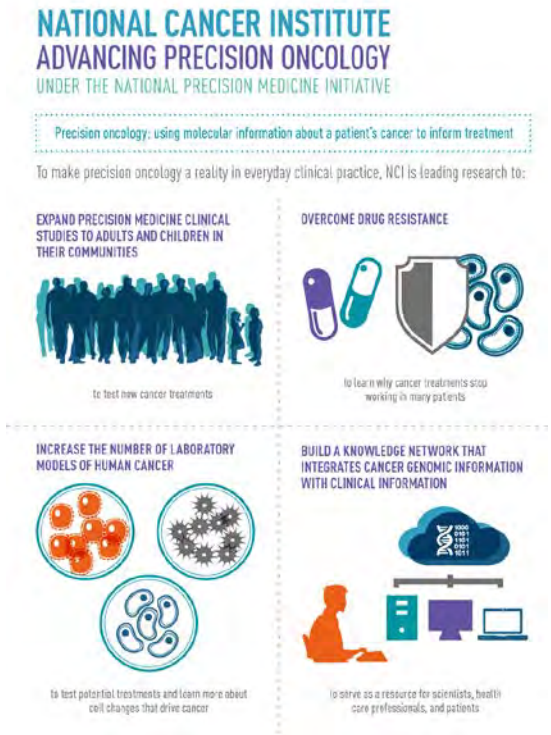
Disease guided approach → **Pathological guided approach** → **Molecular approach**

The NEW ENGLAND JOURNAL of MEDICINE

Perspective

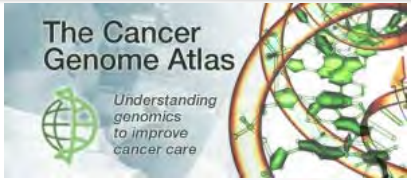
FEBRUARY 26, 2015

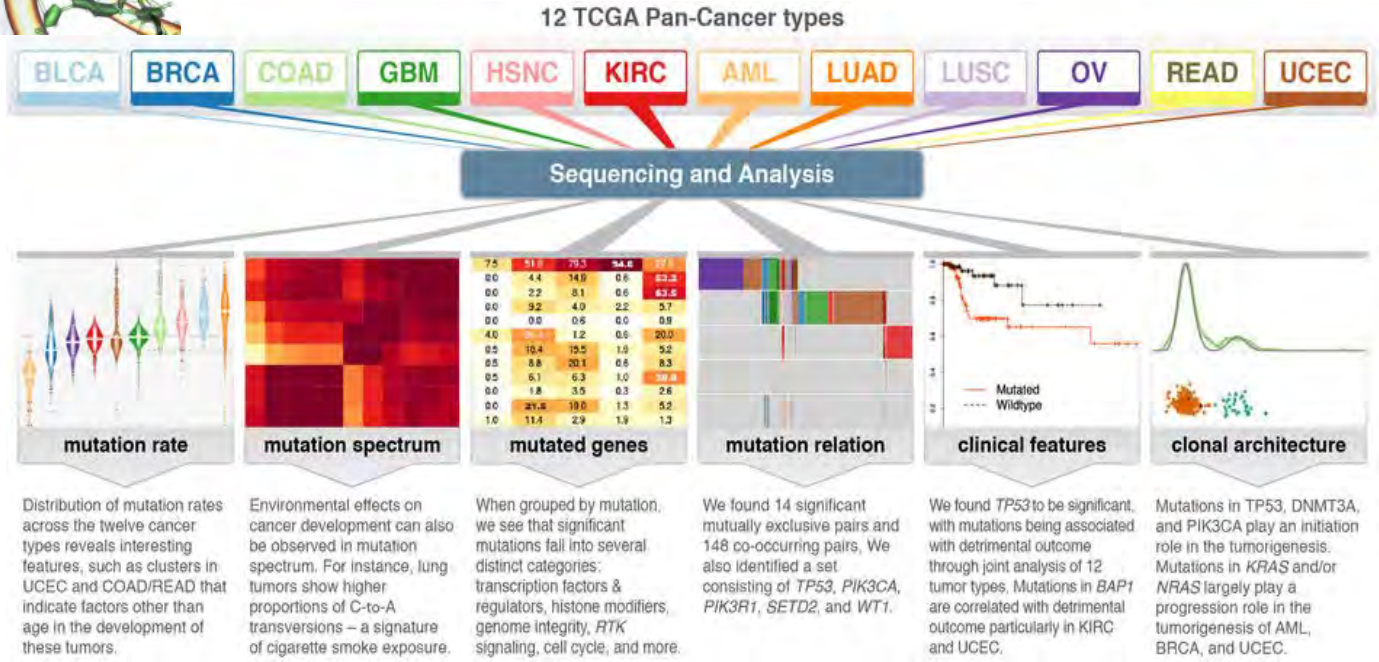
A New Initiative on Precision Medicine



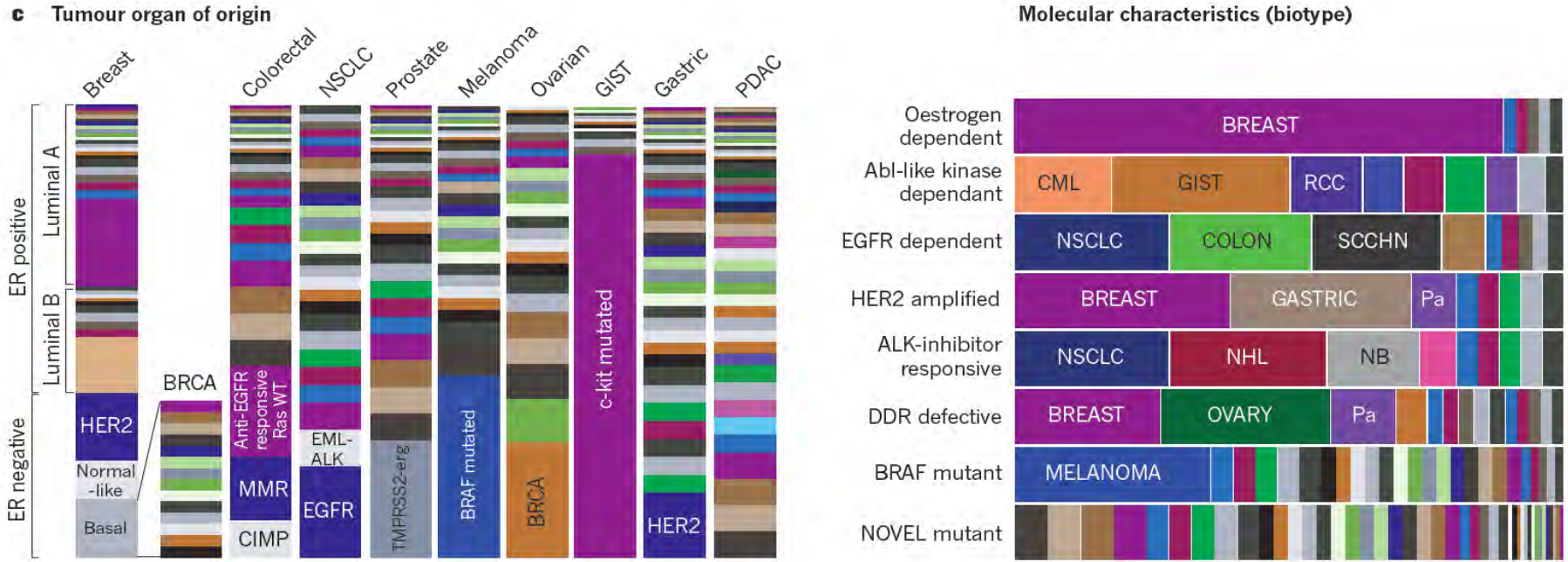
Precision medicine's more individualized, molecular approach to cancer will enrich and modify, but not replace, the successful staples of oncology — prevention, diagnostics, some screening methods, and effective treatments — while providing a strong framework for accelerating the adoption of precision medicine in other spheres.

