

Development of the PI3K Pathway Inhibitors

How to Choose the Right Cell Line

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Sr. Scientist

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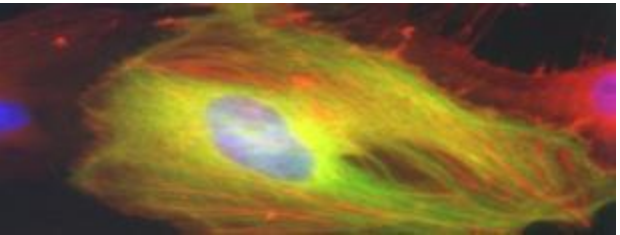
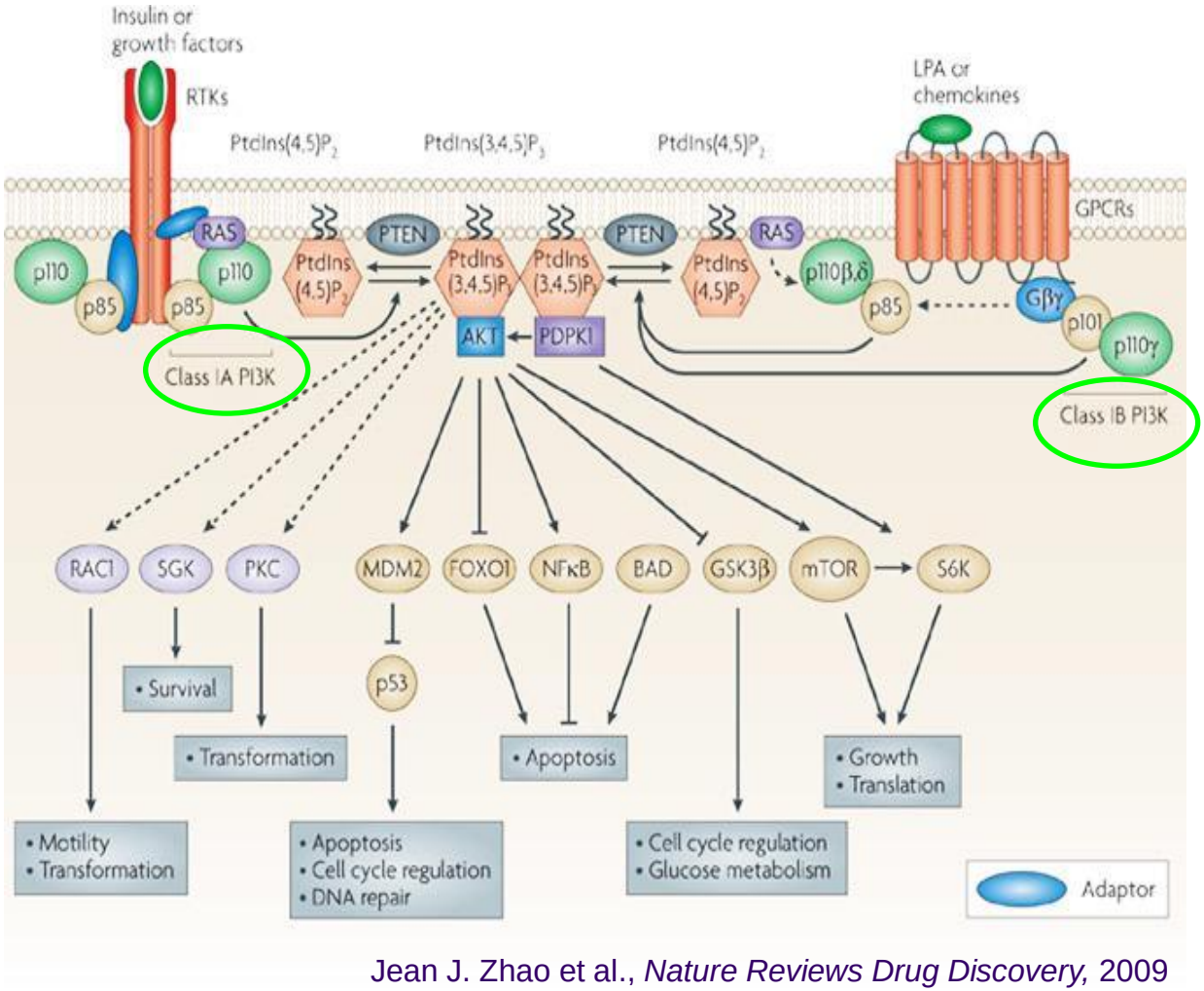


PI3k pathway regulates cell growth and survival

Regulates:
Proliferation
Survival/apoptosis
Metabolism
Angiogenesis
Transformation

