Cancer Bio Course

Session 2: Human observational studies. Sequencing and experimental validation. Breast cancer treatment resistance

August 14th, 2024

Memorial Sloan Kettering Cancer Center

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How do we study cancer?

How do we study cancer?

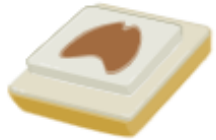
Clinical annotations

Less-invasive technologies

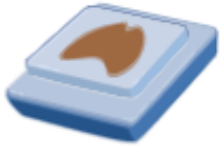
Tumor samples

Blood samples

Other samples



FFPE (Formalin Fixed Paraffin Embedded) Tissue



FF (Fresh Frozen) Tissue

Single-cell Suspensions

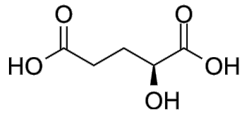
Lysis-Storage Solutions

DNA

Chromatin

RNA

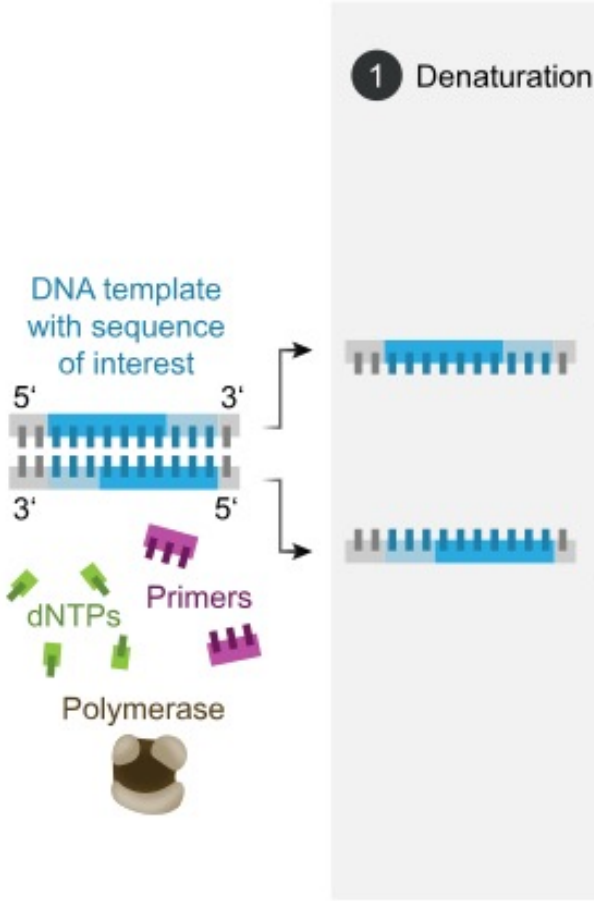
Protein



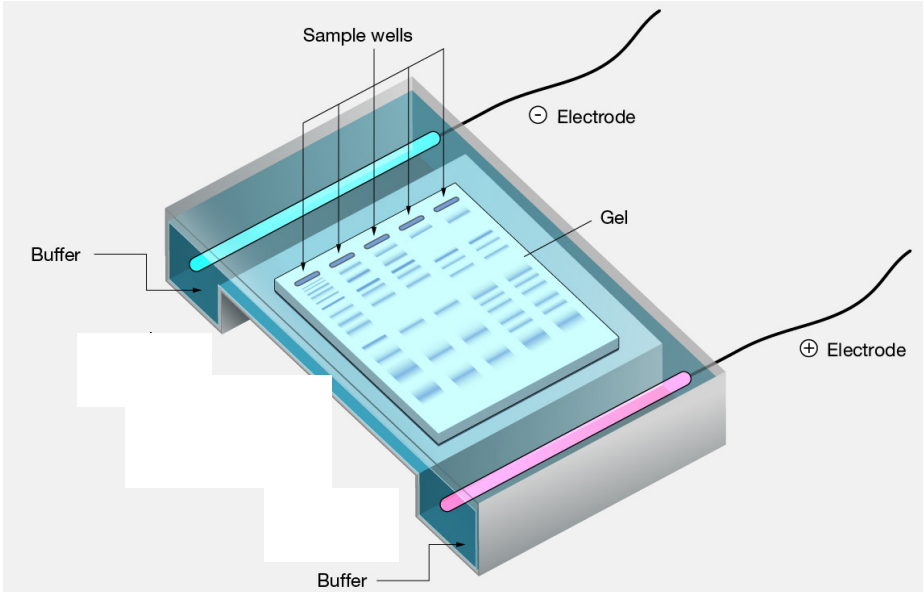
Metabolites

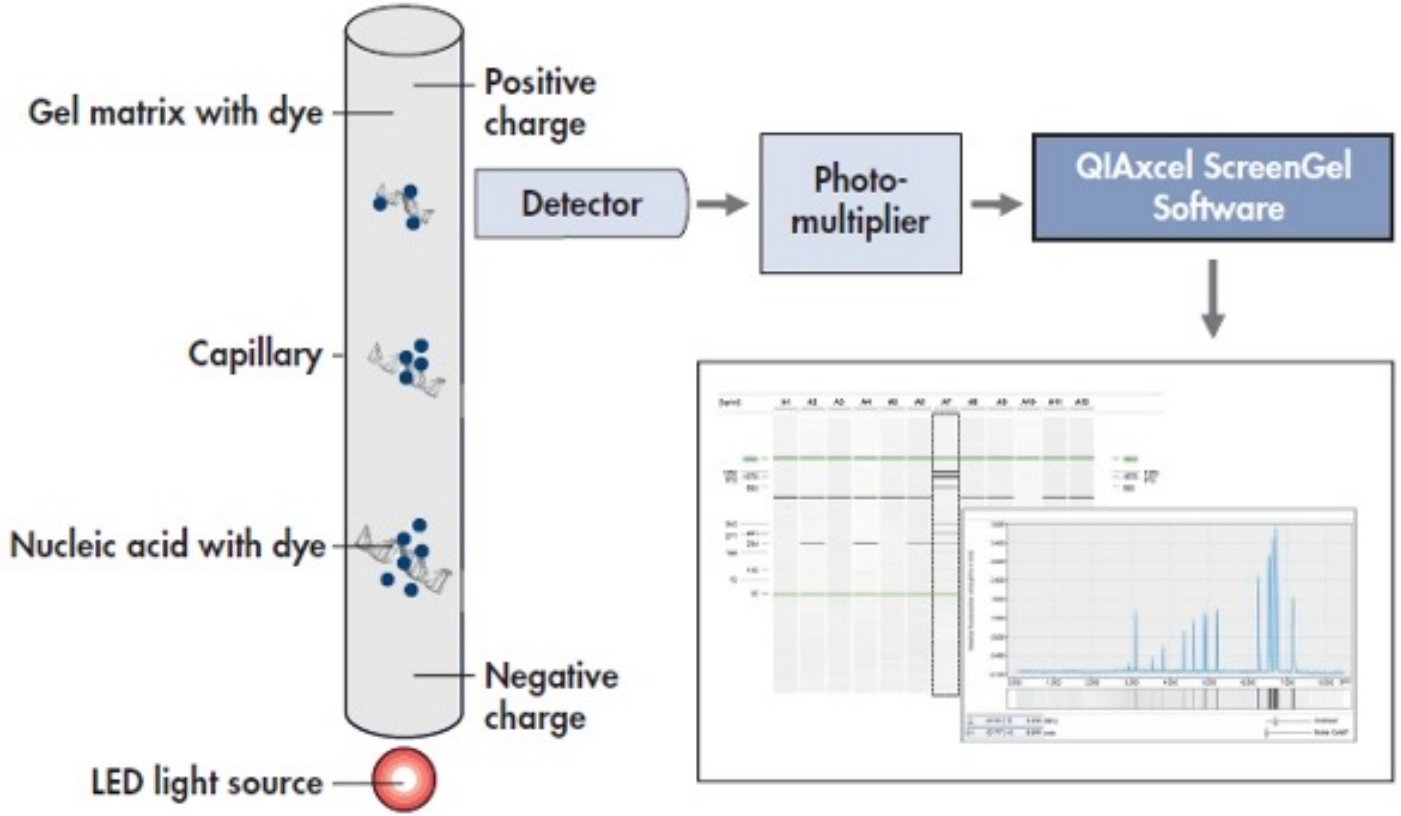
How do we sequence?