Next Generation Sequencing Identifies Driver Mutations and Enable Precision Medicine in Cancer





From histology to molecular definition of cancer subtypes



Biankin, AV et al. Nature 2015

Next Generation Sequencing Enables Precision Medicine

2000
Sanger Sequencing (1977-)

ABIPrism (Applied Biosystems)
Up to 2304 sequences per day (96 per hour)

2017
NGS (2006-)

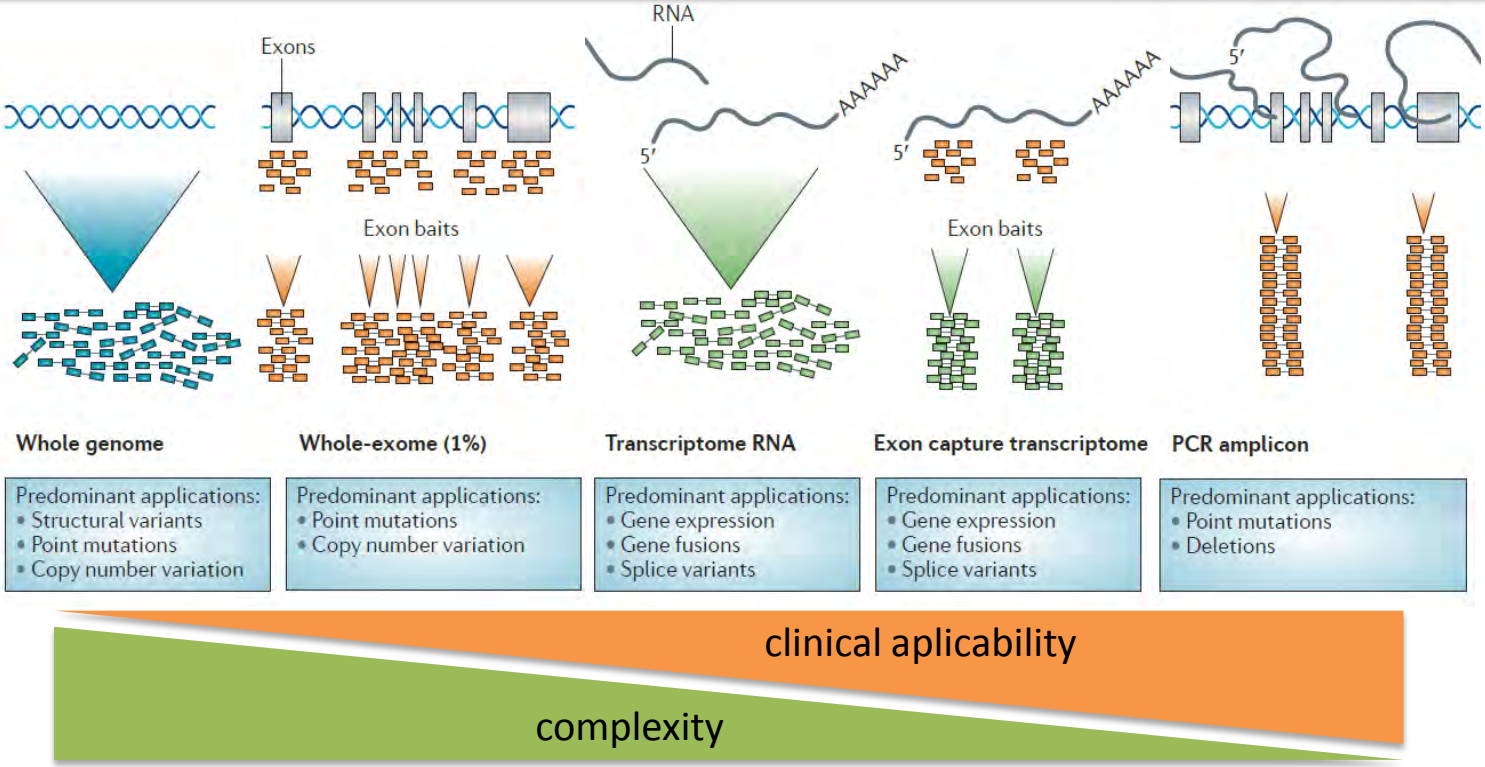
HiSeq (Illumina)
Up to 2 billion sequences per day

MiSeq (Illumina)
Up to 50 million sequences per day

868,000 fold increase per day

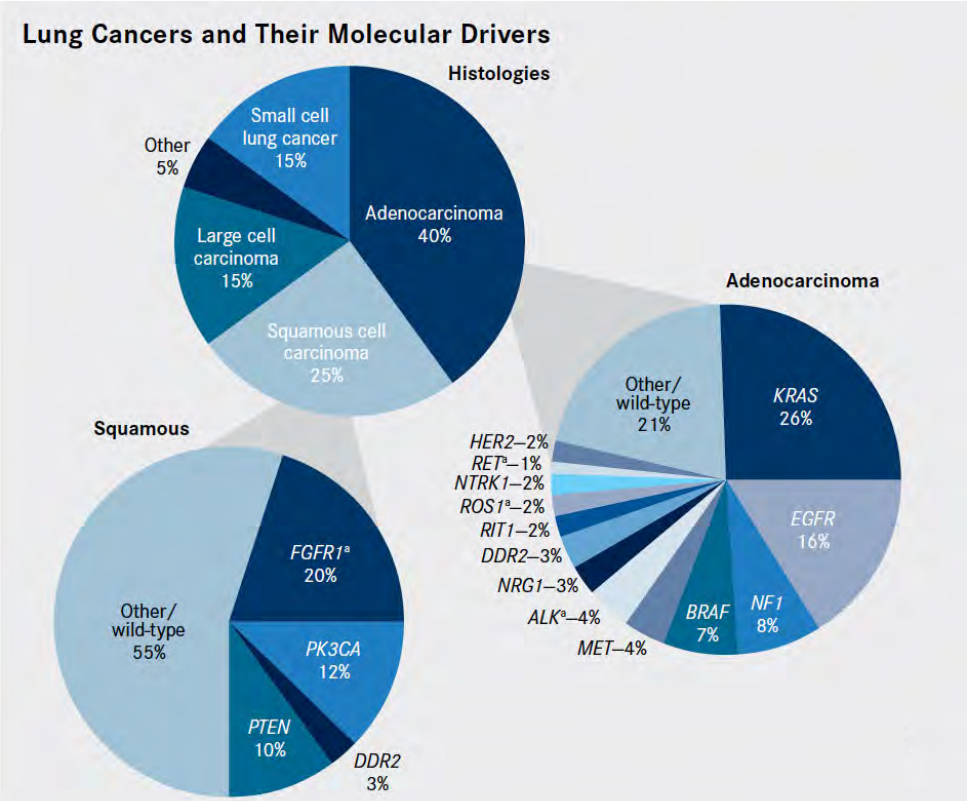
Next Generation Sequencing Enables Precision Medicine

Next Generation Sequencing Enables Precision Medicine



Simon R et al. Nat Rev Drug Discov 2013

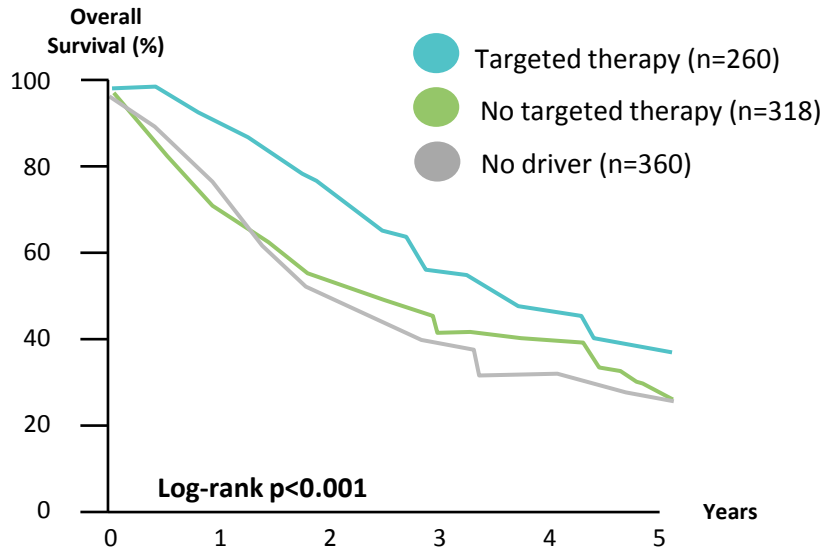
Clinical applications of genomic analysis in cancer