Activated by:
RTKs
GPCR
Integrins
RAS

Complexity:
PI3K isoforms
Pathway component
Feedback loops
Crosstalk between signaling cascades



Jean J. Zhao et al., *Nature Reviews Drug Discovery,* 2009

Nature Reviews | Drug Discovery

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Development of PI3K inhibitors

1st generation

- Wortmannin: a fungal metabolite initially isolated from *Penicillium wortmanni*.
  - poor stability
  - poor selectivity
- LY294002: a synthetic compound derived from quercetin, a broad-spectrum kinase inhibitor.
  - poor solubility
  - poor selectivity

2nd generation

- Class I PI3K inhibitor
- Class I PI3k/ mTOR inhibitor
- Pan-PI3K inhibitor
- Pan-PI3k/ mTOR inhibitor
- PI3K α, δ, γ isoforms inhibitor
- PI3K δ isoform inhibitor

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2nd generation PI3K inhibitors

Agent	Target	Sponsor	Phase	Cancer type or condition
PI3K inhibitors				
BEZ235	Class I PI3K and mTOR	Novartis	Phase I–II	Advanced solid tumours; advanced breast cancer
BGT226	Class I PI3K and mTOR	Novartis	Phase I–II	Solid tumours; advanced breast cancer; Cowden's syndrome
BKM120	Class I PI3K	Novartis	Phase I (in the first quarter of 2009)	Solid tumours
XL765	Class I PI3K and mTOR	Exelixis	Phase I	Solid tumours; non-small-cell lung cancer; malignant gliomas
XL147	Class I PI3K	Exelixis	Phase I	Advanced solid tumours; endometrial carcinoma; ovarian carcinoma; non-small-cell lung cancer
GDC0941	Class I PI3K	Genentech	Phase I	Advanced solid tumours; non-Hodgkin's lymphoma
SF1126	Pan-PI3K and mTOR	Semafore	Phase I	Advanced solid tumours
GSK1059615	Pan-PI3K	GlaxoSmithKline	Phase I	Advanced solid tumours; metastatic breast cancer; endometrial cancer; lymphoma
PX-866	PI3K (α, δ and γ isoforms)	Oncothyreon	Phase I	Advanced solid tumours
CAL-101	PI3K (δ isoform)	Calistoga	Phase I	Chronic lymphocytic leukaemia; acute myeloid leukaemia; non-Hodgkin's lymphoma

Jean J. Zhao *et al*., *Nature Reviews Drug Discovery,* 2009

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Targeting PI3K pathway components

- AKT inhibitors

Perifosine (also known as KRX-0401)	AKT	Keryx	Phase I–II
MK2206	AKT	Merck	Phase I
VQD-002 (also known as API-2 and TCN)	AKT	VioQuest	Phase I
XL418	AKT and S6K	Exelixis	Phase I‡

- mTOR inhibitors

Rapamycin/sirolimus (Rapamune)	mTORC1	Wyeth	Phase I–II
			Approved
Temsirolimus (CCI-779/Torisel)	mTORC1	Wyeth	Phase I–III
			Approved
Everolimus (RAD001/Afinitor)	mTORC1	Novartis	Phase I–III
			Approved
AP23573 (also known as deforolimus and MK-8669)	mTORC1	Merck/Ariad	Phase I–III
AZD8055	mTORC1 and mTORC2	AstraZeneca	Phase I–II
OSI-027	mTORC1 and mTORC2	OSI	Phase I

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Targeting P13K- more complicated than we thought?

- Genetic alteration of PI3K pathway components in cancer
- Feedback loops and signaling pathways crosstalk
- Drug resistance mechanism behind Herceptin and others
- Combination strategies