PCR: Polymerase Chain Reaction

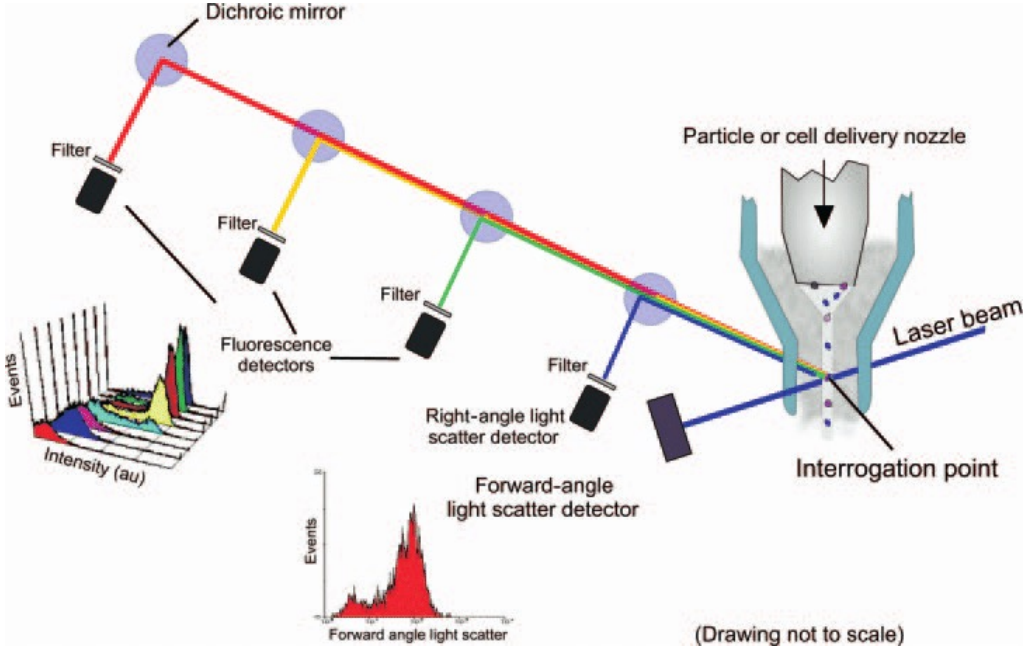


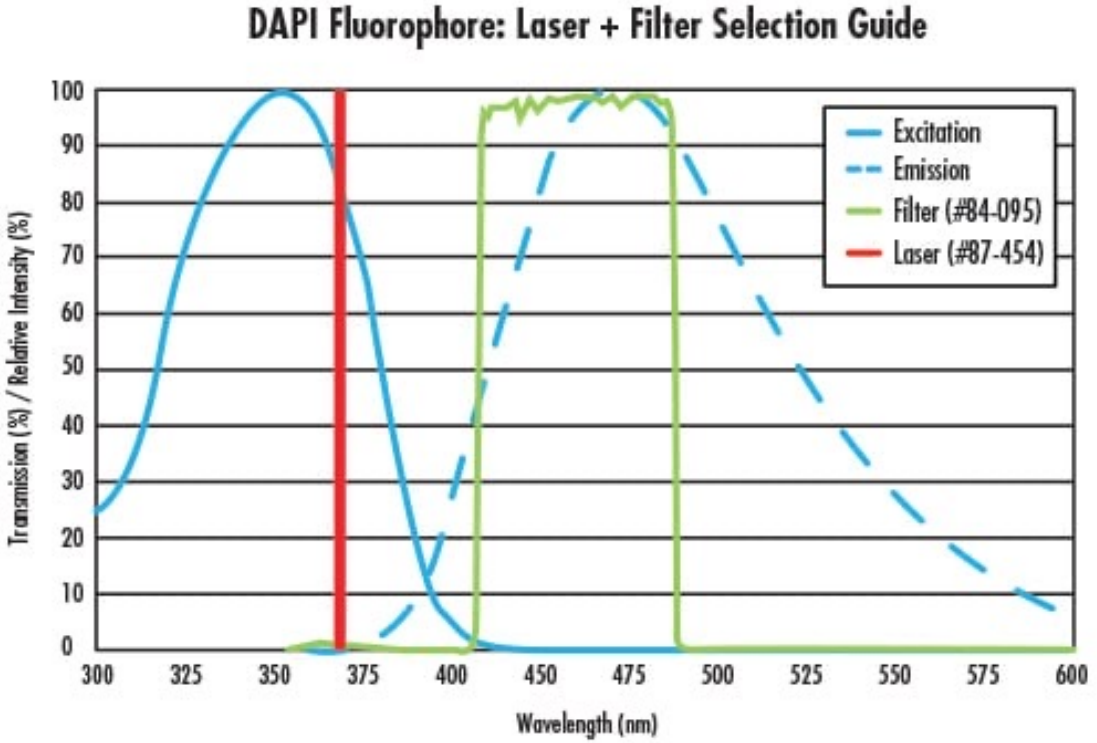
Electrophoresis



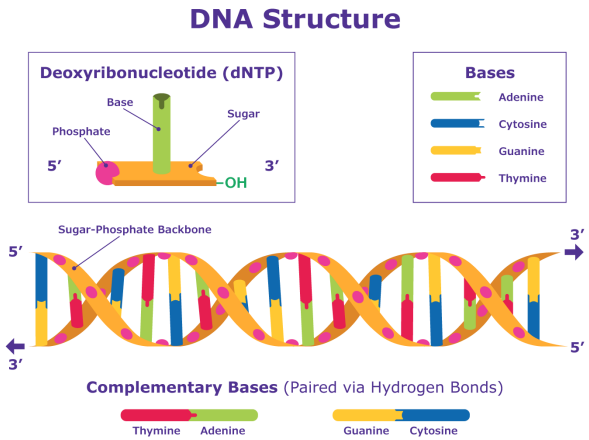


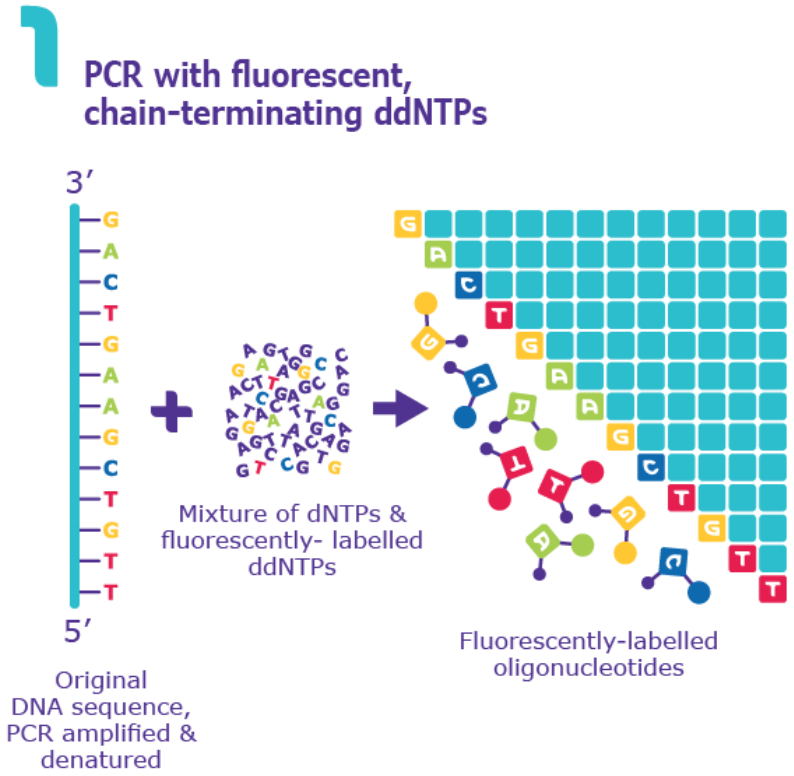
Fluorophores





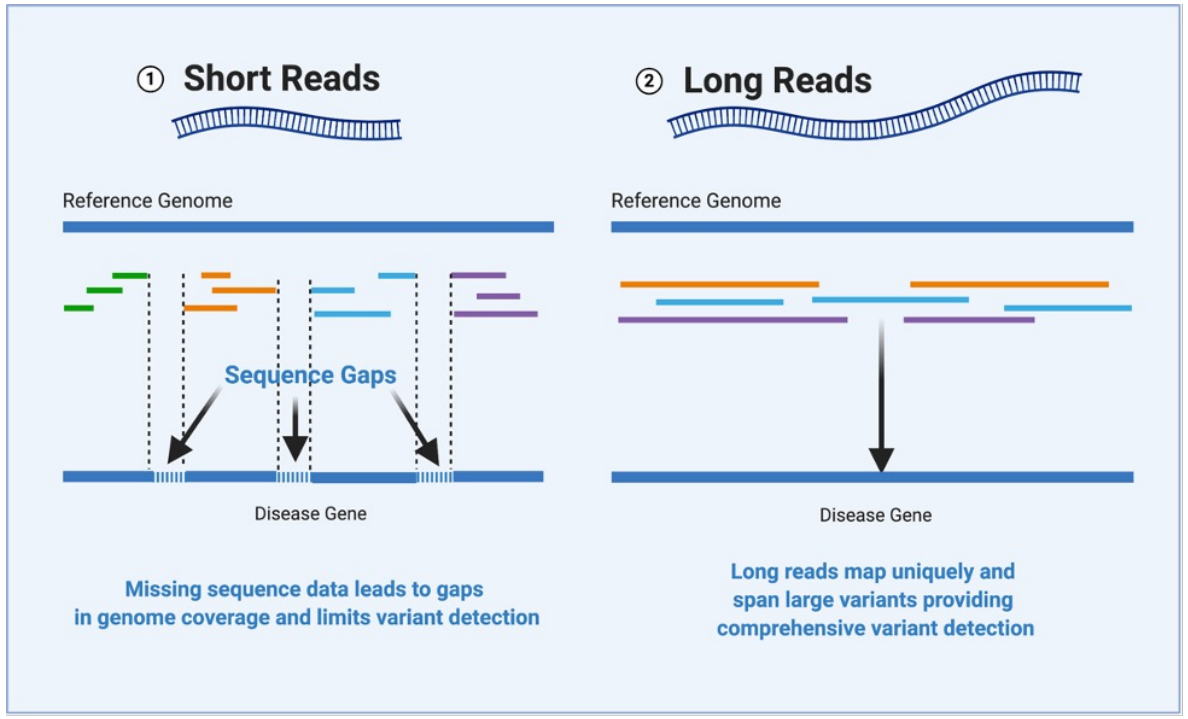
Traditional old school Sanger Sequencing

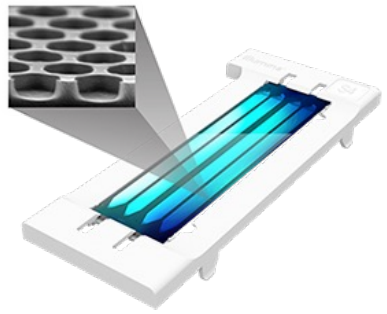


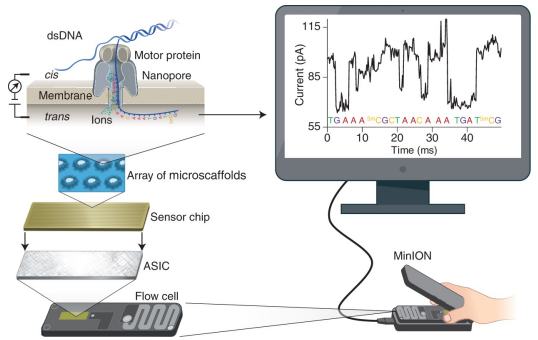


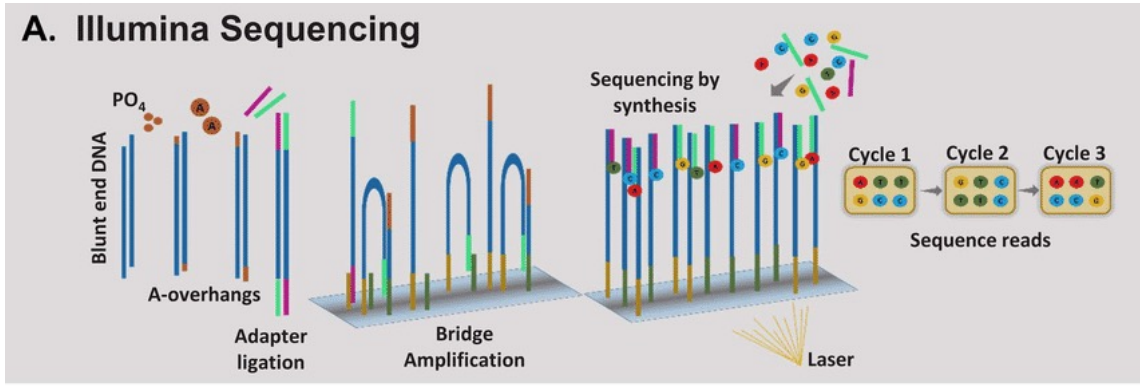
CHAIN TERMINATION PCR

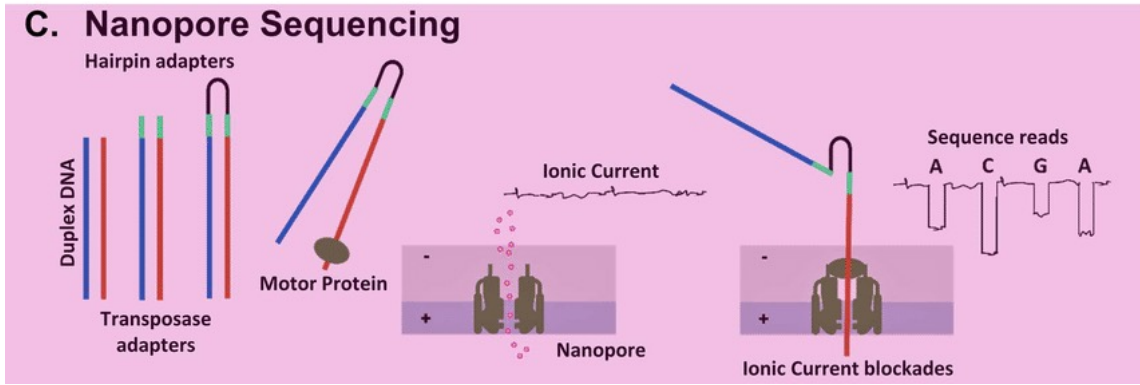
Understanding short read vs long read sequencing