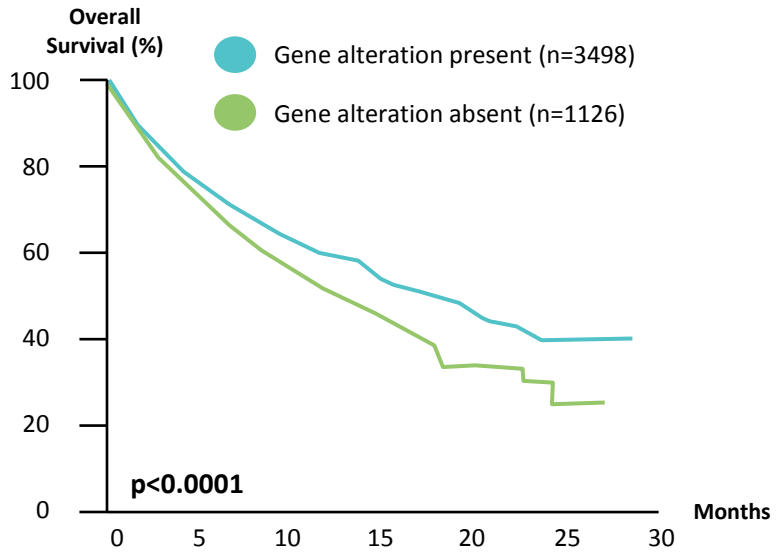
Rosell, R et al. Lancet 2016

Molecular profiling has been shown to improve outcomes in lung cancer patients

Metastatic lung adenocarcinoma patients



Advanced NSCLC patients

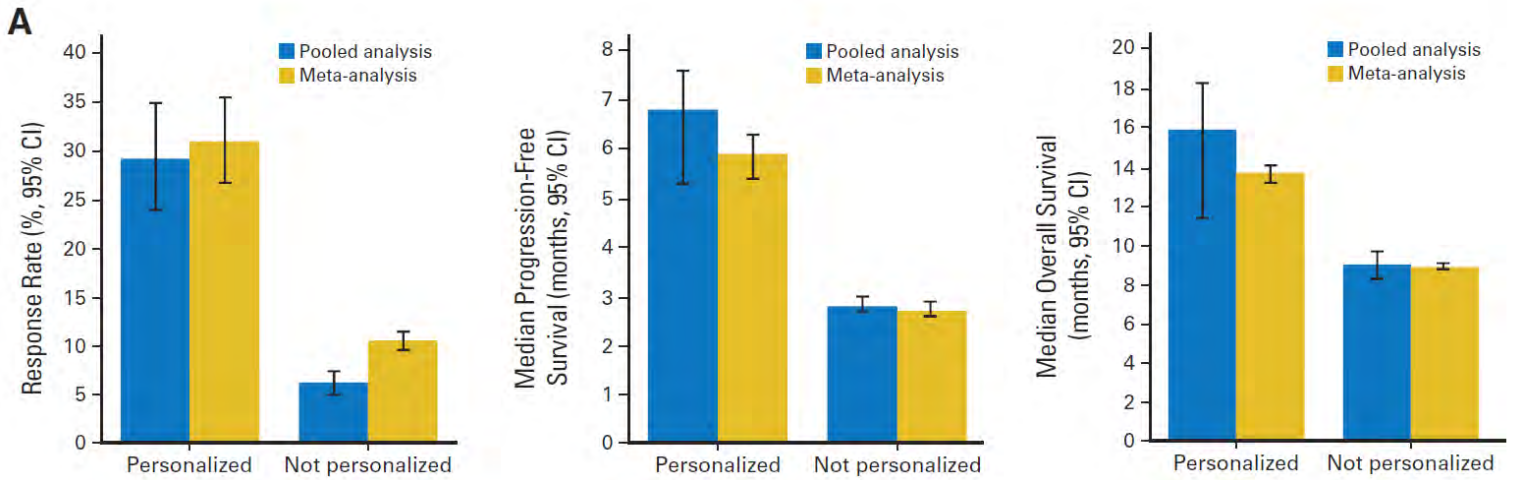


Biomarkers associated with targeted therapies in guidelines: HER2, EGFR, ALK, BRAF, MET
Biomarkers not associated with targeted therapies in guidelines: PIK3CA, MEK, AKT1, KRAS, NRAS

Kris MG et al. (2014) JAMA 311(19):1998-2006
Barlesi F et al. (2016) Lancet S0140-6736(16)

Clinical applications of genomic analysis in cancer

Impact of Precision Medicine in profile-driven clinical research in diverse cancer types



Analysis of phase II, single-agent arms (n=32,149 patients) revealed that, across malignancies,

- **A personalized strategy** was an independent predictor of **better outcomes** and **fewer toxic deaths**
- Non-personalized targeted therapies were associated with poorer outcomes than cytotoxic agents

Schwaederle, M et al. J Clin Oncol 33 2015

NCI-Molecular Analysis for Therapy Choice trial

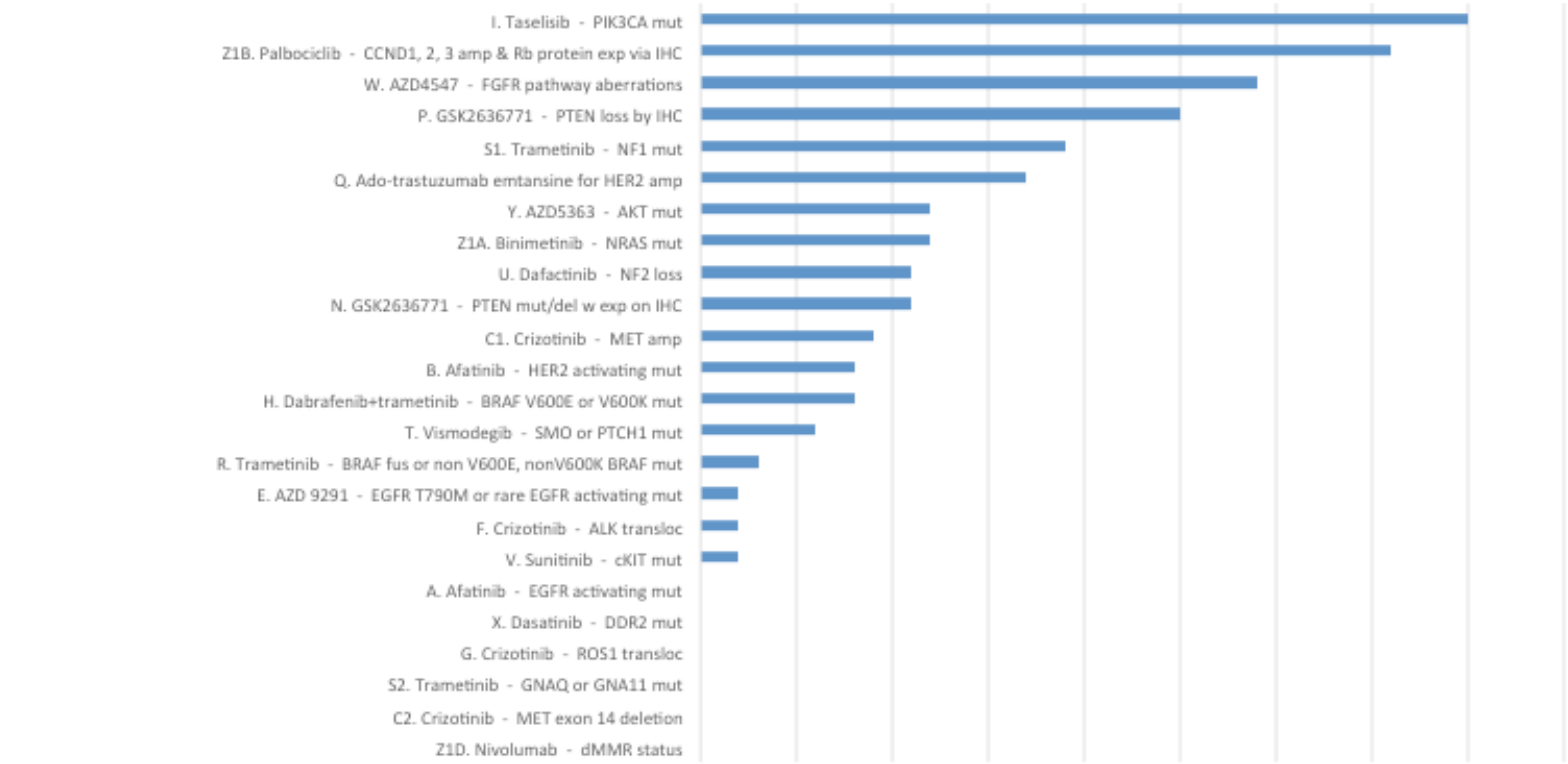
www.cancer.gov/nci-match

ABOUT 6,000 CANCER PATIENTS WILL BE SCREENED WITH A TUMOR BIOPSY

GENE SEQUENCING WILL LOOK FOR CHANGES IN 143 GENES

THE BIOPSIED TUMOR TISSUE WILL UNDERGO GENE SEQUENCING

NCI-Molecular Analysis for Therapy Choice trial



NCI-Molecular Analysis for Therapy Choice trial

NCI-MATCH's Customized Assay

Hotspot Genes, N=73

ABL1
AKT1
ALK
AR
ARAF
BRAF
BTK
CBL
CDK4
CHEK2
CSF1R
CTNNB1
DDR2
DNMT3A
EGFR
ERBB2
ERBB3
ERBB4
ESR1
EZH2
FGFR1
FGFR2
FGFR3
FLT3
FOXL2
GATA2
GNA11
GNAQ
GNAS
HNF1A
HRAS
IDH1
IDH2
IFITM1
IFITM3
JAK1
JAK2
JAK3
KDR
KIT
KNSTRN
KRAS
MAGOH
MAP2K1
MAP2K2
MAPK1
MAX
MED12
MET
MLH1
MPL
MTOR
MYD88
NFE2L2
NPM1
NRAS
PAX5
PDGFRA
PIK3CA
PPP2R1A
PTPN11
RAC1
RAF1
RET
RHEB
RHOA
SF3B1
SMO
SPOP
SRC
STAT3
U2AF1
XPO1