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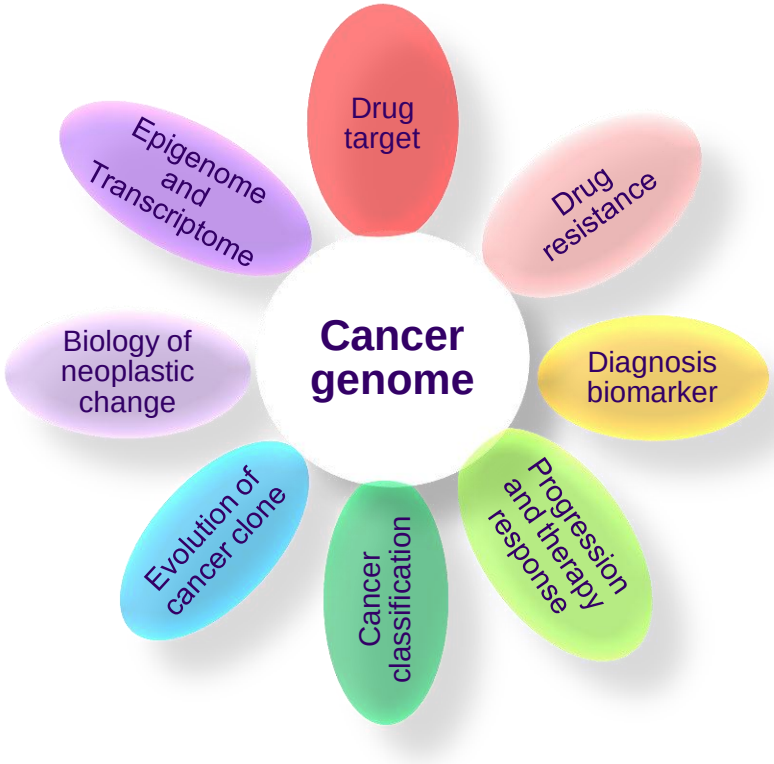
Human cancer genome projects



- The Cancer Genome Atlas
- International Cancer Genome Consortium (ICGC)
- Cancer Cell Line Encyclopedia
- Catalogue of Somatic Mutations in Cancer (COSMIC)

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Genetic alteration of PI3K in cancer

- PIK3CA
  - Mutation
  - Amplification
- PIK3CB
  - Amplification
  - ↑ activity & expression

Genetic alteration	Cancer type	Frequency
p110α (PIK3CA)		
Mutations	Breast	27% (468/1766)
	Endometrial	24% (102/429)
	Colon	15% (448/3024)
	Upper digestive tract	11% (38/352)
	Gastric	8% (29/362)
	Pancreas	8% (8/104)
	Ovarian	8% (61/787)
	Liver	6% (19/303)
	Brain	5.9% (59/996)
	Oesophageal	5% (13/239)
	Lung	3% (28/962)
	Melanoma	9% (24/278)
	Urinary tract	17% (28/162)
	Prostate	2% (1/57)
	Thyroid	2% (7/394)
Amplifications	Lung (squamous cell)	53% (40/75)
	Lung (adenocarcinoma)	12.5% (15/120)
	Lung (small cell)	21.4% (3/14)
	Lung (non-small-cell)	12.0% (11/92)
	Cervical	69% (11/16)
	Breast	8.7% (8/92)
	Head and neck	32.2% (52/161)
	Gastric	36% (20/55)
	Thyroid	9% (12/128)
	Oesophageal	6% (5/87)
	Cervical	9% (2/22)
	Endometrial	10% (3/29)
	Ovarian	11.9% (16/134)
	Glioblastoma	6.1% (21/344)
p110β (PIK3CB)		
Amplifications	Ovarian	5%
	Breast	5%
Increase in activity and expression	Colon	70% (7/10)
	Bladder	89% (8/9)