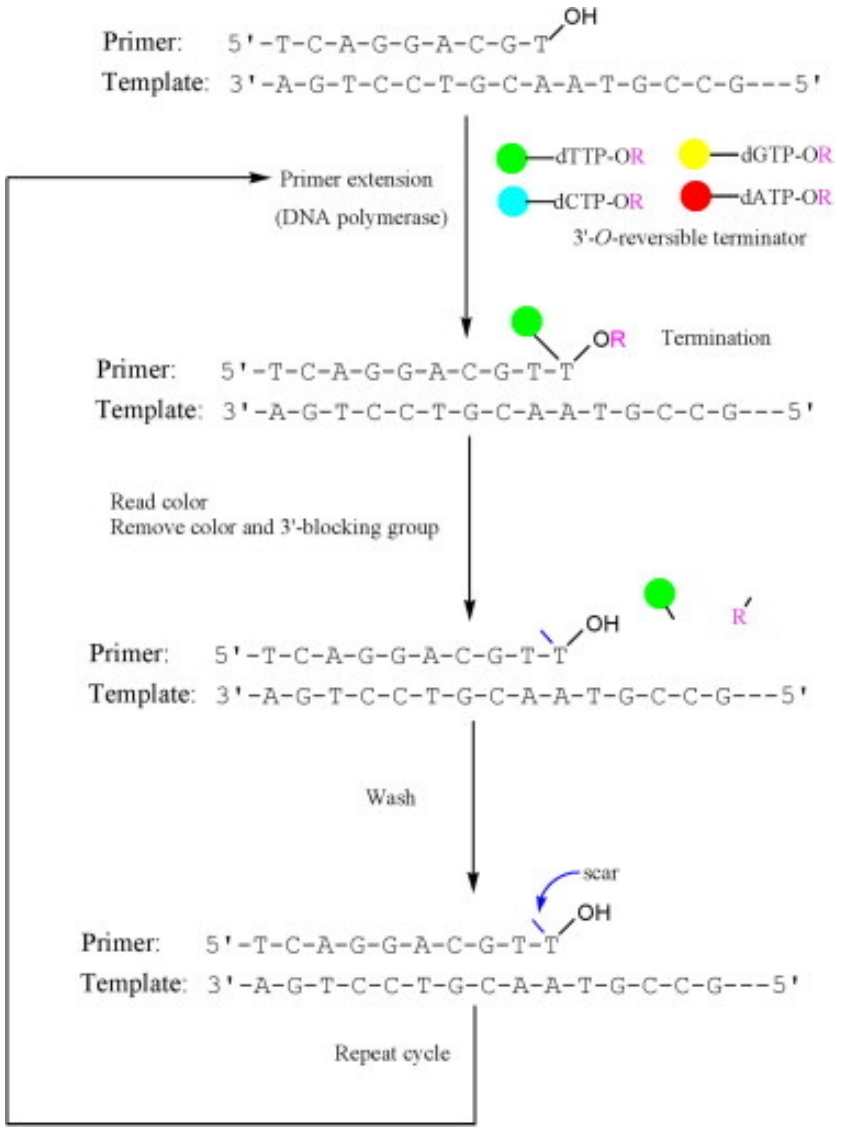


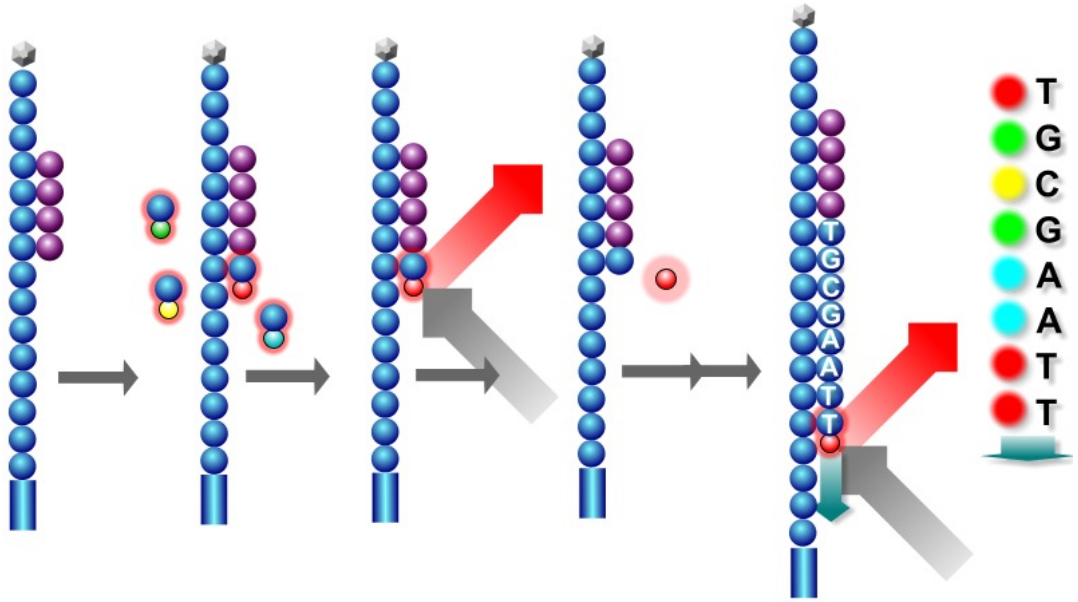




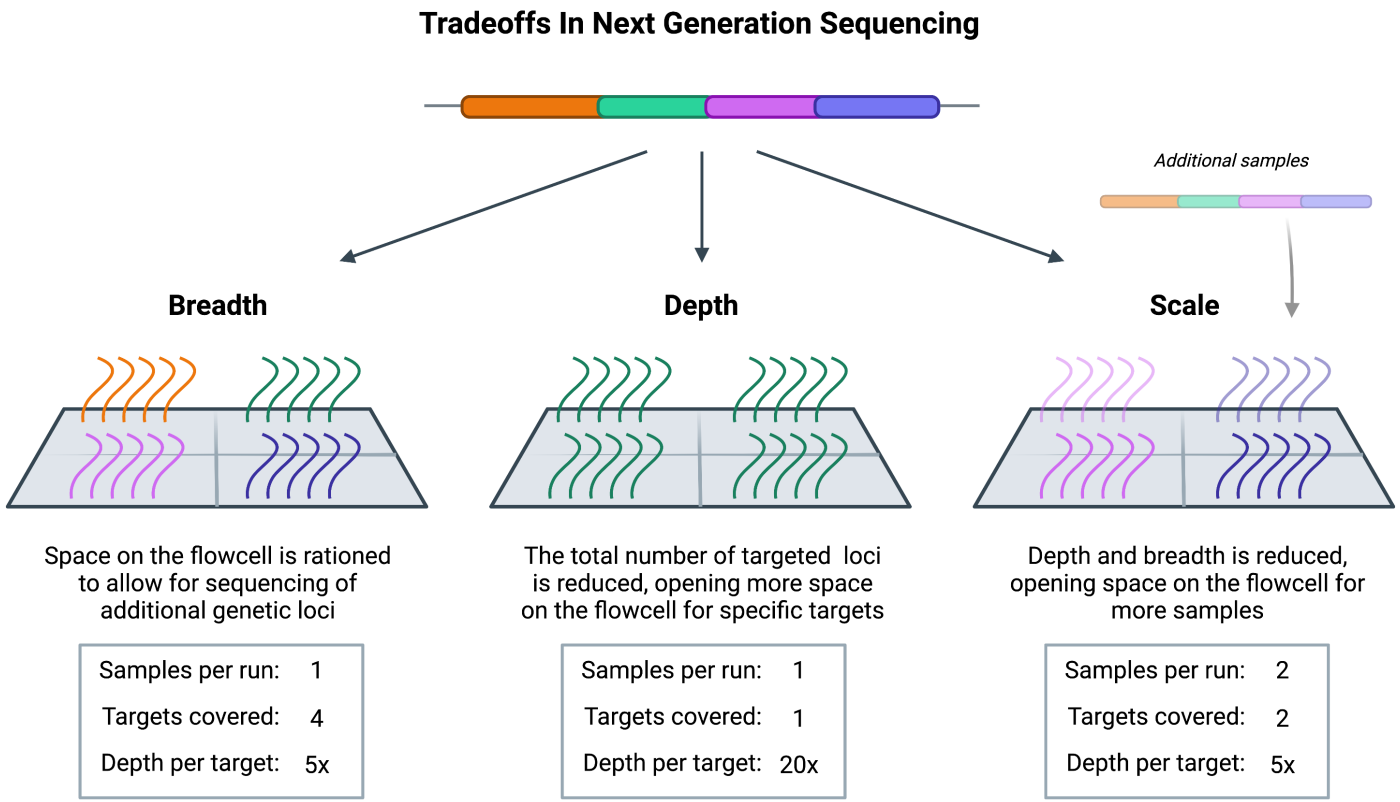


Reversible terminators (Illumina)

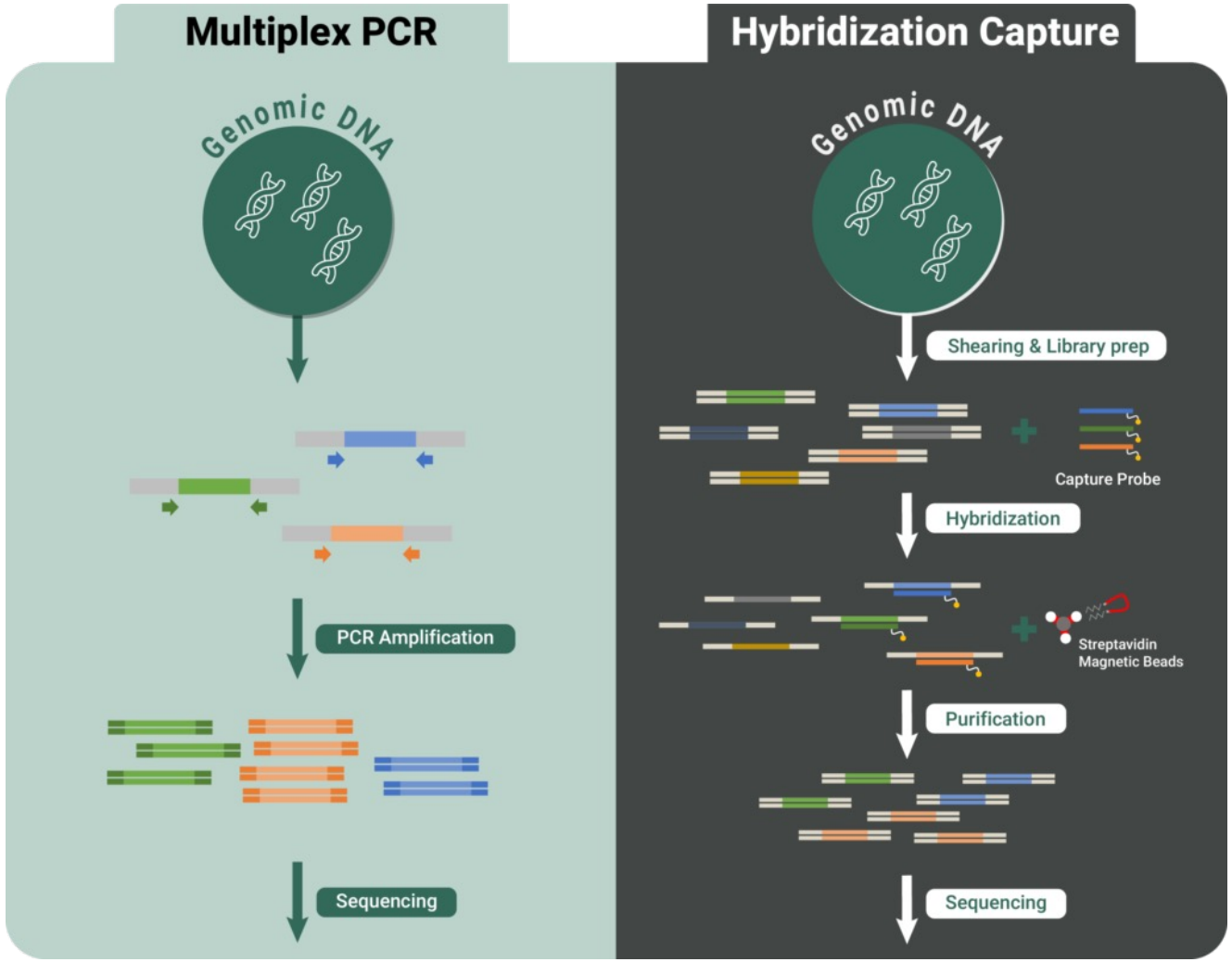




Depth vs Breadth

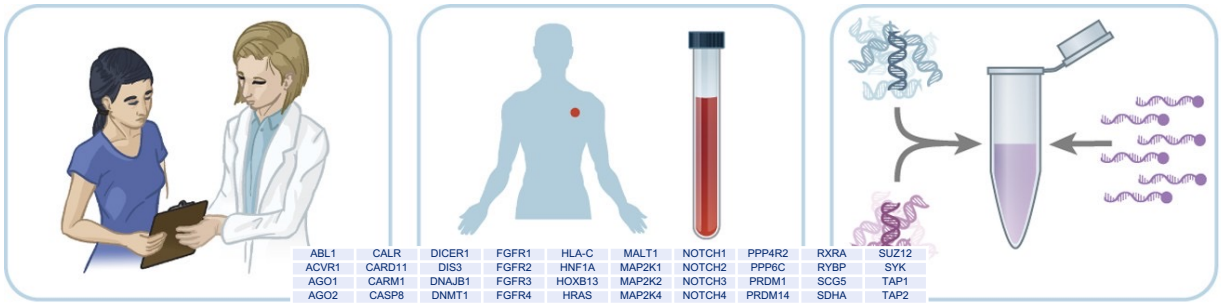


Targeted sequencing



MSK-IMPACT

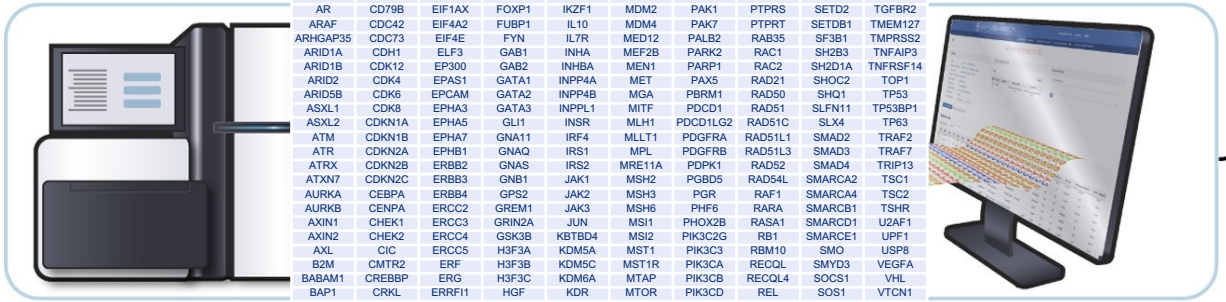
(Integrated Mutation Profiling of Actionable Cancer Targets