Full-Gene Coverage, N=26

APC
ATM
BAP1
BRCA1
BRCA2
CDH1
CDKN2A
FBXW7
GATA3
MSH2
NF1
NF2
NOTCH1
PIK3R1
PTCH1
PTEN
RB1
SMAD4
SMARCB1
STK11
TET2
TP53
TSC1
TSC2
VHL
WT1

Copy Number Variants, N=49

ACVRL1
AKT1
APEX1
AR
ATP11B
BCL2L1
BCL9
BIRC2
BIRC3
CCND1
CCNE1
CD274
CD44
CDK4
CDK6
CSNK2A1
DCUN1D1
EGFR
ERBB2
FGFR1
FGFR2
FGFR3
FGFR4
FLT3
GAS6
IGF1R
IL6
KIT
KRAS
MCL1
MDM2
MDM4
MET
MYC
MYCL
MYCN
MYO18A
NKX2-1
NKX2-8
PDCD1LG2
PDGFRA
PIK3CA
PNP
PPARG
RPS6KB1
SOX2
TERT
TIAF1
ZNF217

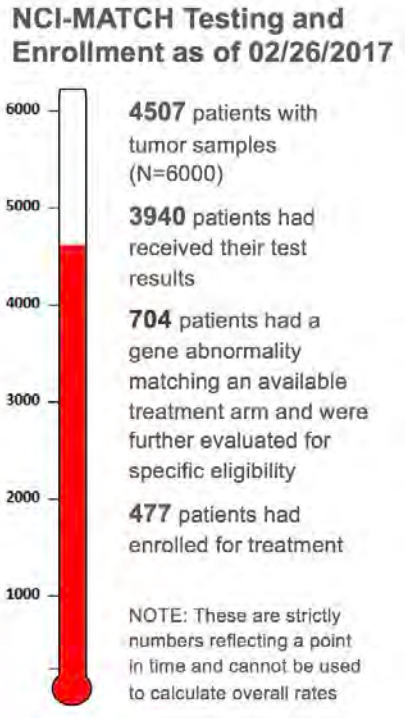
Fusion Drivers, N=22

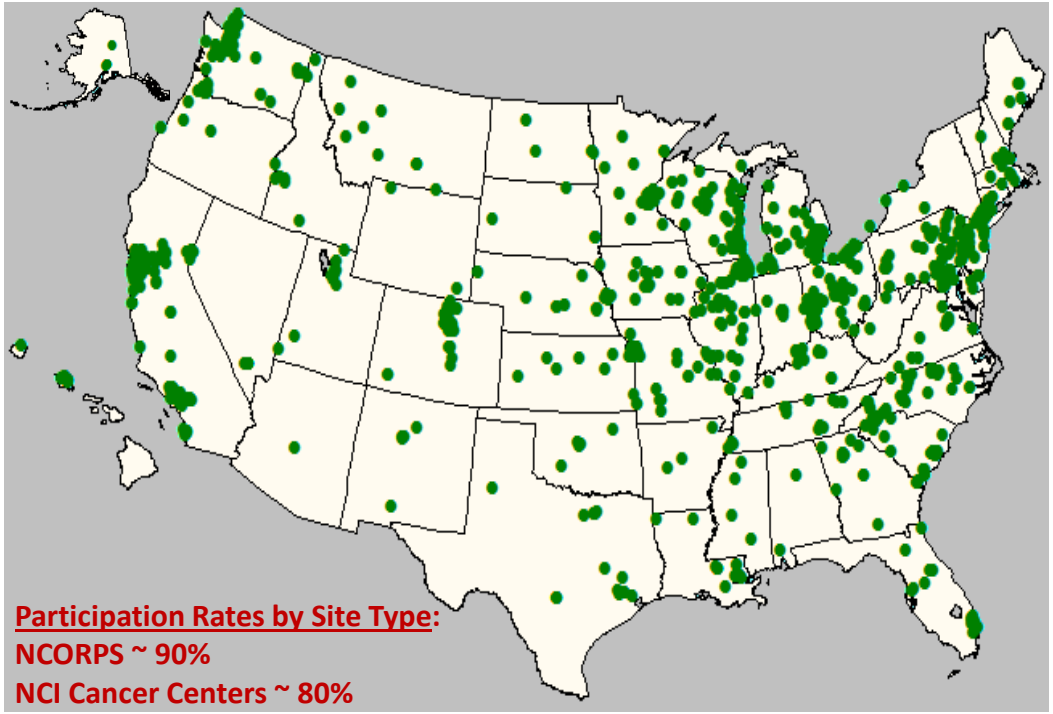
ALK
RET
ROS1
NTRK1
NTRK3
FGFR1
FGFR2
FGFR3
BRAF
RAF1
ERG
ETV1
ETV4
ETV5
ABL1
AKT3
AXL
EGFR
ERBB2
PDGFRA
PPARG

- 143 genes
- 2530 amplicons in DNA panel
- 207 amplicons in RNA panel

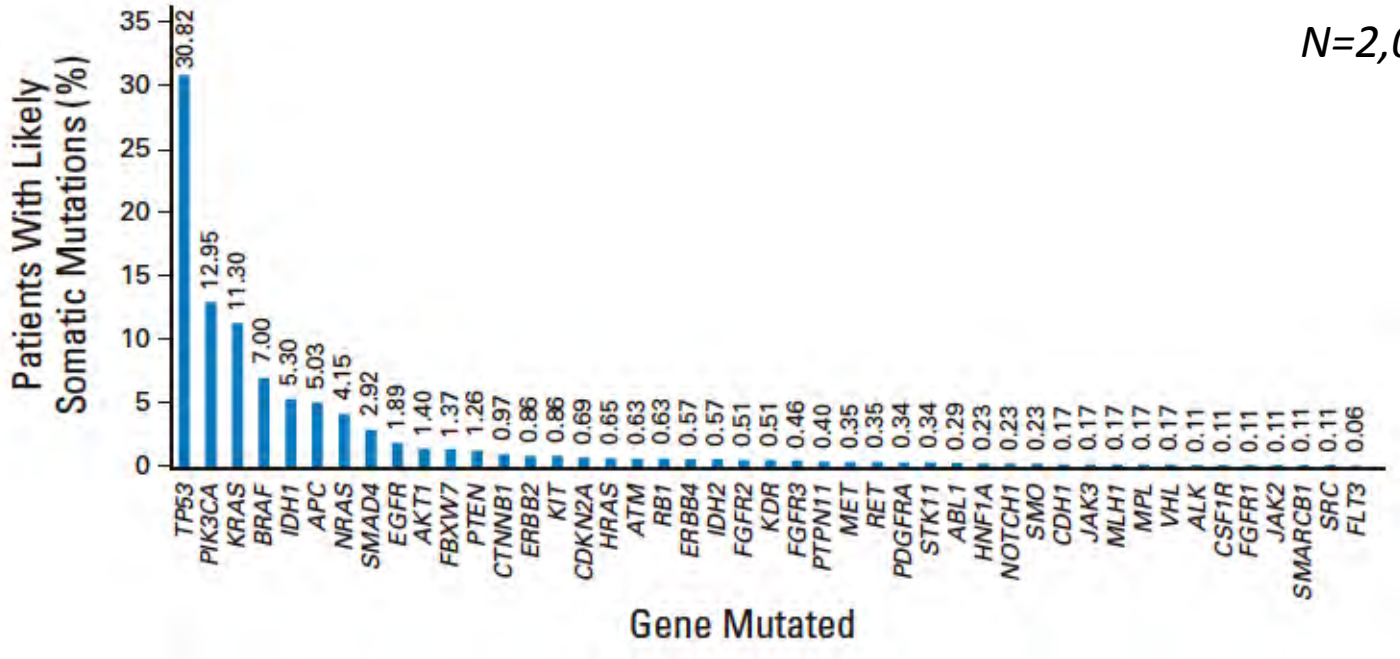
NCI-Molecular Analysis for Therapy Choice trial