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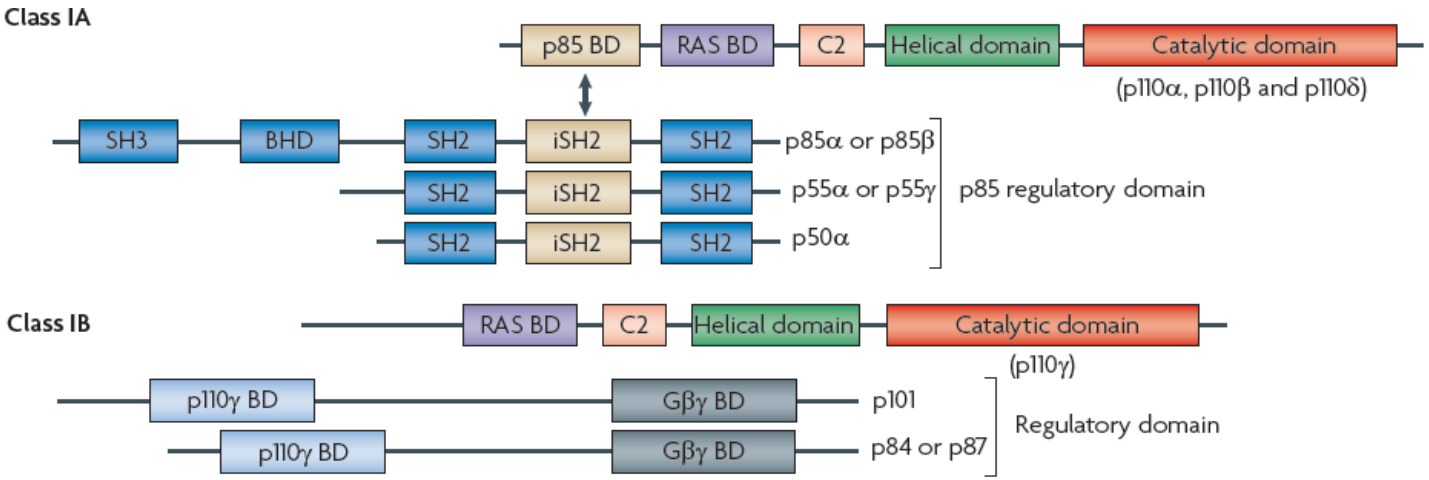
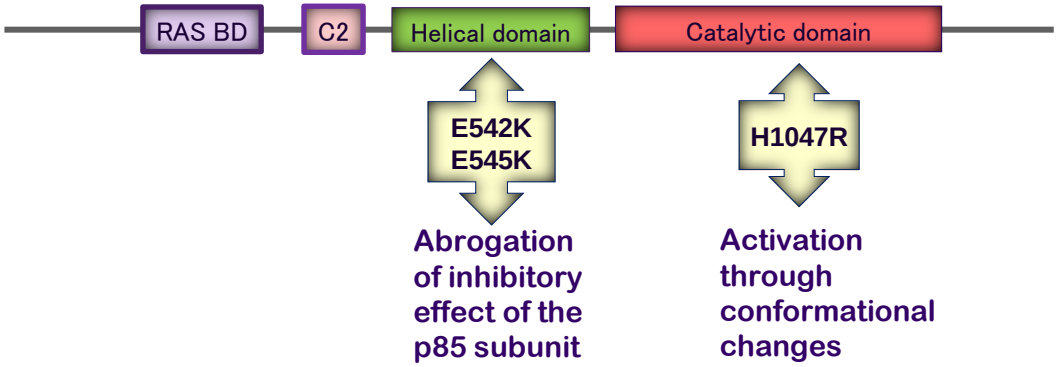
PIK3CA somatic mutation

PI3K family



most frequent mutation
p110α
(PIK3CA)



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Genetic alteration of PI3K pathway component

- PDPK1
  - Amplification
  - ↑ expression
- AKT
  - Mutation
  - Amplification
- PTEN
  - Loss of heterozygosity
  - Deletion

Genetic alteration	Cancer type	Frequency
PDPK1		
Amplifications and overexpression	Breast	20%
AKT		
AKT1 mutation (E17K)	Breast Colon Ovarian Lung	3.7% (31/845) 2.8% (4/139) 2% (1/50) 1.9% (2/105)
AKT1 amplifications	Gastric	20% (1/5)
AKT2 amplifications	Ovarian Pancreas Head and neck Breast	14.1% (30/213) 20% (7/35) 30% (12/40) 3% (3/106)
AKT3 mutation (E17K)	Skin	1.5% (2/137)
AKT3 amplifications	Glioblastoma	2% (4/205)
p85α (PIK3R1)		
Mutations	Glioblastoma Ovarian Colon	9.9% (9/91); 8% (8/105) 4% (3/80) 2% (1/60)
PTEN		
Loss of heterozygosity	Gastric Breast Melanoma Prostate Glioblastoma	25.3% (84/332) 24.9% (99/398) 37% (53/143) 30% (70/230) 28% (113/404)
Mutations	Endometrial Brain Skin Prostate Colon Ovary Breast Haematopoietic and lymphoid tissue Stomach Liver Kidney Vulva Urinary tract Thyroid Lung	38% (604/1569) 21% (611/2913) 17% (96/555) 14% (51/371) 13% (53/416) 9% (55/645) 6% (34/561) 6% (54/866) 6% (28/499) 5% (20/372) 5% (14/294) 65% (17/26) 9% (13/142) 5% (27/591) 9% (48/548)

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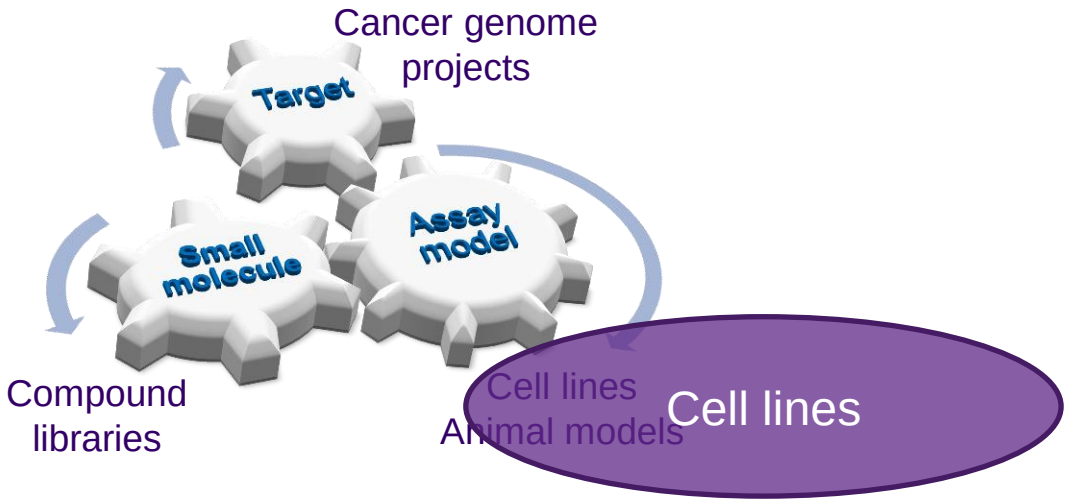
Opportunities emerge with cancer genome projects