1. Patient consen

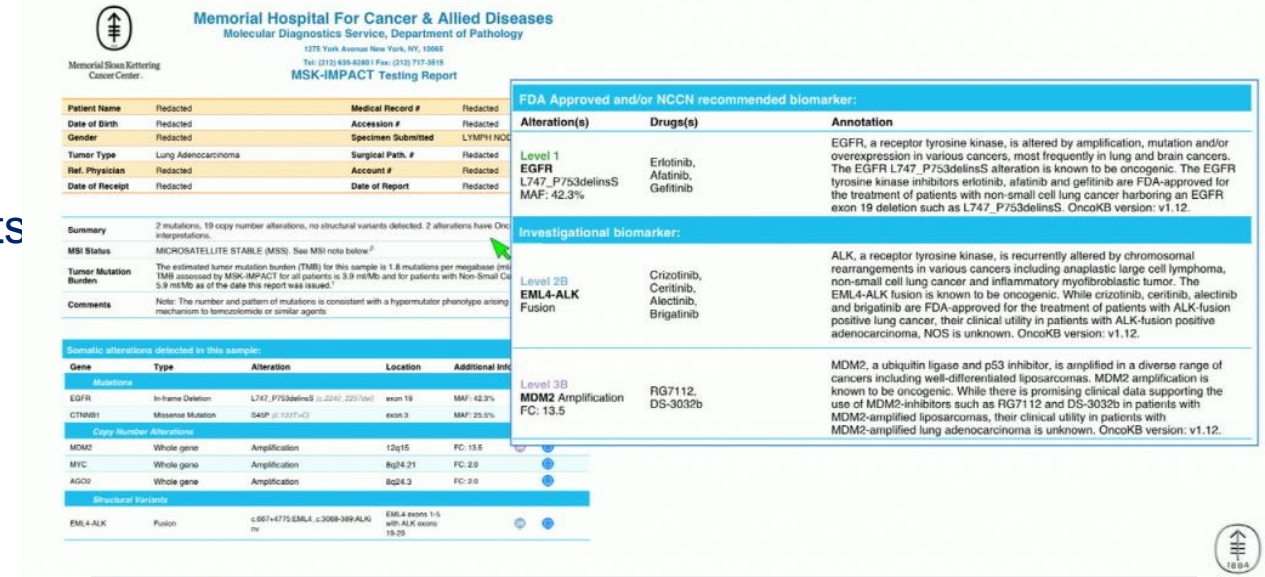
Sample preparation

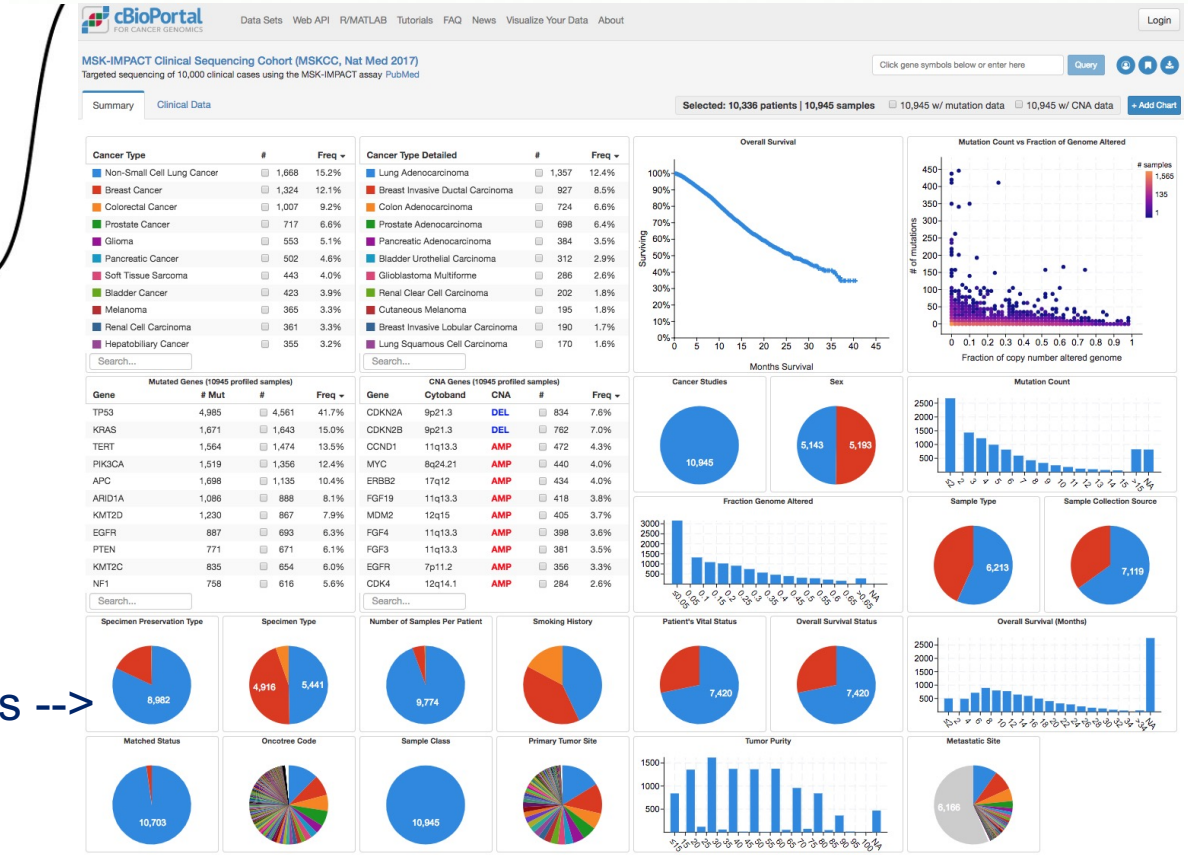
ABL1	CALR	DICER1	FGFR1	HLA-C	MALT1	NOTCH1	PPP4R2	RXRA	SUZ12
ACVR1	CARD11	DIS3	FGFR2	HNF1A	MAP2K1	NOTCH2	PPP6C	RYBP	SYK
AGO1	CARM1	DNAJB1	FGFR3	HOXB13	MAP2K2	NOTCH3	PRDM1	SCG5	TAP1
AGO2	CASP8	DNMT1	FGFR4	HRAS	MAP2K4	NOTCH4	PRDM14	SDHA	TAP2
AKT1	CBFB	DNMT3A	FH	ICOSLG	MAP3K1	NPM1	PREX2	SDHAF2	TBX3
AKT2	CBL	DNMT3B	FLCN	ID3	MAP3K13	NRAS	PRKAR1A	SDHB	TCEB1
AKT3	CCND1	DOT1L	FLT1	IDH1	MAP3K14	NSD1	PRKCI	SDHC	TCF3
ALB	CCND2	DROSHA	FLT3	IDH2	MAPK1	NTHL1	PRKD1	SDHD	TCF7L2
ALK	CCND3	DUSP4	FLT4	IFNGR1	MAPK3	NTRK1	PTCH1	SERPINB3	TEK
ALOX12B	CCNE1	E2F3	FOXA1	IGF1	MAPKAP1	NTRK2	PTEN	SERPINB4	TERT
ANKRD11	CD274	EED	FOXF1	IGF1R	MAX	NTRK3	PTP4A1	SESN1	TET1
APC	CD276	EGFL7	FOXL2	IGF2	MCL1	NUF2	PTPN11	SESN2	TET2
APLNR	CD79A	EGFR	FOXO1	IKBKE	MDC1	NUP93	PTPRD	SESN3	TGFBR1
AR	CD79B	EIF1AX	FOXP1	IKZF1	MDM2	PAK1	PTPRS	SETD2	TGFBR2
ARAF	CDC42	EIF4A2	FUBP1	IL10	MDM4	PAK7	PTPRT	SETDB1	TMEM127
ARHGAP35	CDC73	EIF4E	FYN	IL7R	MED12	PALB2	RAB35	SF3B1	TMPRSS2
ARID1A	CDH1	ELF3	GAB1	INHA	MEF2B	PARK2	RAC1	SH2B3	TNFAIP3
ARID1B	CDK12	EP300	GAB2	INHBA	MEN1	PARP1	RAC2	SH2D1A	TNFRSF14
ARID2	CDK4	EPAS1	GATA1	INPP4A	MET	PAX5	RAD21	SHOC2	TOP1
ARID5B	CDK6	EPCAM	GATA2	INPP4B	MGA	PBRM1	RAD50	SHQ1	TP53
ASXL1	CDK8	EPHA3	GATA3	INPPL1	MITF	PDCD1	RAD51	SLFN11	TP53BP1
ASXL2	CDKN1A	EPHA5	GLI1	INSR	MLH1	PDCD1LG2	RAD51C	SLX4	TP63
ATM	CDKN1B	EPHA7	GNA11	IRF4	MLLT1	PDGFRA	RAD51L1	SMAD2	TRAF2
ATR	CDKN2A	EPHB1	GNAQ	IRS1	MPL	PDGFRB	RAD51L3	SMAD3	TRAF7
ATRX	CDKN2B	ERBB2	GNAS	IRS2	MRE11A	PDPK1	RAD52	SMAD4	TRIP13
ATXN7	CDKN2C	ERBB3	GNB1	JAK1	MSH2	PGBD5	RAD54L	SMARCA2	TSC1
AURKA	CEBPA	ERBB4	GPS2	JAK2	MSH3	PGR	RAF1	SMARCA4	TSC2
AURKB	CENPA	ERCC2	GREM1	JAK3	MSH6	PHF6	RARA	SMARCB1	TSHR
AXIN1	CHEK1	ERCC3	GRIN2A	JUN	MSI1	PHOX2B	RASA1	SMARCD1	U2AF1
AXIN2	CHEK2	ERCC4	GSK3B	KBTBD4	MSI2	PIK3C2G	RB1	SMARCE1	UPF1
AXL	CIC	ERCC5	H3F3A	KDM5A	MST1	PIK3C3	RBM10	SMO	USP8
B2M	CMTR2	ERF	H3F3B	KDM5C	MST1R	PIK3CA	RECQL	SMYD3	VEGFA
BABAM1	CREBBP	ERG	H3F3C	KDM6A	MTAP	PIK3CB	RECQL4	SOCS1	VHL
BAP1	CRKL	ERRFI1	HGF	KDR	MTOR	PIK3CD	REL	SOS1	VTCN1
BARD1	CRLF2	ESR1	HIST1H1C	KEAP1	MUTYH	PIK3CG	REST	SOX17	WHSC1
BBC3	CSDE1	ETAA1	HIST1H2BD	KIT	MYC	PIK3R1	RET	SOX2	WHSC1L1
BCL10	CSF1R	ETV1	HIST1H3A	KLF4	MYCL1	PIK3R2	RFWD2	SOX9	WT1
BCL2	CSF3R	ETV6	HIST1H3B	KLF5	MYCN	PIK3R3	RHEB	SPEN	WWTR1
BCL2L1	CTCF	EZH1	HIST1H3C	KMT2A	MYD88	PIM1	RHOA	SPOP	XIAP
BCL2L11	CTLA4	EZH2	HIST1H3D	KMT2B	MYOD1	PLCG2	RICTOR	SPRED1	XPO1
BCL6	CTNNB1	FAM123B	HIST1H3E	KMT2C	NADK	PLK2	RIT1	SPRTN	XRCC2
BCOR	CTR9	FAM175A	HIST1H3F	KMT2D	NBN	PMAIP1	RNF43	SRC	YAP1
BIRC3	CUL3	FAM46C	HIST1H3G	KMT5A	NCOA3	PMS1	ROS1	SRSF2	YES1
BLM	CXCR4	FAM58A	HIST1H3H	KNSTRN	NCOR1	PMS2	RPS6KA4	STAG2	ZFHX3
BMPR1A	CXORF67	FANCA	HIST1H3I	KRAS	NEGR1	PNRC1	RPS6KB2	STAT3	ZNRF3
BRAF	CYLD	FANCC	HIST1H3J	LATS1	NF1	POLD1	RPTOR	STAT5A	ZRSR2
BRCA1	CYP19A1	FAT1	HIST2H3C	LATS2	NF2	POLE	RRAGC	STAT5B	
BRCA2	CYSLTR2	FBXW7	HIST2H3D	LMO1	NFE2L2	POT1	RRAS	STK11	
BRD4	DAXX	FGF19	HIST3H3	LYN	NFKBIA	PPARG	RRAS2	STK19	
BRIP1	DCUN1D1	FGF3	HLA-A	LZTR1	NKX2-1	PPM1D	RTEL1	STK40	
BTK	DDR2	FGF4	HLA-B	MAD2L2	NKX3-1	PPP2R1A	RUNX1	SUFU	



4. Sequencing

. Case review and sign out





cBioPortal for Cancer Genomics -->

https://www.cbioportal.org

Paper discussion

Cancer Cell
Article

The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers

Pedram Razavi,[1,2,6] Matthew T. Chang,[1,3,6] Guotai Xu,[1] Chaitanya Bandlamudi,[4] Dara S. Ross,[5] Neil Vasan,[1,2] Yanyan Cai,[5] Craig M. Bielski,[4] Mark T.A. Donoghue,[4] Philip Jonsson,[1,3] Alexander Penson,[1,3] Ronglai Shen,[3] Fresia Pareja,[5] Ritika Kundra,[4] Sumit Middha,[5] Michael L. Cheng,[2] Ahmet Zehir,[5] Cyriac Kandoth,[4] Ruchi Patel,[4] Kety Huberman,[4] Lillian M. Smyth,[2] Komal Jhaveri,[2] Shanu Modi,[2] Tiffany A. Traina,[2] Chau Dang,[2] Wen Zhang,[2] Britta Weigelt,[5] Bob T. Li,[2] Marc Ladanyi,[1,5] David M. Hyman,[2] Nikolaus Schultz,[3,4] Mark E. Robson,[2] Clifford Hudis,[2] Edi Brogi,[5] Agnes Viale,[4] Larry Norton,[2] Maura N. Dickler,[2] Michael F. Berger,[4,5] Christine A. Iacobuzio-Donahue,[1,5] Sarat Chandarlapaty,[1,2] Maurizio Scaltriti,[1,5] Jorge S. Reis-Filho,[1,5] David B. Solit,[1,2,4,*] Barry S. Taylor,[1,3,4,*] and José Baselga[1,2,7,*]
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https://doi.org/10.1016/j.ccell.2018.08.008

Paper discussion

- **Explanation of the question under research** - *why did they decide to do this?*
- **Discussion figure by figure –** *is this paper not as good as authors think?:*
  - What is the point of each figure/panel?
  - Are there any missing experimental conditions?
  - Are results interpretable?
  - Do the results support the conclusions by the authors?
  - Would you have done anything differently?
  - Are there any missing experiments?
  - What are the limitations of the work?
  - What experiments could be done as a follow-up to the paper?

Research Question

- **Explanation of the question under research** - *why did they decide to do this?*

“The genomic evolution of breast cancers exposed to systemic therapy and its effects on clinical outcome have not been broadly characterized.”

“In contrast to the abundance of genomic information about primary breast cancer, far less is known about the genomic alterations in metastatic tumors, the ultimate cause of death in most breast cancer patients.”

What is the impact of the study?

“A detailed characterization of the genomic landscape of breast cancer metastasis could provide important insights including identifying:

(1) genomic drivers of metastatic disease progression,

(2) the extent and clinical impact of tumoral heterogeneity,

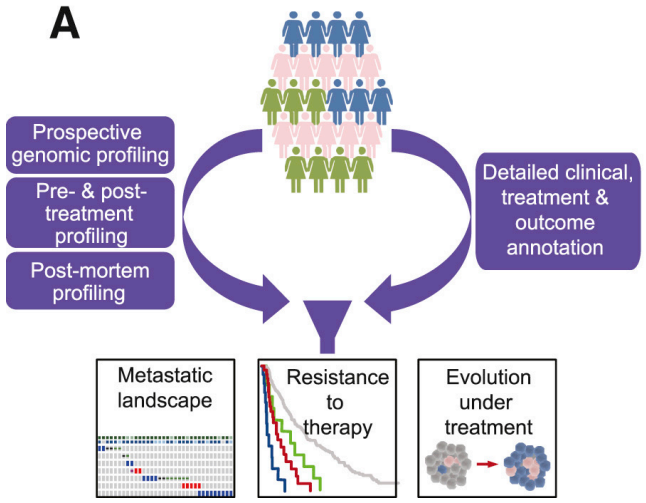
(3) the biologic determinants of variable response of individual patients to different therapies, and

(4) additional potential therapeutic targets.”

Objective and Study design

Objective:

“In this study, we aimed to perform a large clinico-genomic analysis to identify additional genomic alterations that might mediate resistance to hormonal therapy and provide a rationale for the development of therapeutic approaches to overcome resistance.”



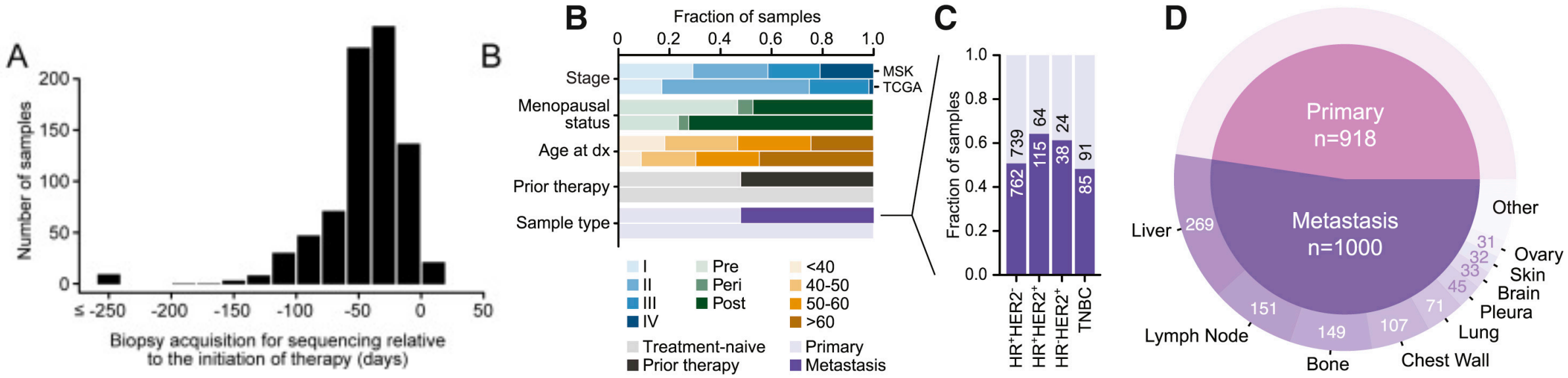
Justification for patient selection:

“While encompassing all breast cancer subtypes, we have focused our efforts on HR+ tumors since they represent the largest subset and because they are frequently treated with defined lines of hormonal therapy, thereby enabling the study of resistance mechanisms.”