NCI-MATCH Distribution of 1089 Sites



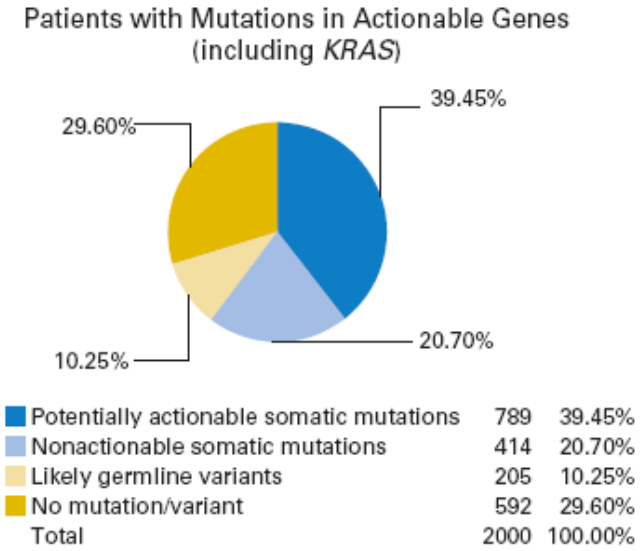


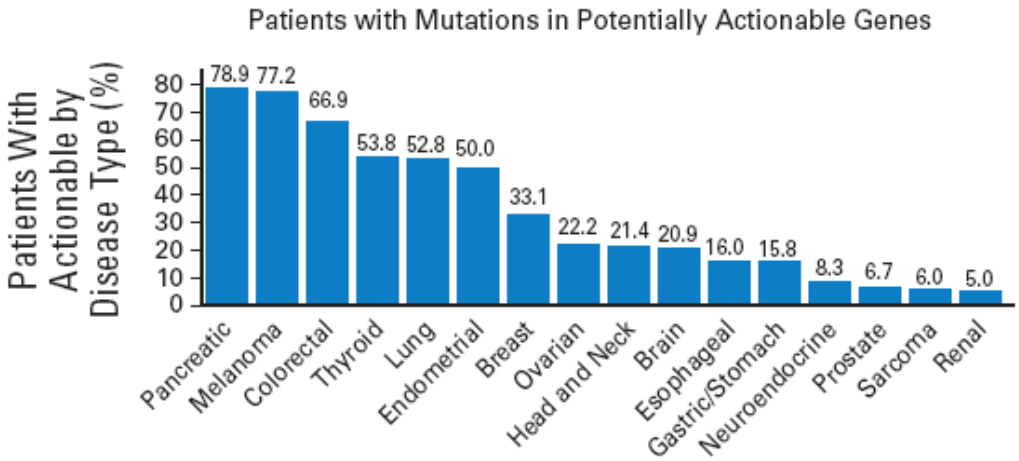
Low prevalence of mutation drivers in diverse tumors



Meric-Bernstam, F et al. J Clin Oncol 2015

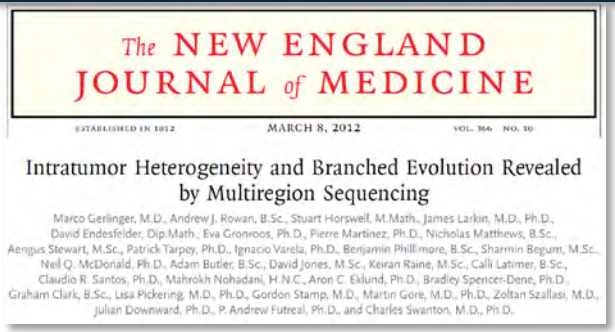
Low prevalence of mutation drivers in diverse tumors





Meric-Bernstam, F et al. J Clin Oncol 2015

Tumour spatial and temporal molecular heterogeneity

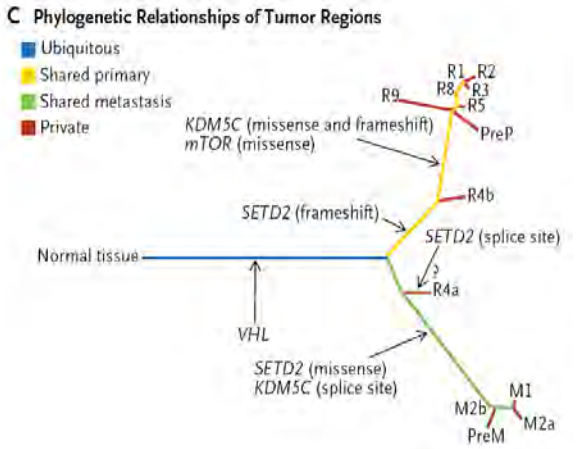


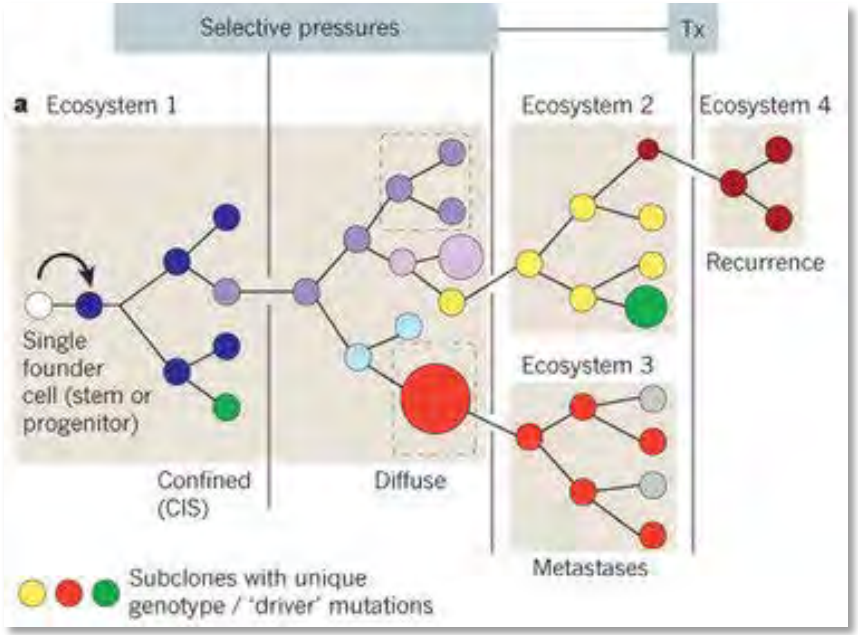
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 8, 2012 VOL. 366 NO. 10

Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing

Marco Gerlinger, M.D., Andrew J. Rowan, B.Sc., Stuart Horswell, M.Math., James Larkin, M.D., Ph.D., David Endesfelder, Dip.Math., Eva Gronroos, Ph.D., Pierre Martinez, Ph.D., Nicholas Matthews, B.Sc., Aengus Stewart, M.Sc., Patrick Tarpey, Ph.D., Ignacio Varela, Ph.D., Benjamin Phillimore, B.Sc., Sharmin Begum, M.Sc., Neil Q. McDonald, Ph.D., Adam Butler, B.Sc., David Jones, M.Sc., Keiran Raine, M.Sc., Calli Latimer, B.Sc., Claudio R. Santos, Ph.D., Mahrokh Nohadani, H.N.C., Aron C. Eklund, Ph.D., Bradley Spencer-Dene, Ph.D., Graham Clark, B.Sc., Lisa Pickering, M.D., Ph.D., Gordon Stamp, M.D., Martin Gore, M.D., Ph.D., Zoltan Szallasi, M.D., Julian Downward, Ph.D., P. Andrew Futreal, Ph.D., and Charles Swanton, M.D., Ph.D.