Sequencing data has spawned

- New fields of inquiry
- New treatment targets
- New drug development paradigms



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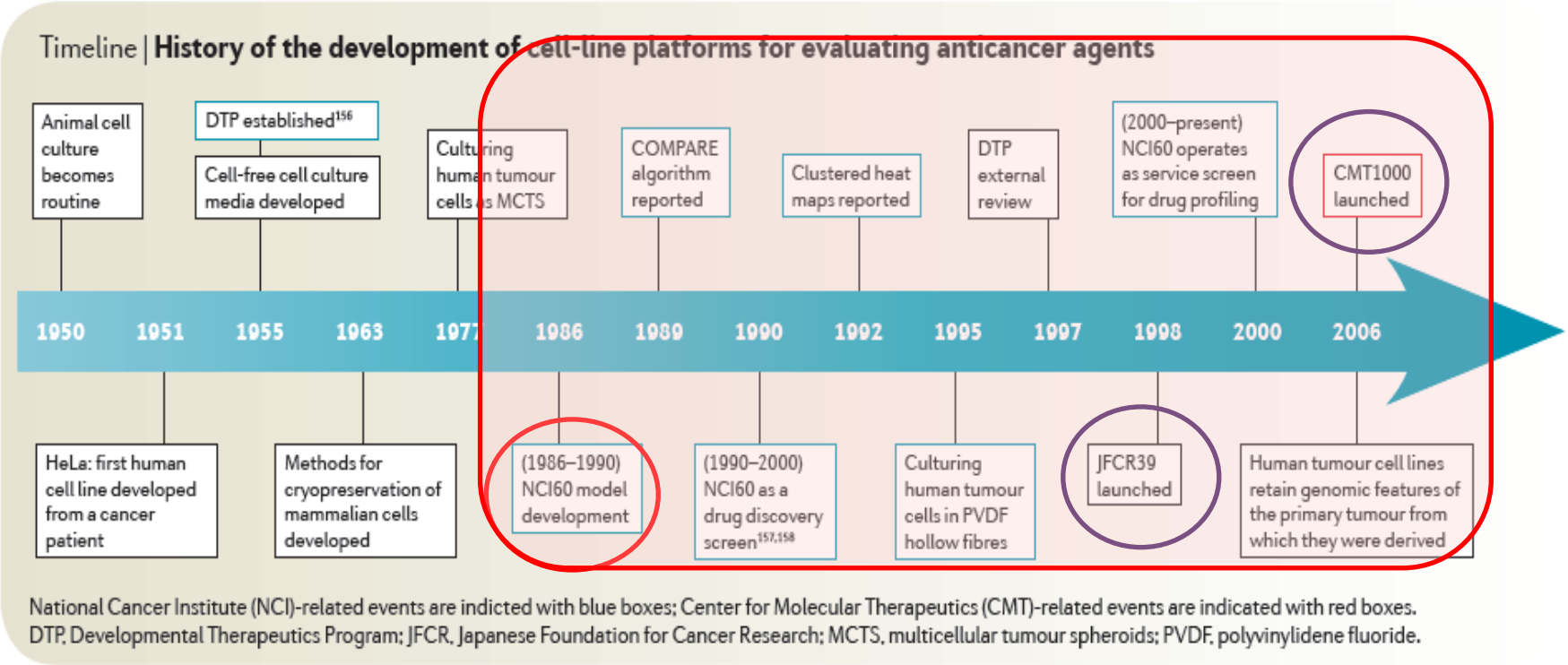
Cell line platforms for evaluating anticancer agents

Traditional tumor cell line panels developmental timeline



Sreenath V. Sharma *et al.*, *Nature Reviews Cancer* , 2010

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Cell line platforms for evaluating anticancer agents