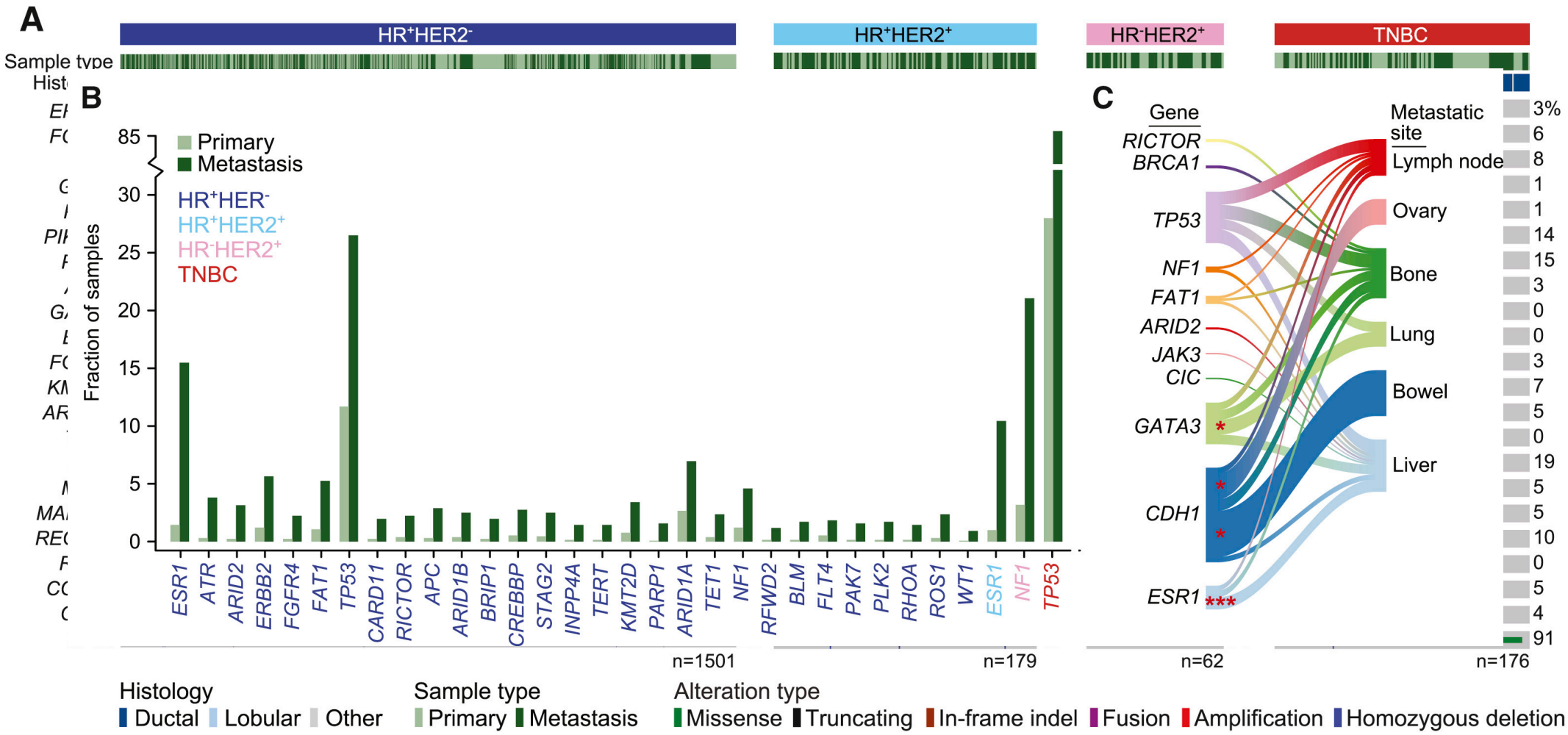
Justification for test selection:

“While not designed for breast cancer gene discovery, sequencing was performed at high depth of coverage (771-fold average coverage) providing greater sensitivity than typical broader-scale sequencing approaches for the detection of subclonal mutational events (those present in only a subset of cancer cells)”

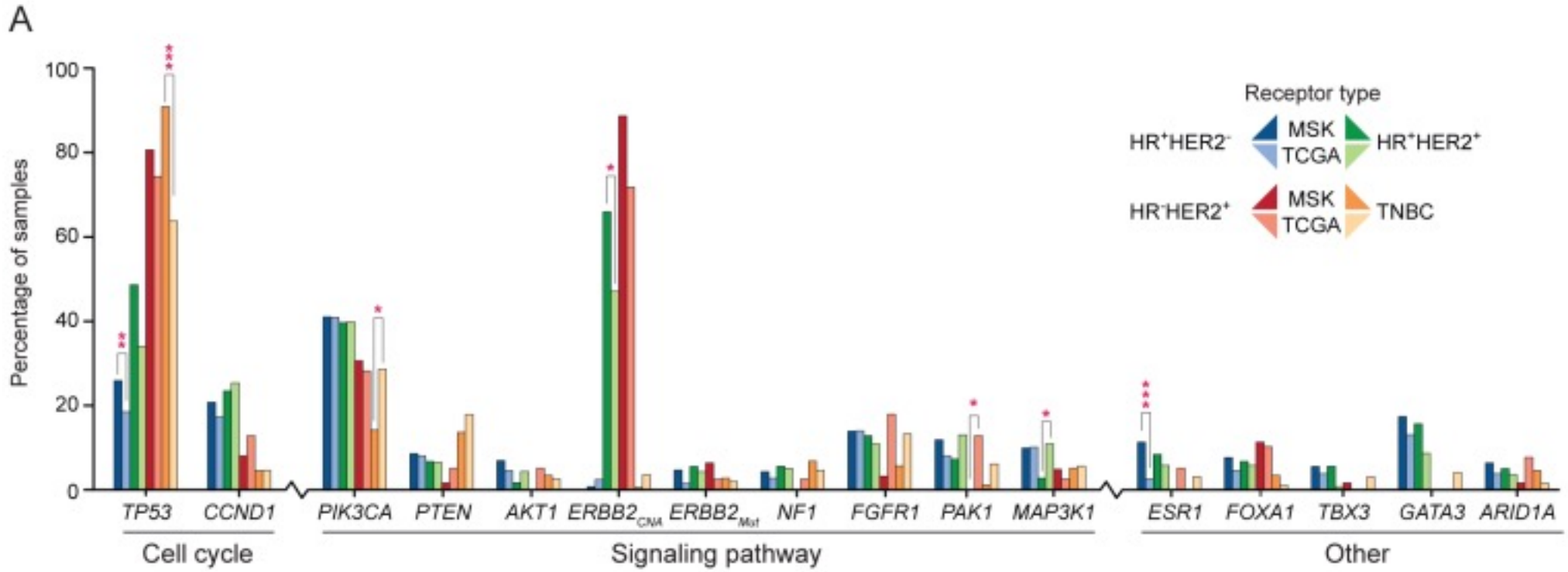
Patient and sample selection



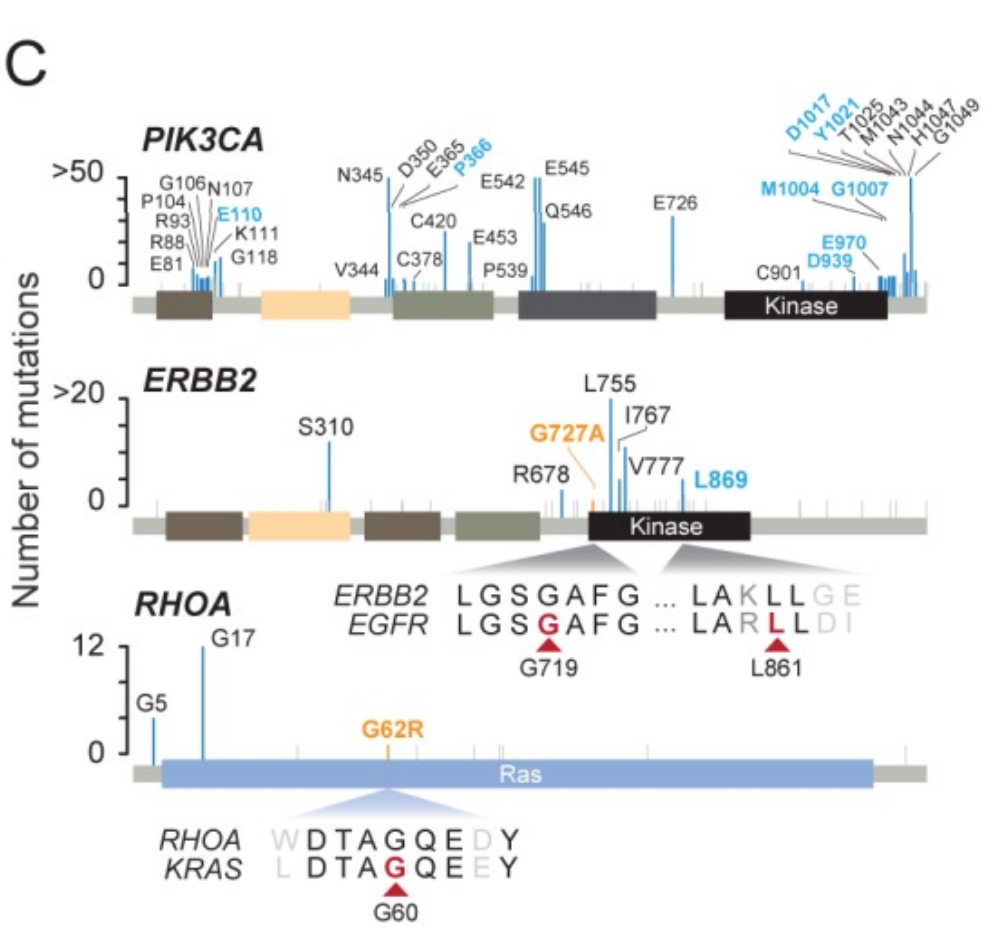
Results#1

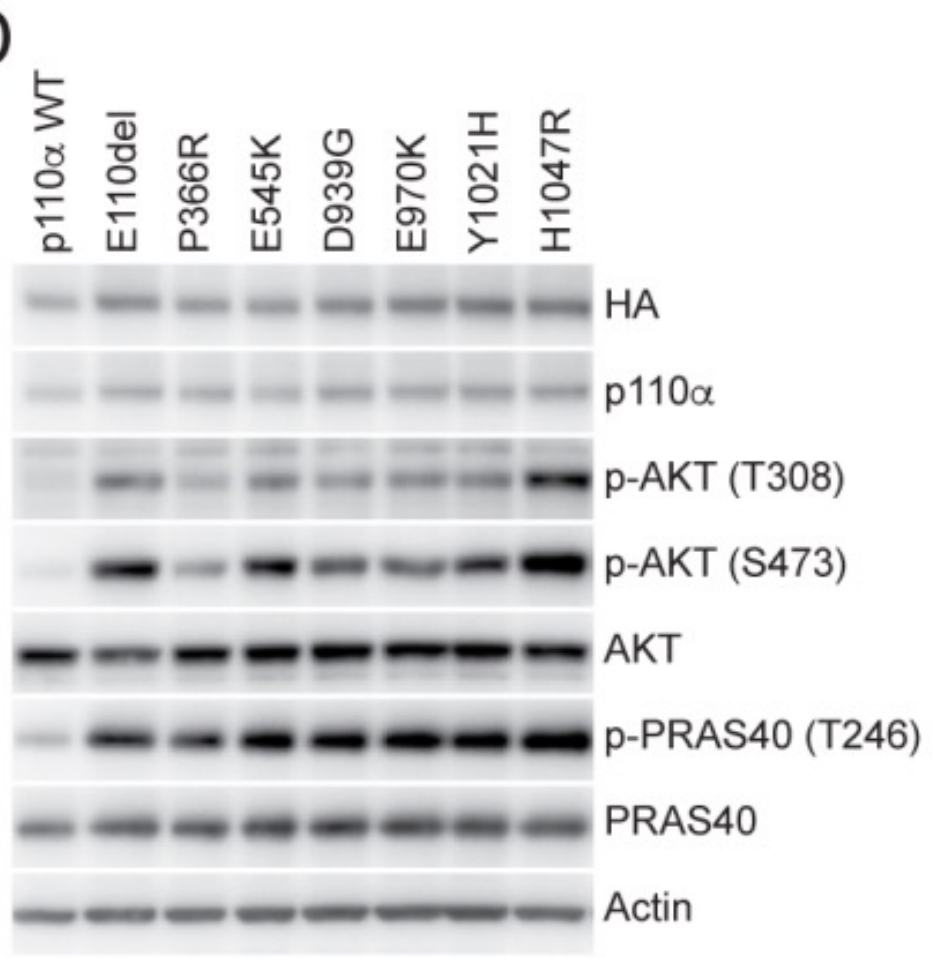

A
HR+HER2-
HR+HER2+
HR-HER2+
TNBC
Sample type
Hist
B
Primary
Metastasis
HR+HER-
HR+HER2+
HR-HER2+
TNBC
Fraction of samples
85
30
25
20
15
10
5
0
ESR1 ATR ARID2 ERBB2 FGFR4 FAT1 TP53 CARD11 RICTOR APC ARID1B BRIP1 CREBBP STAG2 INPP4A TERT KMT2D PARP1 ARID1A TET1 NF1 RFWD2 BLM FLT4 PAK7 PLK2 RHOA ROS1 WT1 ESR1 NF1 TP53
n=1501
n=179
C
Gene
RICTOR
BRCA1
TP53
NF1
FAT1
ARID2
JAK3
CIC
GATA3
CDH1
ESR1
Metastatic site
Lymph node
Ovary
Bone
Lung
Bowel
Liver
3%
6
8
1
1
14
15
3
0
0
3
7
5
0
19
5
5
10
0
5
4
91
n=62
n=176
Histology
Ductal
Lobular
Other
Sample type
Primary
Metastasis
Alteration type
Missense
Truncating
In-frame indel
Fusion
Amplification
Homozygous deletion

Results#3S


A
Percentage of samples
100
80
60
40
20
0
Receptor type
HR+HER2-
MSK
TCGA
HR+HER2+
HR-HER2+
MSK
TCGA
TNBC
TP53
CCND1
PIK3CA
PTEN
AKT1
ERBB2CNA
ERBB2Mut
NF1
FGFR1
PAK1
MAP3K1
ESR1
FOXA1
TBX3
GATA3
ARID1A
Cell cycle
Signaling pathway
Other