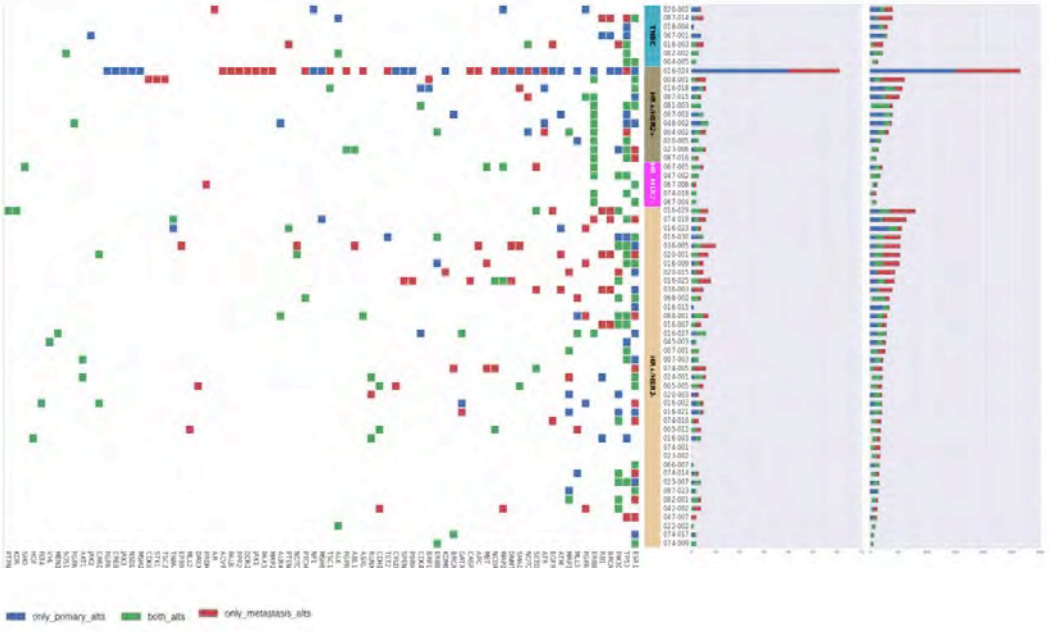




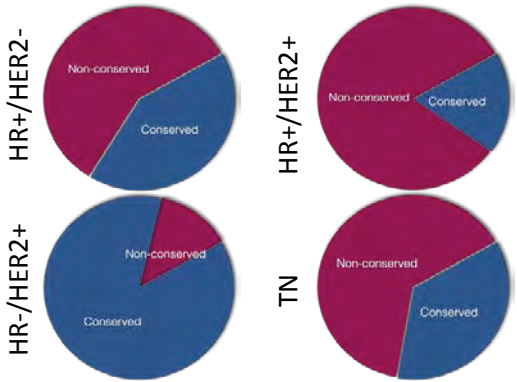
Yates, LR et al. Nat Med 2015

Tumour spatial and temporal molecular heterogeneity

Temporal intratumor molecular heterogeneity in breast cancer GEICAM 2009/03 trial

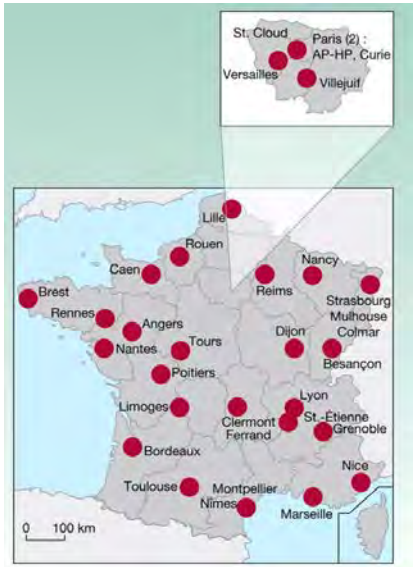


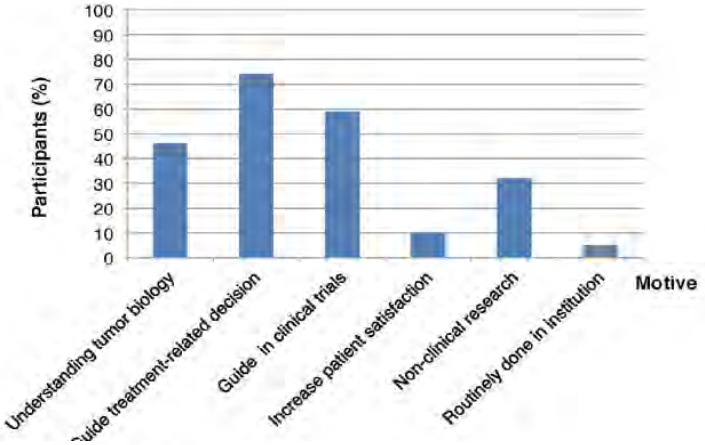
- *1340 mutations in 156 genes and 888 CNA in 171 genes*
- *Non-conserved driver alterations are significantly more frequent in discordant tumors…*
- *and the effect is due to lost primary driver alterations*
- *Lost primary alterations are significantly different distributed along subtypes: more frequent in luminal B-HER2 and less frequent in HER2*

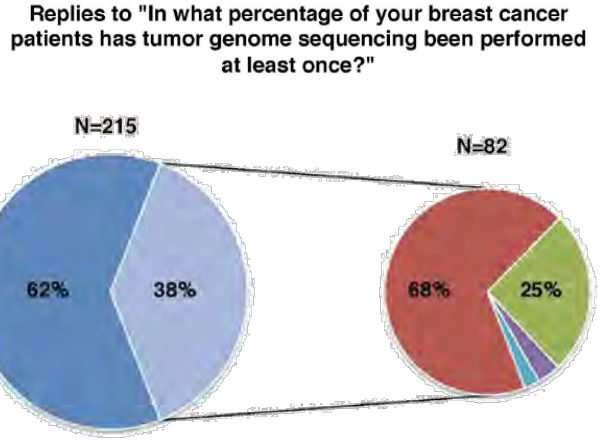


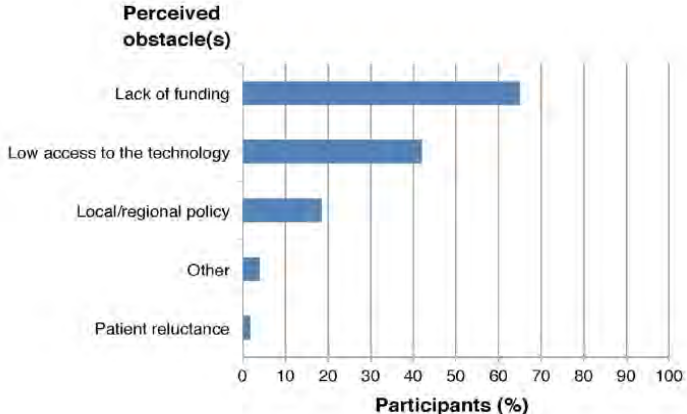
p<0.001

Clinical applications of genomic analysis in cancer









Gingras, I et al. Sci Rep 2016

Local vs Central NGS approach

Illumina MiSeq

Nanostring nCounter

Life Technologies Ion Torrent

Life Technologies Proton

Helicos HeliScope

illumina®

SOPHiA™

iontorrent

Multiplicom
A part of Agilent Technologies

Enabling personalized medicine

TruSight® Tumor 15

Table 1: Gene Content on TruSight Tumor 15

AKT1	GNA11	NRAS
BRAF	GNAQ	PDGFRA
EGFR	KIT	PIK3CA
ERBB2	KRAS	RET
FOXL2	MET	TP53

Agilent Technologies

TruSight® Tumor

Table 1: TruSight Tumor 26 Genes

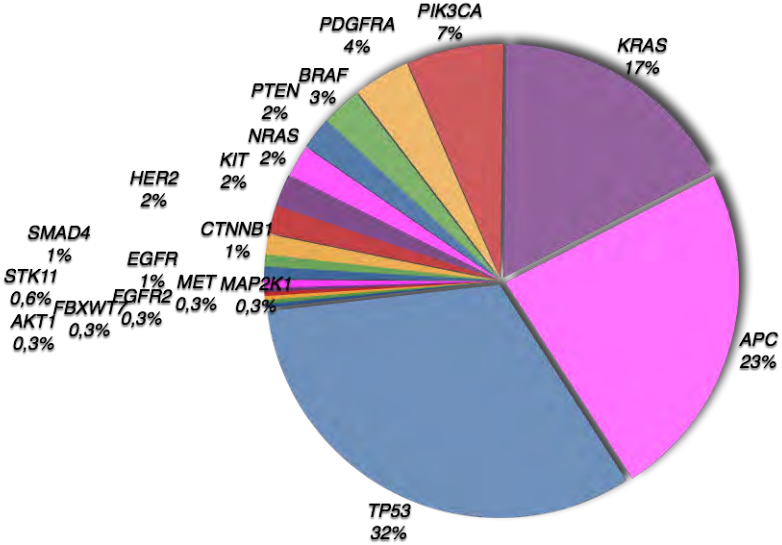
AKT1	EGFR	GNAS	NRAS
ALK	ERBB2	KIT	PDGFRA
APC	FBXW7	KRAS	PIK3CA
BRAF	FGFR2	MAP2K1	PTEN
CDH1	FOXL2	MET	SMAD4
CTNNB1	GNAQ	MSH6	SRC

Hotspot genes		Copy number variants	Fusion drivers
35 genes		19 genes	23 genes
DNA			RNA
AKT1	JAK1	ALK	ABL1
ALK	JAK2	AR	ALK
AR	JAK3	BRAF	AKT3
BRAF	KIT	CCND1	AXL
CDK4	KRAS	CDK4	BRAF
CTNNB1	MAP2K1	CDK6	EGFR
DDR2	MAP2K2	EGFR	ERBB2
EGFR	MET	ERBB2	EPG
ERBB2	MTOR	FGFR1	ETV1
ERBB3	NRAS	FGFR2	ETV4
ERBB4	PDGFRA	FGFR3	ETV5
ESR1	PIK3CA	FGFR4	FGFR1
FGFR2	RAF1	KIT	FGFR2
FGFR3	RET	KRAS	FGFR3
GNA11	ROS1	MET	MET
GNAQ	SMO	MYC	NTRK1
HRAS		MYCN	NTRK2
IDH1		PDGFRA	NTRK3
IDH2		PIK3CA	PDGFRA
			PPARG
			RAF1
			RET
			ROS1