NCI60 Cell Panel

Leukaemia
CCRF-CEM
K-562
MOLT-4
RPMI-8226
SR
Melanoma
LOX IMVI
MALME-3M
M14
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62
Ovarian cancer
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3

Non-small-cell lung cancer
A549
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
Colon cancer
COLO 205
HCC-2998
BCT-116
HCT-15
HT29
KM12
SW-620
CNS cancer
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251

Renal cancer
786-0
A498
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31
Prostate cancer
PC-3
DU-145
Breast cancer
MCF7
NCI/ADR-RES
MDA-MB-231
S 578T
MDA-MB-435
BT-549
T-47D

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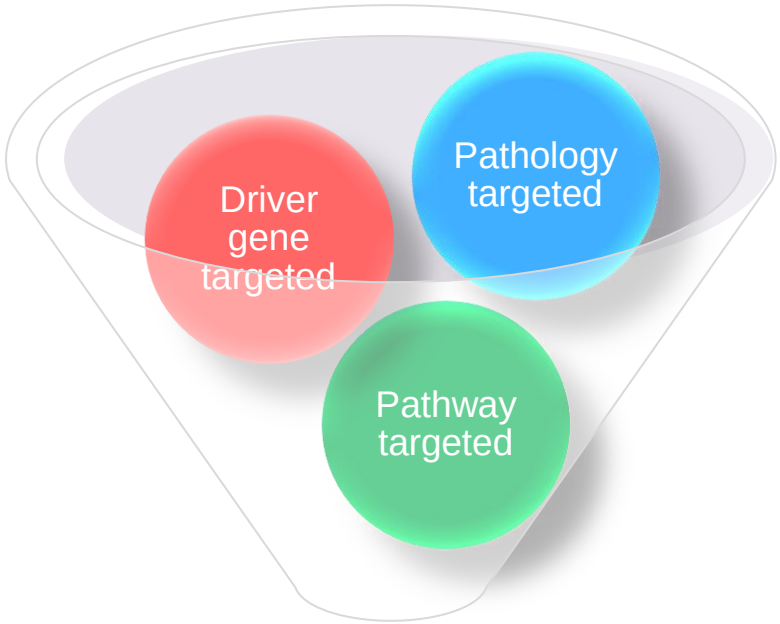
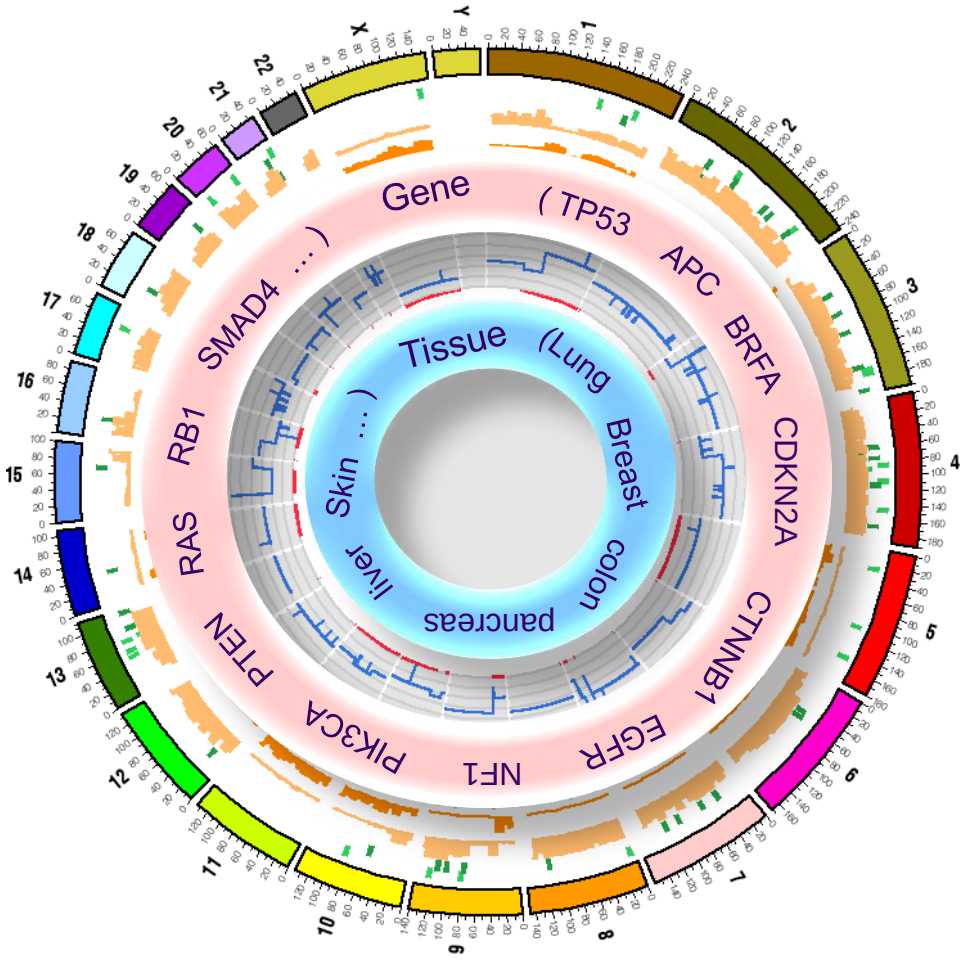
ATCC Tumor Cell Panels Initiative