Results#3S

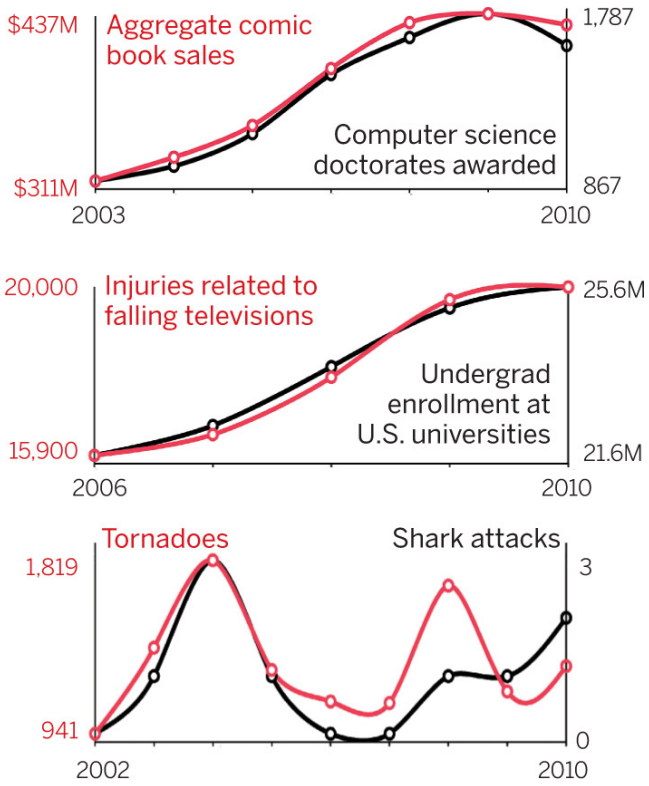

C
PIK3CA
ERBB2
RHOA
Number of mutations
Kinase
Ras
ERBB2 LGSGAFG ... LAKLLGE
EGFR LGSGAFG ... LARLLDI
G719
L861
RHOA WDTAGQEDY
KRAS LDTAGQEEY
G60

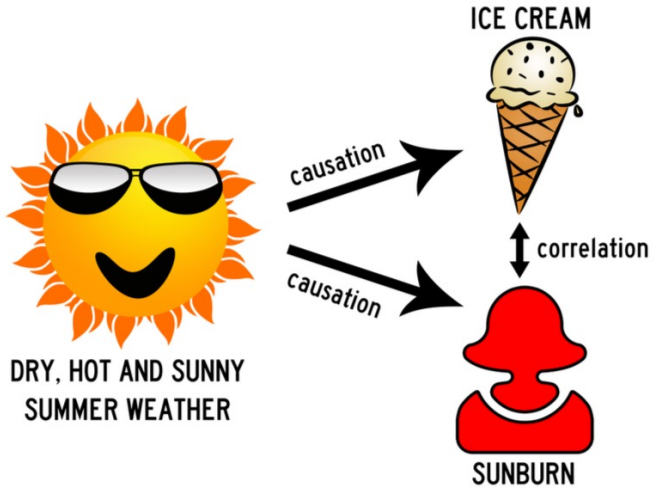

D
p110α WT
E110del
P366R
E545K
D939G
E970K
Y1021H
H1047R
HA
p110α
p-AKT (T308)
p-AKT (S473)
AKT
p-PRAS40 (T246)
PRAS40
Actin

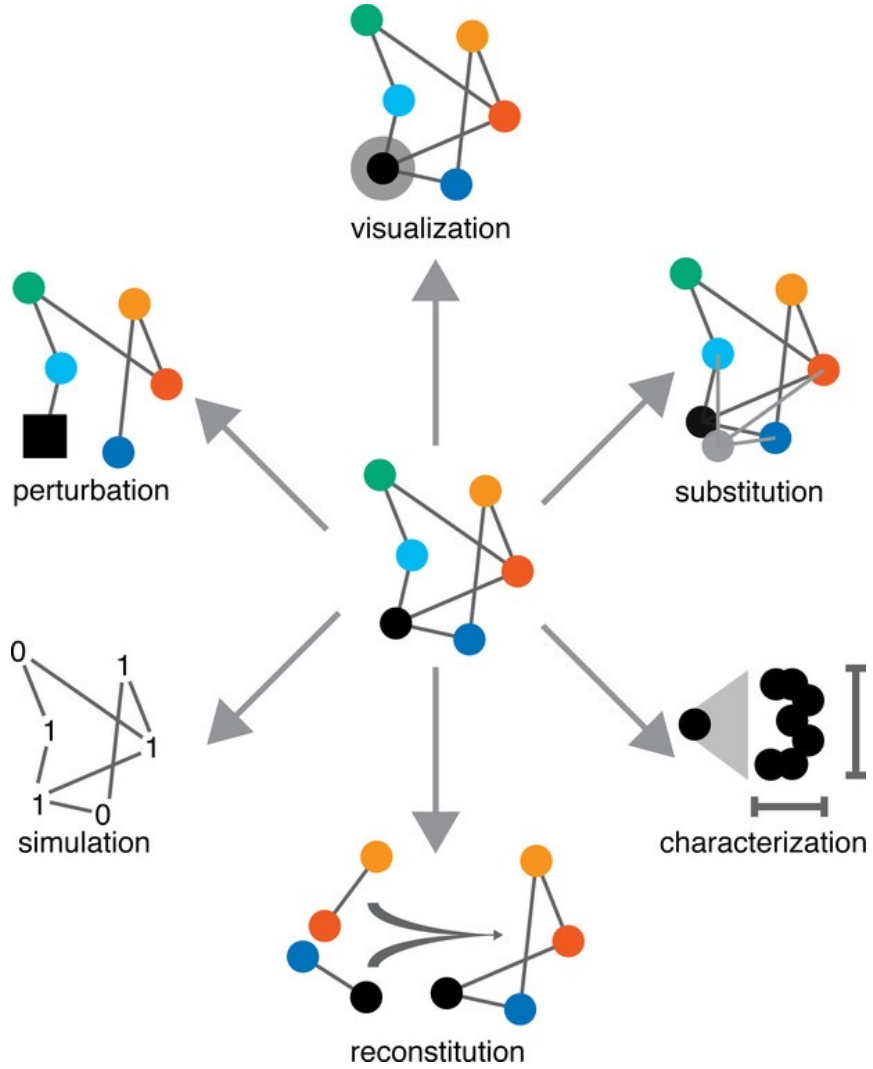
How do we study cancer?

Basics about preclinical validation

Correlation versus causation



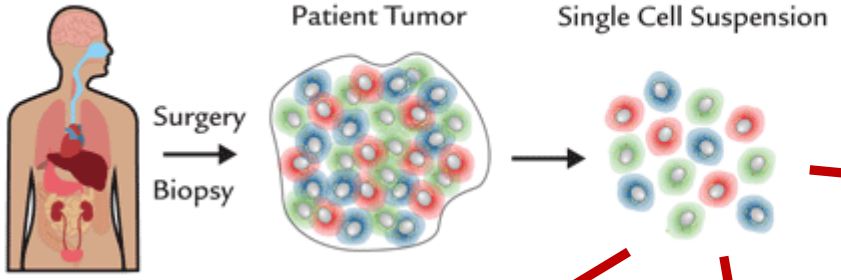




eLife 2020

Basics about preclinical validation

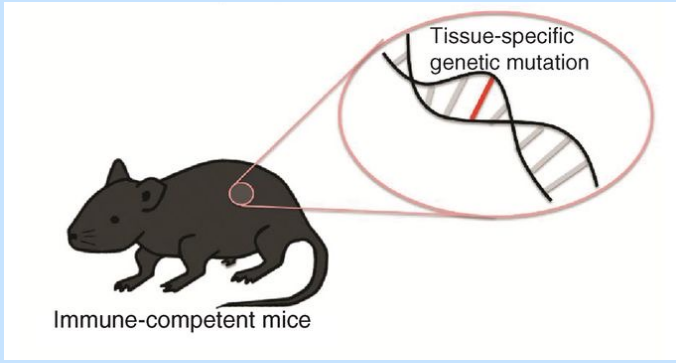
Preclinical models

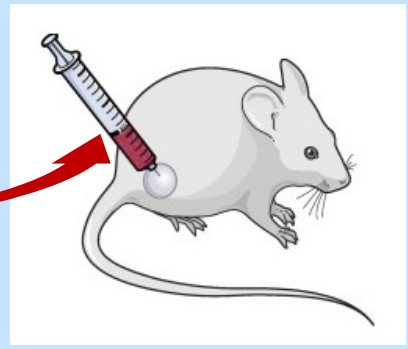


In vivo models

Patient-derived xenografts
(3D system)

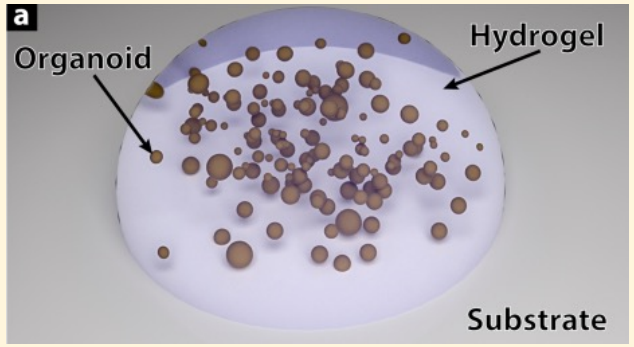
Genetically
engineered
mouse models
(GEMMs)





Cell line xenografts
(3D system)

Cell line (2D system)



Organoids (3D system)

In vitro models

Basics about preclinical validation

Some **pros** and **cons**

In vitro models

Cell lines

- Very easy to work with, quicker and cheaper
- Allow easy genetic manipulation
- Very simplified model, 2D, no tumor microenvironment (TME)

Organoids

- Relatively easy to work with, quick and cheap.
- Allow relatively easy genetic manipulation
- 3D system that reproduces fairly well the behavior of tumors
- Simplified model, no TME

In vivo models

Patient-derived xenografts (PDXs)

- Reproduce very well the behavior of tumors (specially in treatment response)
- As close as you can get to an actual human tumor
- No TME
- Expensive, time-consuming
- Very difficult genetic manipulation

GEMMs

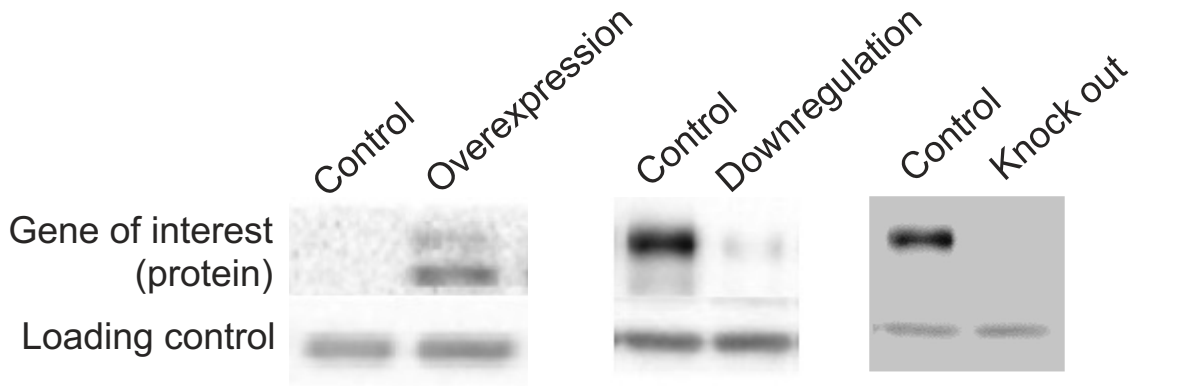
- Can reproduce well the biology of human tumors
- TME
- Expensive and time-consuming
- **Not human!**

Cell line xenografts

- Allow easy genetic manipulation (cell line) and in vivo study (xenograft)
- No TME
- Derived from a very simplified model (cell line)

Basics about preclinical validation

Dysregulating gene expression in cell lines



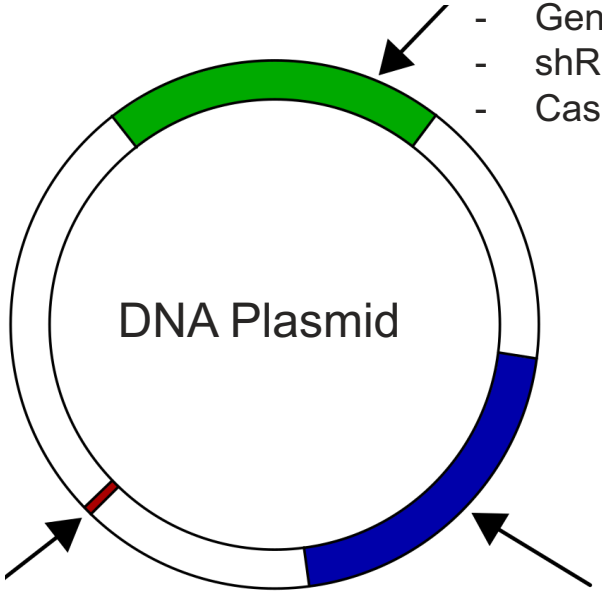
Overexpression vectors, CRISPRa

shRNA/ siRNA, CRISPR-repression

CRISPR/Cas9 KO

Basics about preclinical validation

Genetic engineering of cells



Element of interest to be expressed:
- Gene
- shRNA
- Cas9

Other elements:
- Constitutive promoter
- Inducible promoter
- Genetic elements to generate virus

Selection marker:
- Gene for a fluorescent protein
- Gene for antibiotic resistance

Transfection/ electroporation

Plasmid with element of interest

Plasmid needed to produce virus

Transfection

Cells

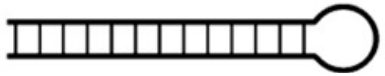
Viruses

Transduction (infection)