Local vs Central NGS approach

	Caris	OncoDNA	Guardant Health	Foundation Medicine
Lab Certifications	✓	✓	✓	✓
Technology NGS	Agilent custom SureSelect NextSeq Illumina	Ion torrent	Digital Seq, HiSeq Illumina	HiSeq Illumina
Genes	592	475	70	315+28 (fusions)
Sensitivity	98% SNP at 10% of tumor cells, 95% CNV (8 copies)	NA	99% SNP	99% SNP at 5% tumor cells, 95% CNV (8 copies)
Tumor Mutational Burden	✓	✓	✘	✓
Other than NGS	MSI (7 markers), 19 IHC, 6 ISH, 1 methyl, 1 sanger	180 IHC, 20 ISH	No	No
Clinical experience	~80.000	~140	~1.000	~110.000
Case reports	✓	✓	✓	✓
Clinical evidence	✓	✘	✘	✓
Results interpretation	✓	✓	✓	✓
Sample requeriments	55 slides	25 slides	2 Blood tubes	14 slides

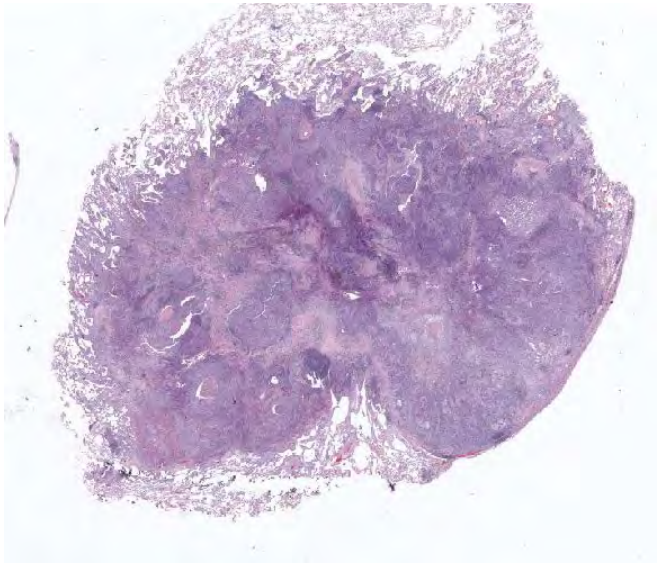
The FJD experience: AmpliSeq



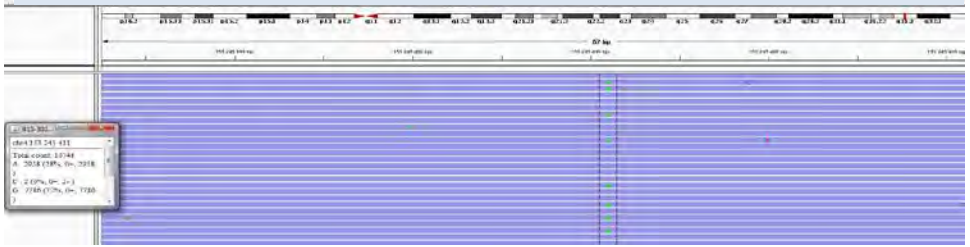
- ✓69/351 (19.6%) non-informative cases
- ✓112/271 (39.8%) no mutation detected
- ✓APC (23%), TP53 (32%) and KRAS (17%), more prevalent mutations

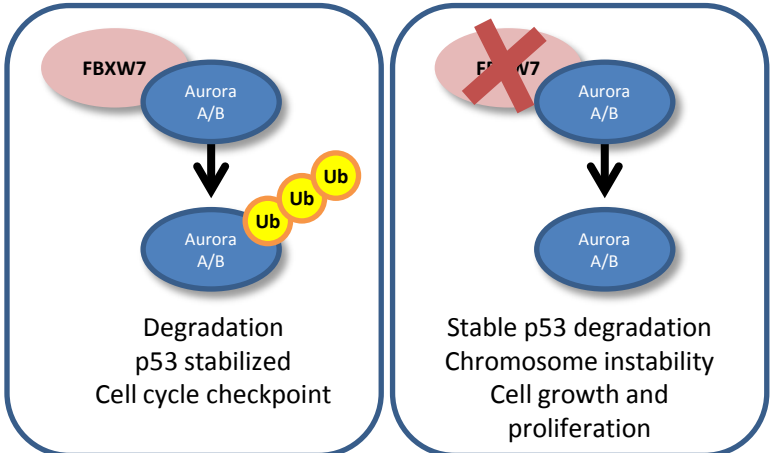
The FJD experience: AmpliSeq

Advanced EGFR, ALK, ROS1 wt lung adenocarcinoma, in non-smoking 47y female



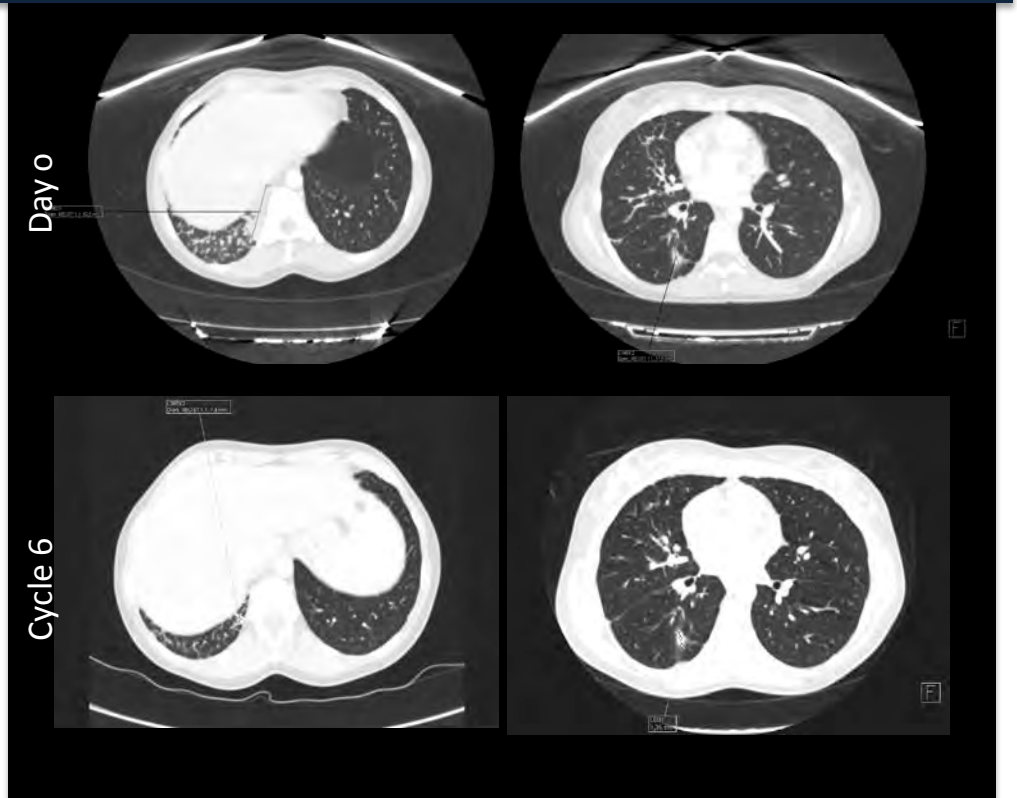
FBXW7 L594F (Exon 11) (28%) AA**G**/AA**A**