Unique tools

- Time savings
- Convenience



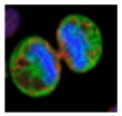
-modified circos cancer genome display

ATCC Tumor Cell Panels

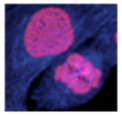
The value of tumor cell lines, as research models and drug discovery tools, is greatly enhanced when there is an understanding of the underlying genetic abnormalities that drive their phenotype. ATCC has taken the first step for your research by annotating our tumor cell lines with gene mutation data from the Sanger Institute COSMIC database,[1] additional in-house testing, and collaboration with Horizon Discovery Ltd. Choose from:

Tissue-Specific Tumor Cell Panels
Choose from a wide selection of Tumor Cell Panels organized by pathology and annotated with published information relevant to your research, such as gene mutation data from the Sanger Institute COSMIC database.[1]

Molecular Signature Panels
Focusing on cell signaling pathways, ATCC has performed additional testing to verify the genomic alteration, gene expression, protein expression and bio-function of key molecular components of cell signaling cascades, oncogenes, and tumor suppressors such as p53.



Horizon® Isogenic Cell Lines Panels – ***Coming Soon!***
In conjunction with Horizon®, a leading developer of isogenic cell lines and related technology, ATCC offers isogenic cell lines in panels organized by driver gene mutations.



Cell Lines Organized by Specific Gene Mutation
Find the individual cell lines you need with annotated gene mutation data from the Sanger Institute COSMIC database.[1]