Basics about preclinical validation

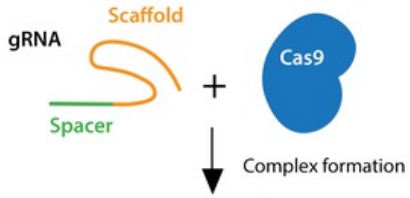
shRNA/siRNA technology for gene expression downregulation

shRNA



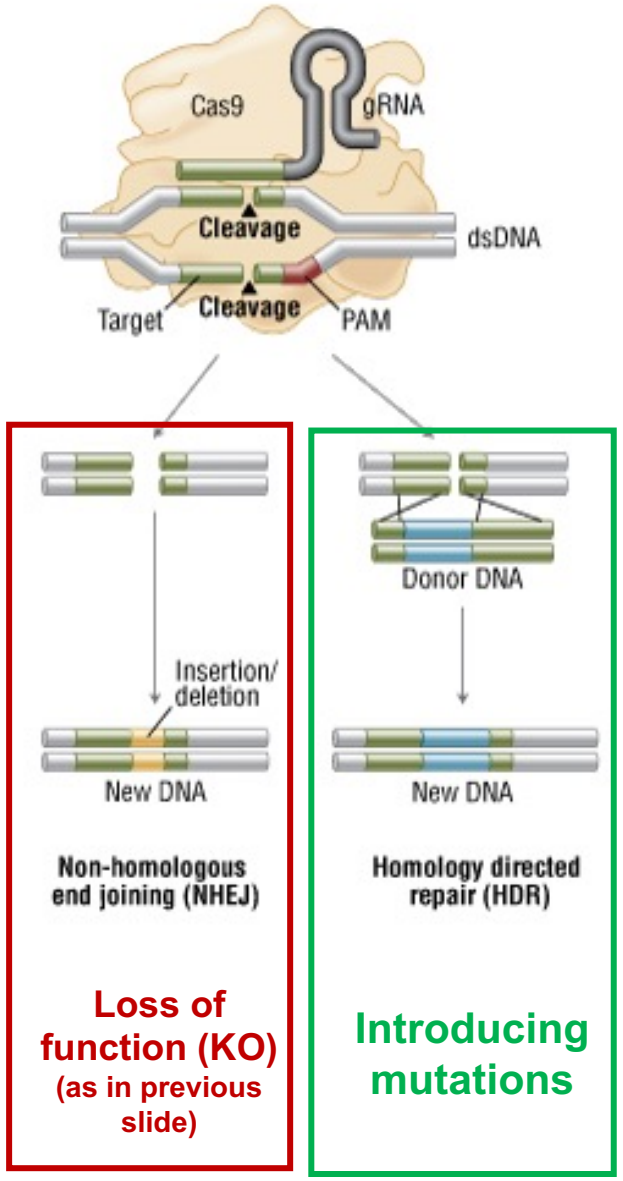
Basics about pre-clinical validation

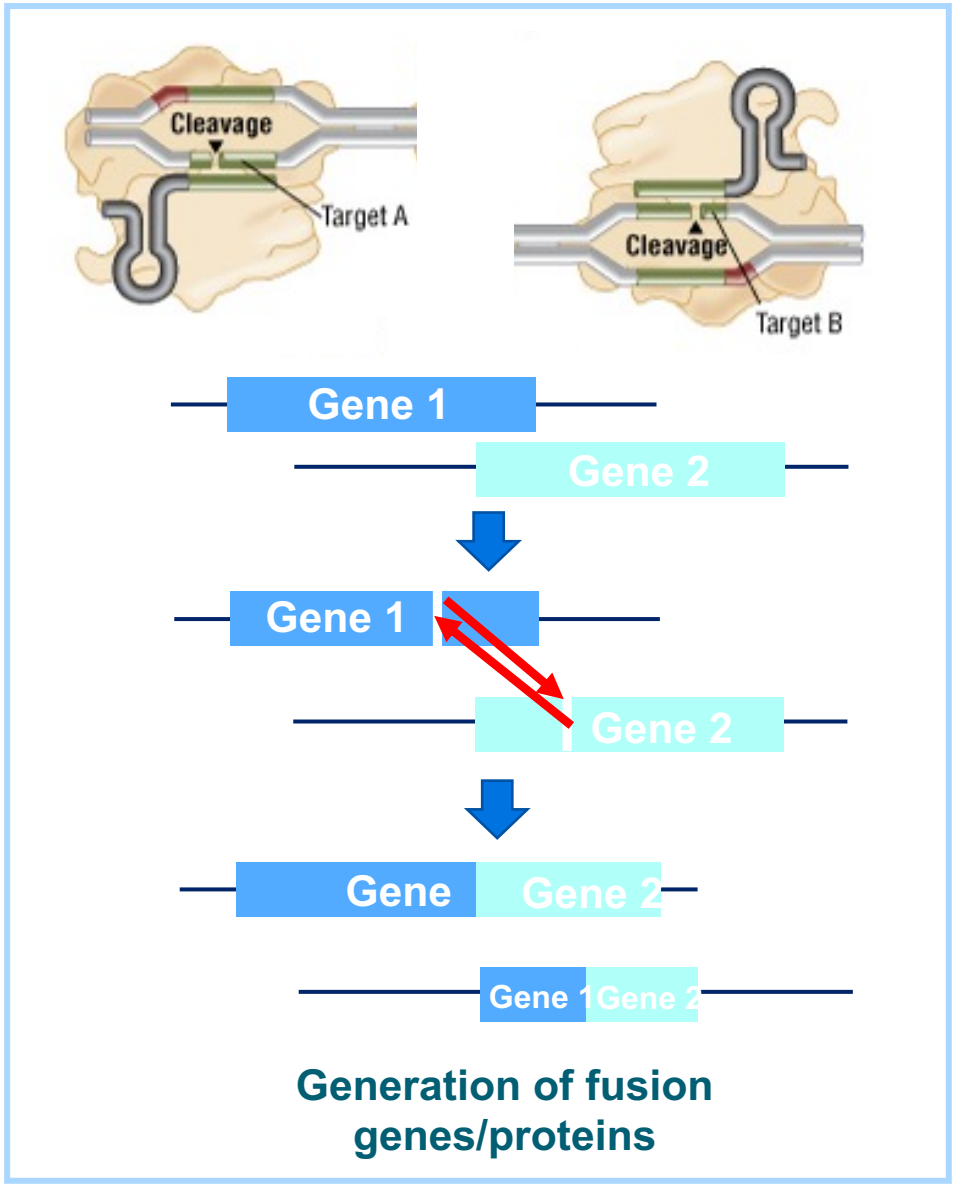
CRISPR-Cas9 technology for gene knock out

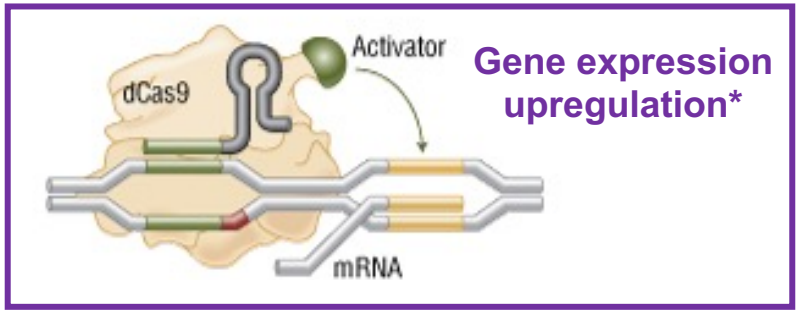


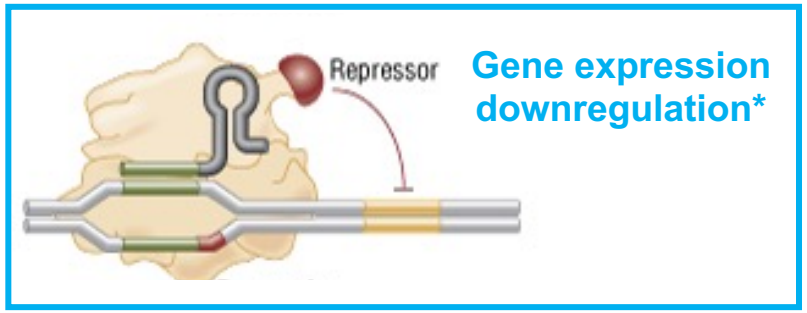
Basics about pre-clinical validation

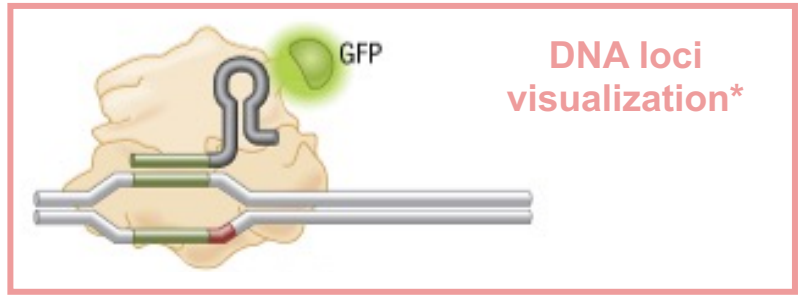
Other applications of CRISPR technology











*For these applications, different Cas9 variants with no cutting function are used

Adapted from New England Biolabs webpage

Paper discussion

Cancer Cell
Article

The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers

Pedram Razavi,[1,2,6] Matthew T. Chang,[1,3,6] Guotai Xu,[1] Chaitanya Bandlamudi,[4] Dara S. Ross,[5] Neil Vasan,[1,2] Yanyan Cai,[5] Craig M. Bielski,[4] Mark T.A. Donoghue,[4] Philip Jonsson,[1,3] Alexander Penson,[1,3] Ronglai Shen,[3] Fresia Pareja,[5] Ritika Kundra,[4] Sumit Middha,[5] Michael L. Cheng,[2] Ahmet Zehir,[5] Cyriac Kandoth,[4] Ruchi Patel,[4] Kety Huberman,[4] Lillian M. Smyth,[2] Komal Jhaveri,[2] Shanu Modi,[2] Tiffany A. Traina,[2] Chau Dang,[2] Wen Zhang,[2] Britta Weigelt,[5] Bob T. Li,[2] Marc Ladanyi,[1,5] David M. Hyman,[2] Nikolaus Schultz,[3,4] Mark E. Robson,[2] Clifford Hudis,[2] Edi Brogi,[5] Agnes Viale,[4] Larry Norton,[2] Maura N. Dickler,[2] Michael F. Berger,[4,5] Christine A. Iacobuzio-Donahue,[1,5] Sarat Chandarlapaty,[1,2] Maurizio Scaltriti,[1,5] Jorge S. Reis-Filho,[1,5] David B. Solit,[1,2,4,*] Barry S. Taylor,[1,3,4,*] and José Baselga[1,2,7,*]
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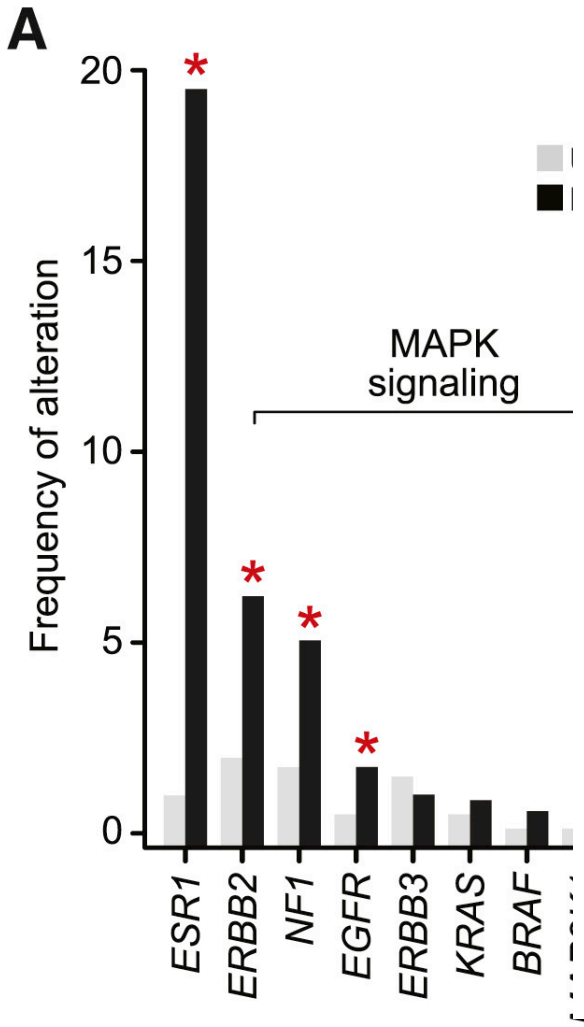
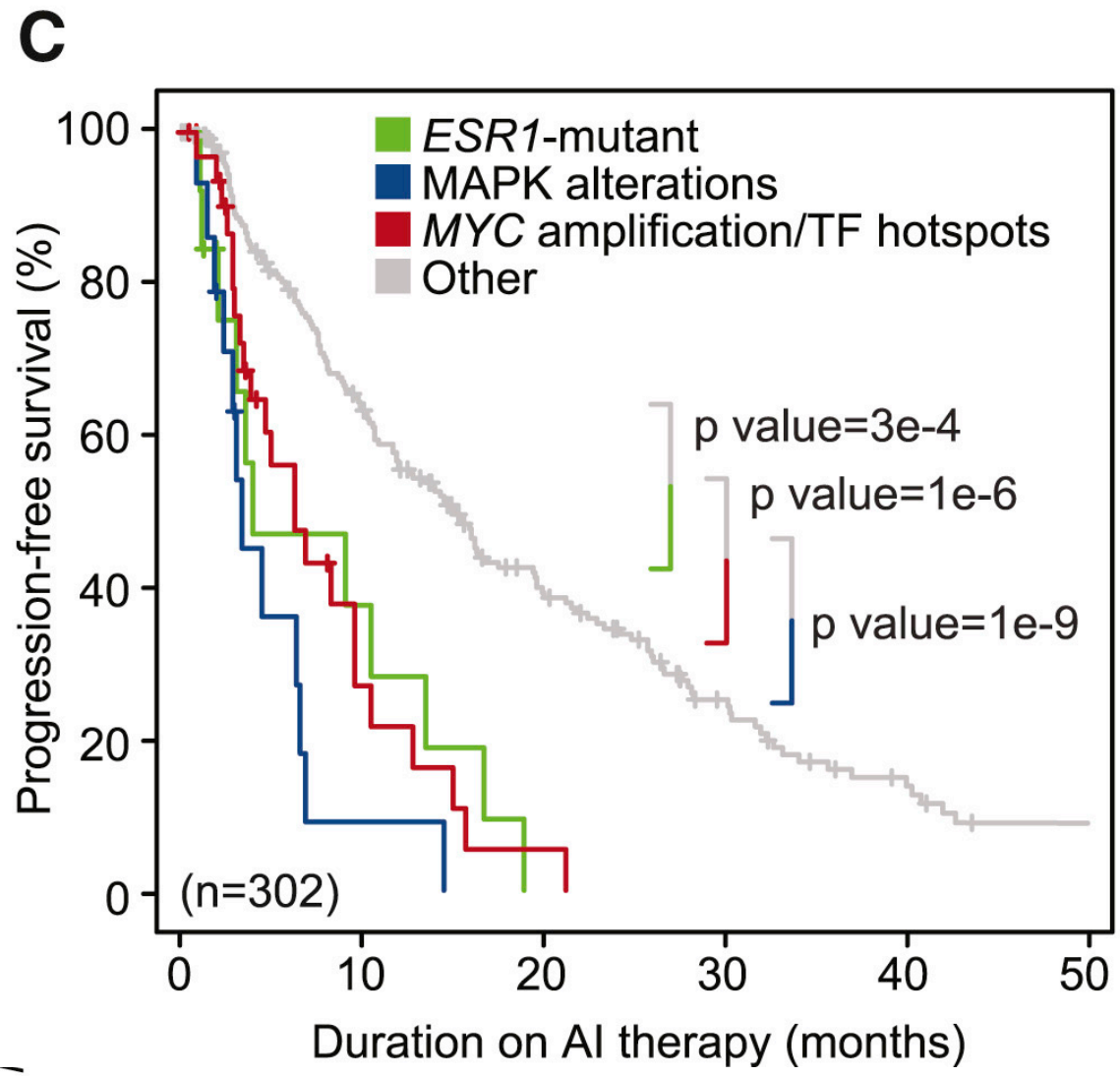
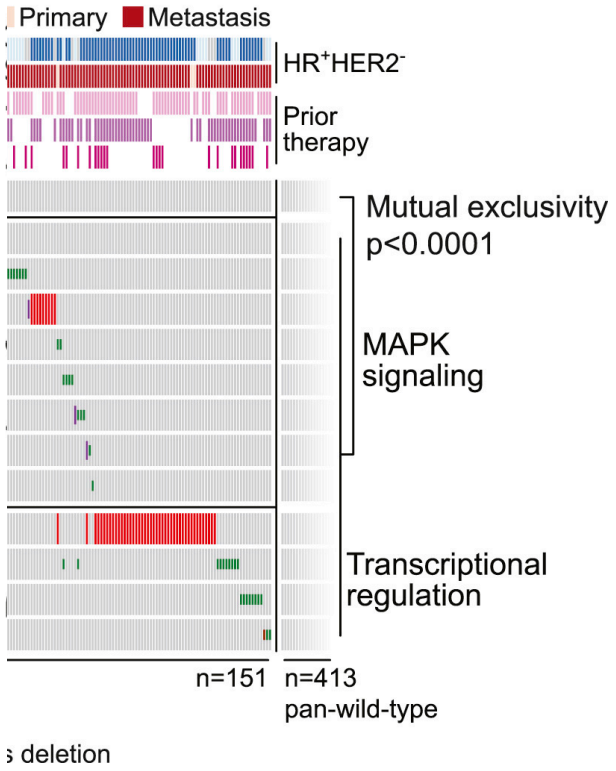
https://doi.org/10.1016/j.ccell.2018.08.008