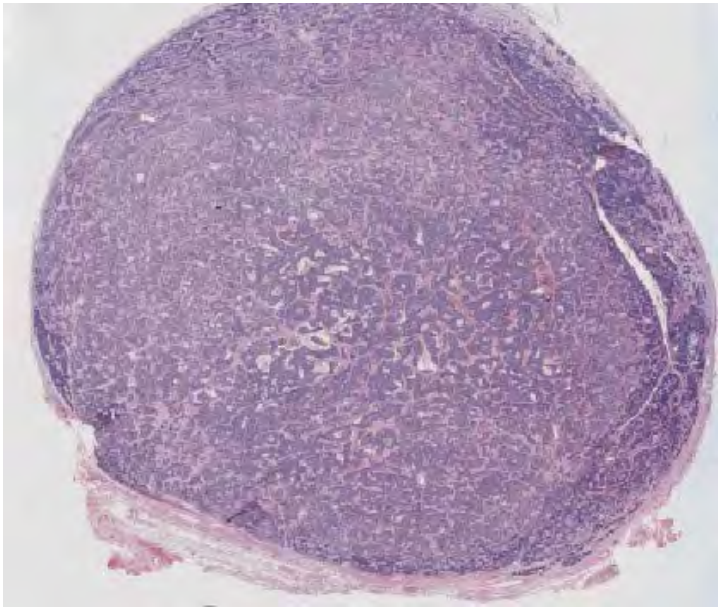
The FJD experience: AmpliSeq

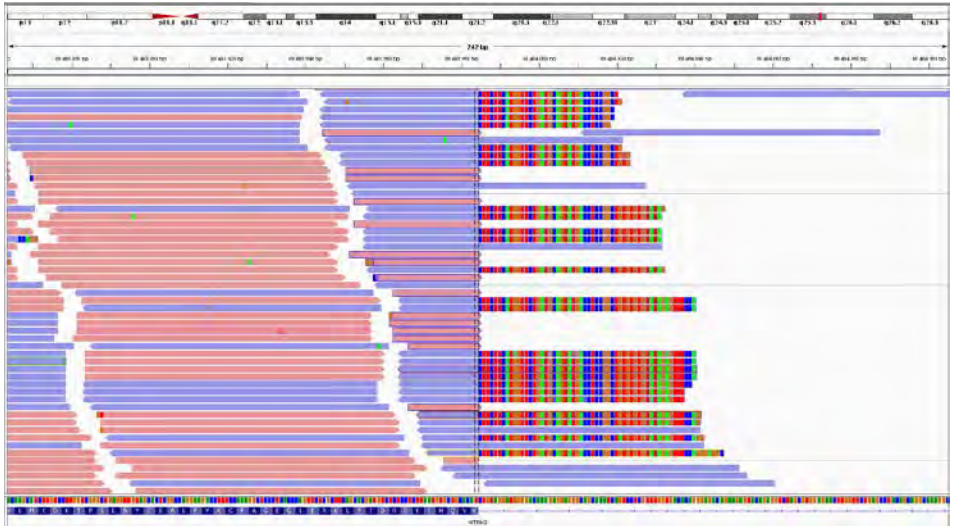
Phase 1, open-label, non-randomized, dose-escalating safety, tolerability, pharmacokinetic, and pharmacodynamic study of TAS-119 in patients with advanced solid tumors



The FJD experience: RNAseq

Supraclavicular lymph node metastasis, triple negative breast carcinoma
51y female



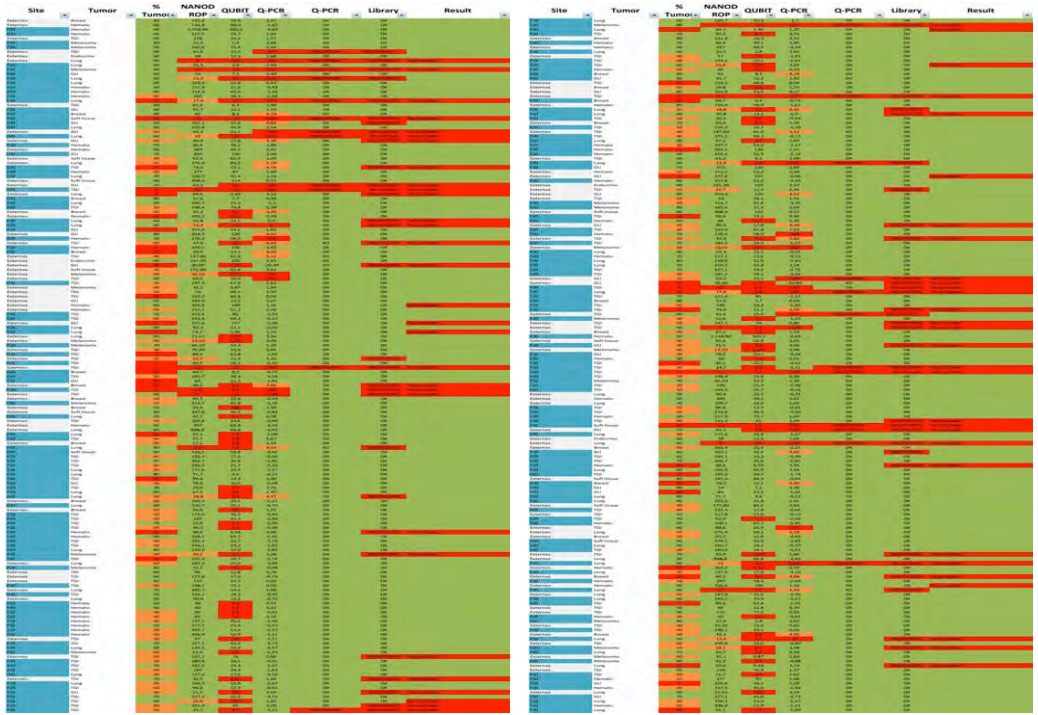


Fusion Calls

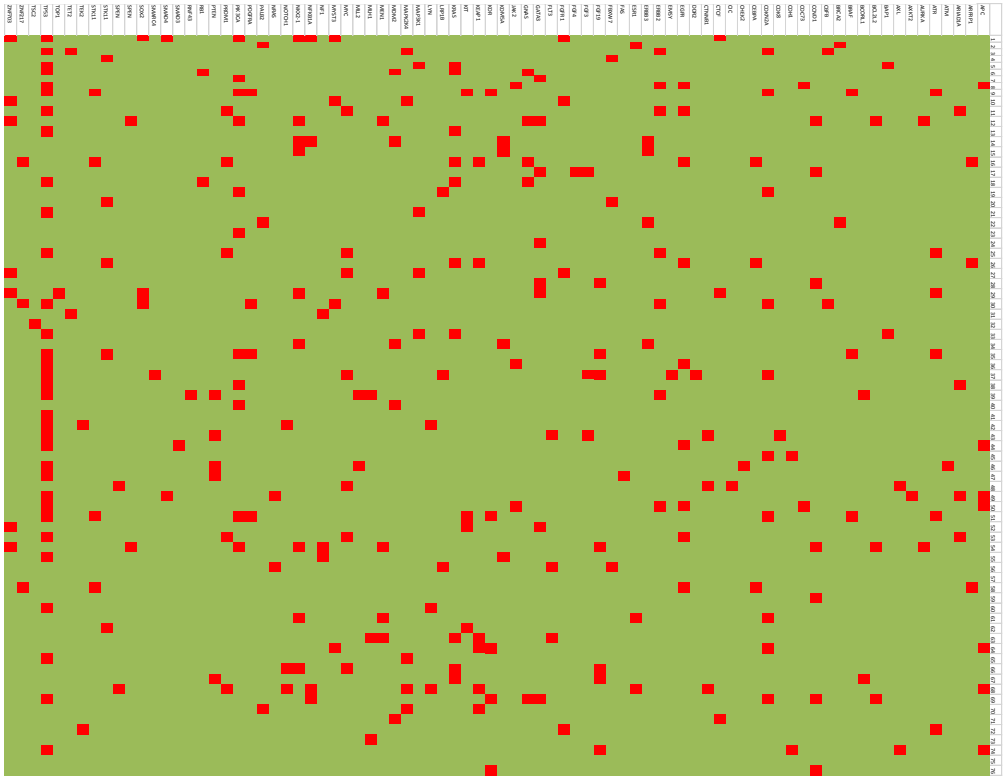
Gene1	Chr1	Pos1	Str1	Gene2	Chr2	Pos2	Str2	Paired Read	Split Read
ETV6	chr12	12,006,495	+	NTRK3	chr15	88,483,984	-	86	560

The FJD experience: pre-analytical sample conditions

		Proportion of non informatives
Site		
	FJD	16,0
	Outside	25,9
Tumor		
	<30% tumor	54,2
	>30% tumor	13,4
Nanodrop		
	<20ng/ul	50,0
	>20ng/ul	11,9
Qubit		
	<5ng/ul	51,5
	>5ng/ul	11,2
QPCR		
	>6	29,4
	4-6	30,8
	<4	11,1



The FJD experience: FoundationOne summary



- ✓ 73 advanced solid tumors
- ✓ All cases presented at least 1 alteration (range 1-10)
- ✓ Mean number of alterations: 4.3 per tumor
- ✓ Most frequent alterations:
  - TP53, 43.4%
  - PIK3CA, 14.5%
  - KRAS, 13.6%
  - CDKN2A, 13,6%
  - NKX2-1, 11.8%
  - GATA3, 10.5%
  - APC, 9.2%
  - CCND1, 9.2%
  - ZNF703, 9.2%
  - FGF19, 9.2%

Reflexiones y mensajes finales

✓ El cáncer es una enfermedad compleja, heterogénea y dinámica desde el punto de vista molecular

✓ El número de alteraciones de interés terapéutico en cáncer y su baja prevalencia obliga a una extensa caracterización molecular

✓La NGS puede ser altamente informativa en mutaciones puntuales, indels y fusiones a partir de escaso material diagnóstico

✓Es una técnica relativamente compleja y de alto coste, que probablemente no podrá ser implementada en la mayoría de los centros