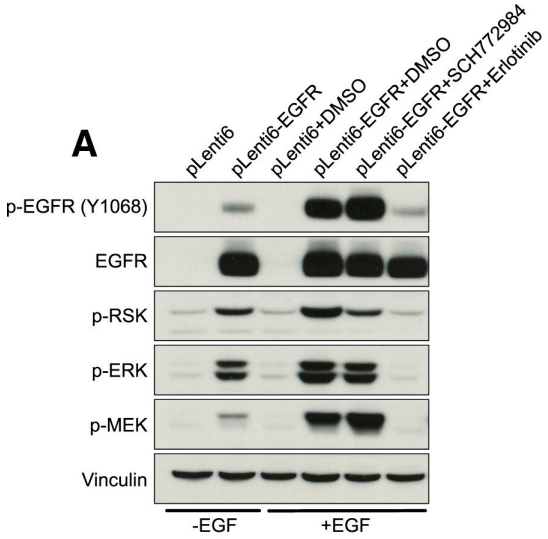
Results#3

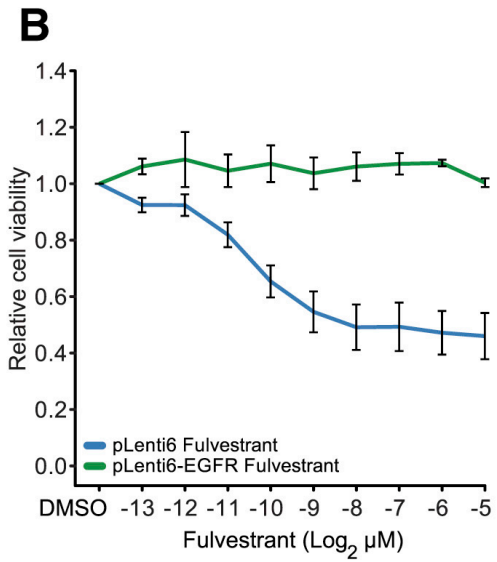

A
Frequency of alteration
20
15
10
5
0
MAPK
signaling
ESR1
ERBB2
NF1
EGFR
ERBB3
KRAS
BRAF

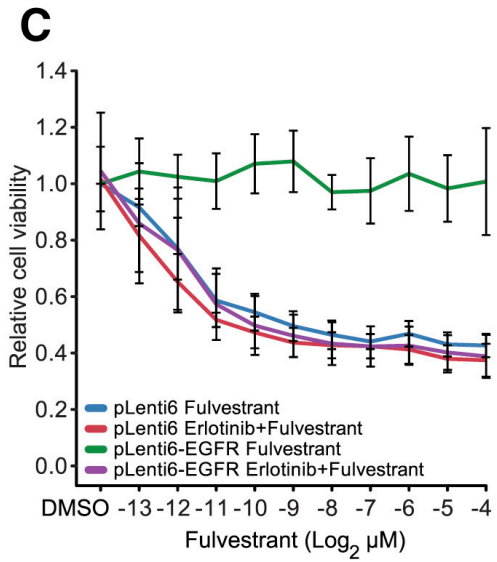

C
Progression-free survival (%)
100
80
60
40
20
0
ESR1-mutant
MAPK alterations
MYC amplification/TF hotspots
Other
p value=3e-4
p value=1e-6
p value=1e-9
(n=302)
0
10
20
30
40
50
Duration on AI therapy (months)

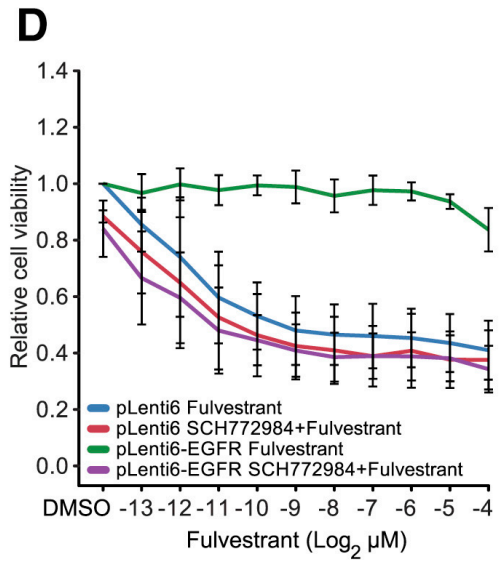

Primary
Metastasis
HR+HER2-
Prior therapy
Mutual exclusivity
p<0.0001
MAPK signaling
Transcriptional regulation
n=151
n=413
pan-wild-type
s deletion

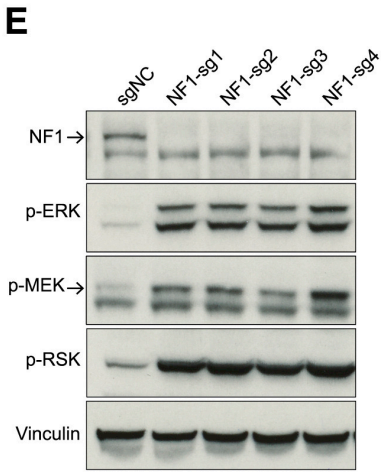
Results#4

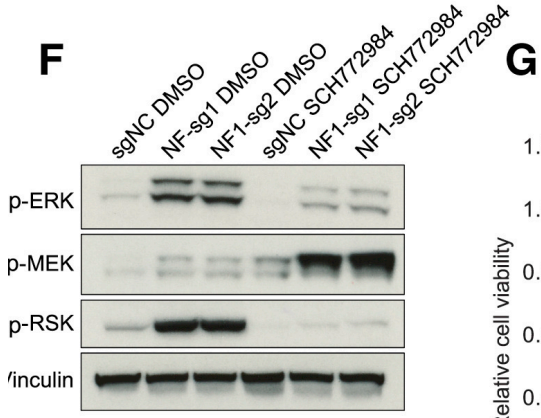


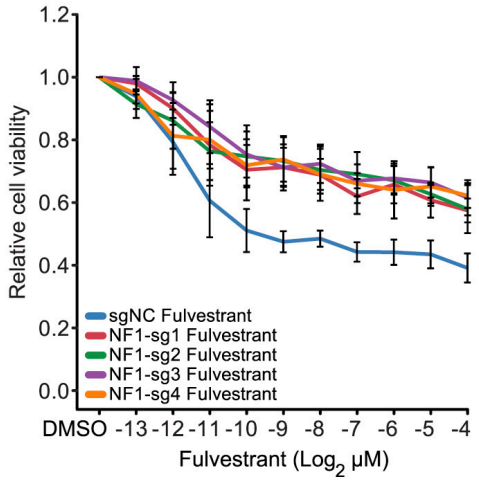


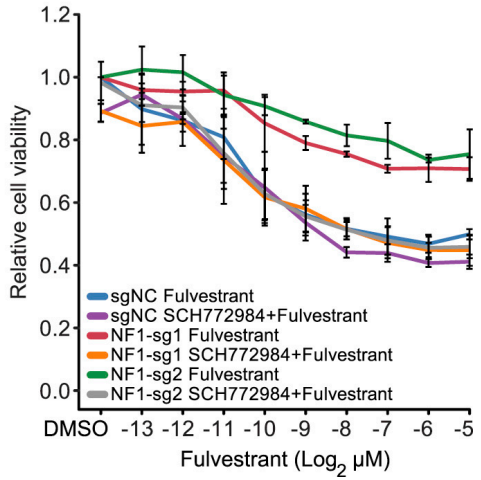




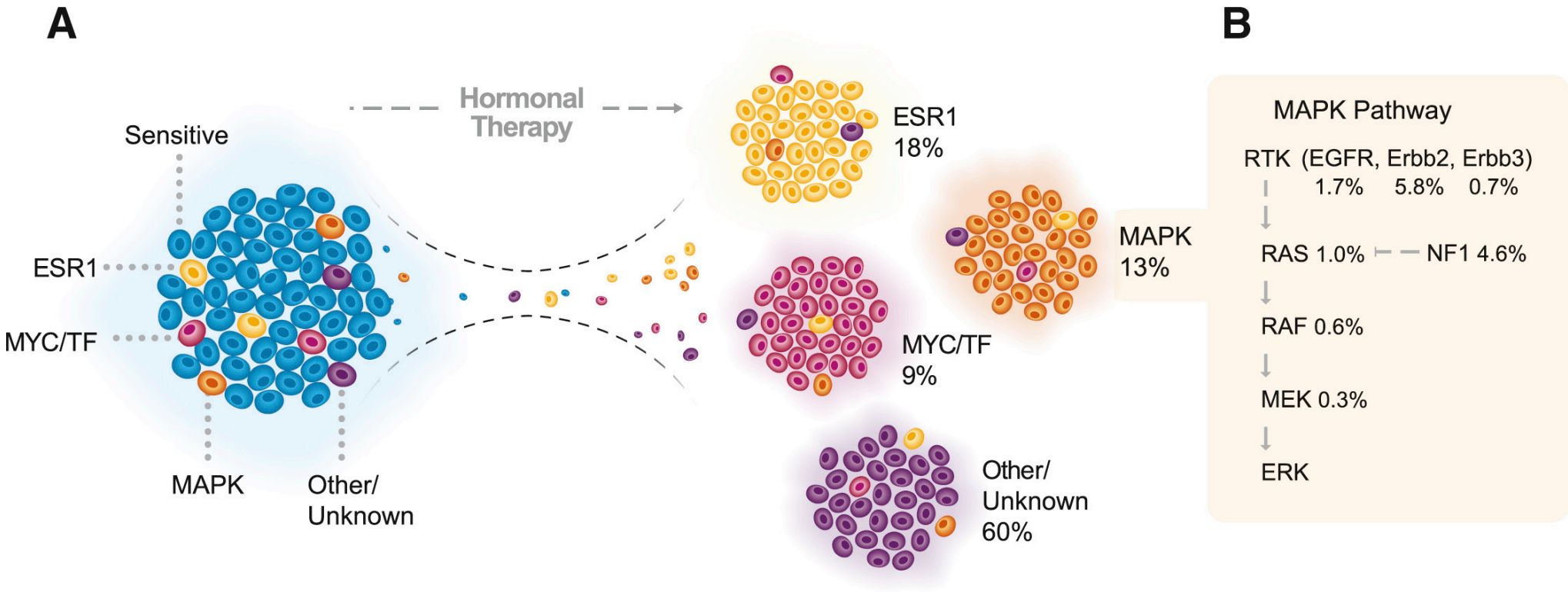








Paper summary



Thanks for your attention!

Any questions?

Memorial Sloan Kettering
Cancer Center