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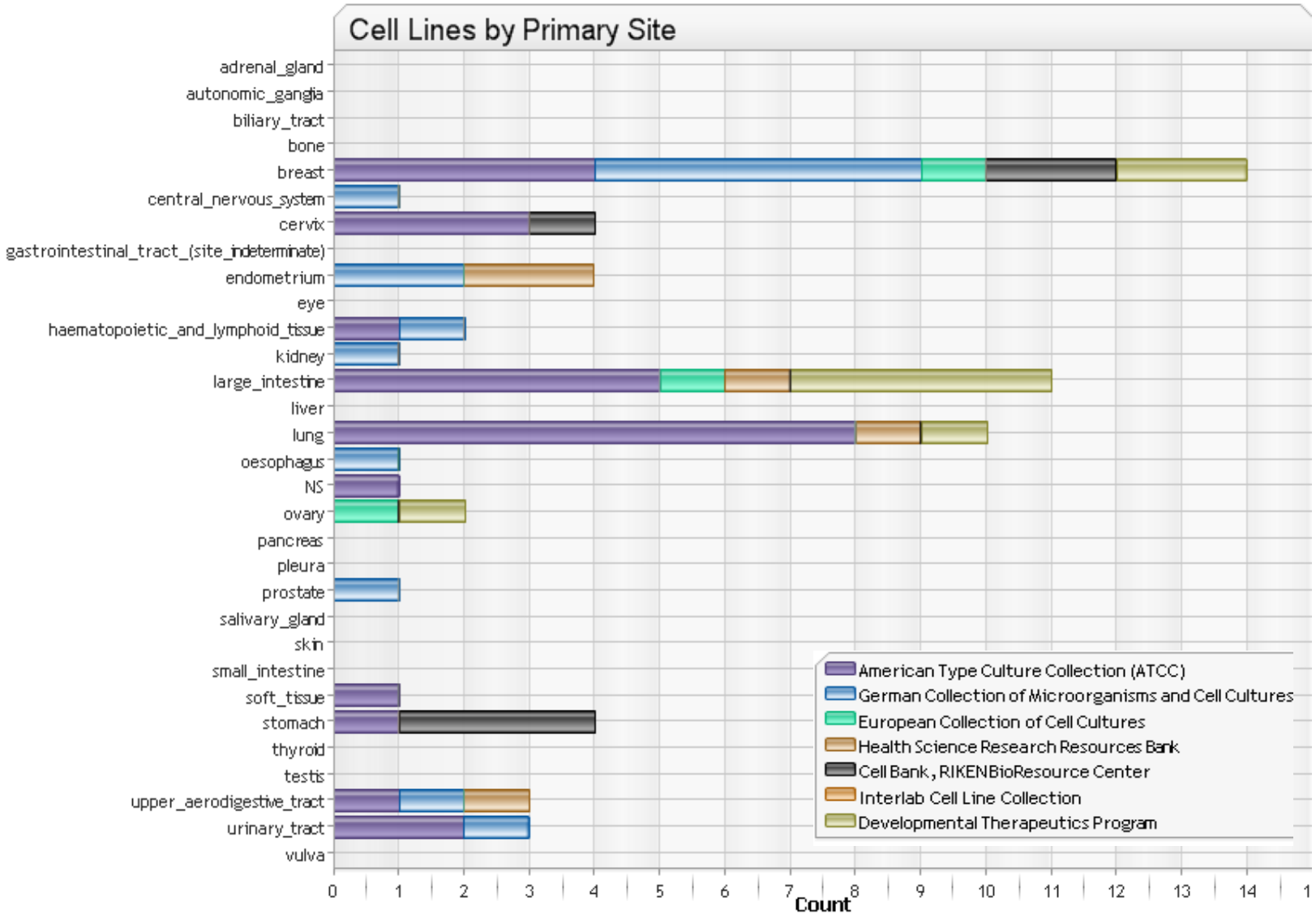
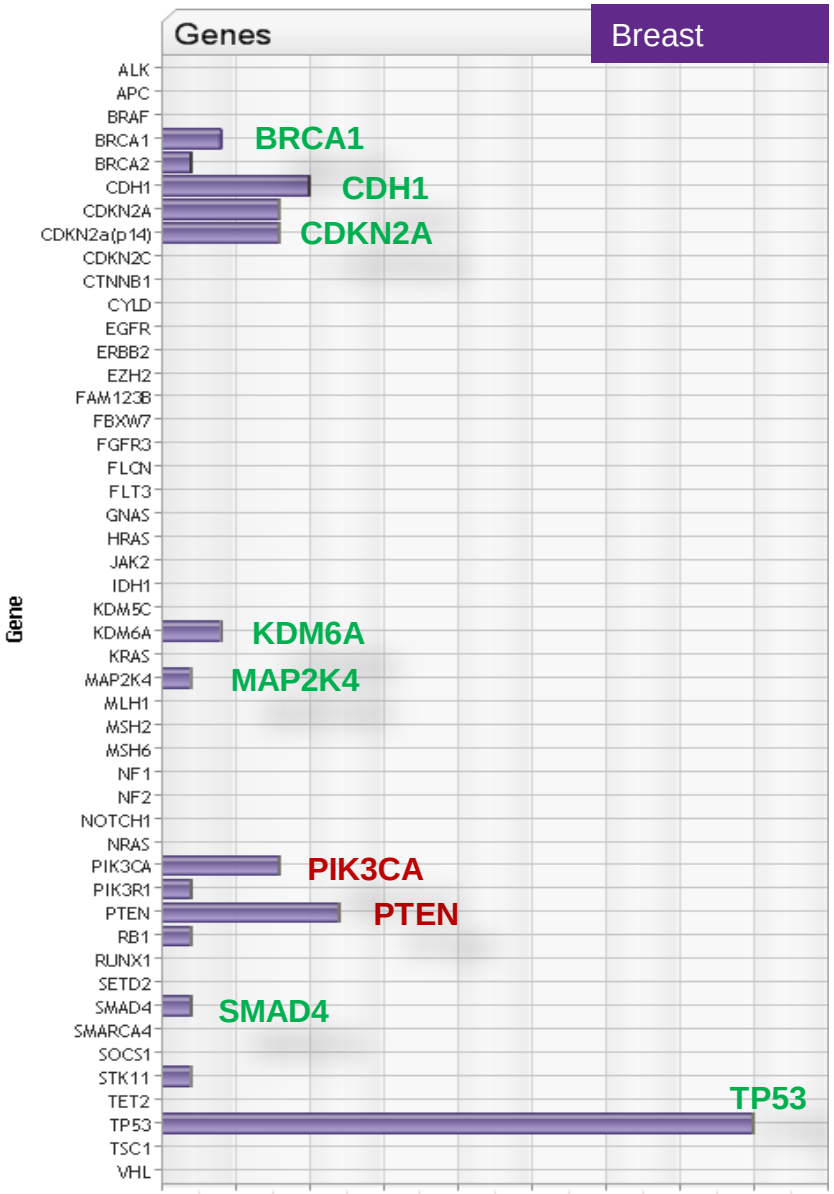
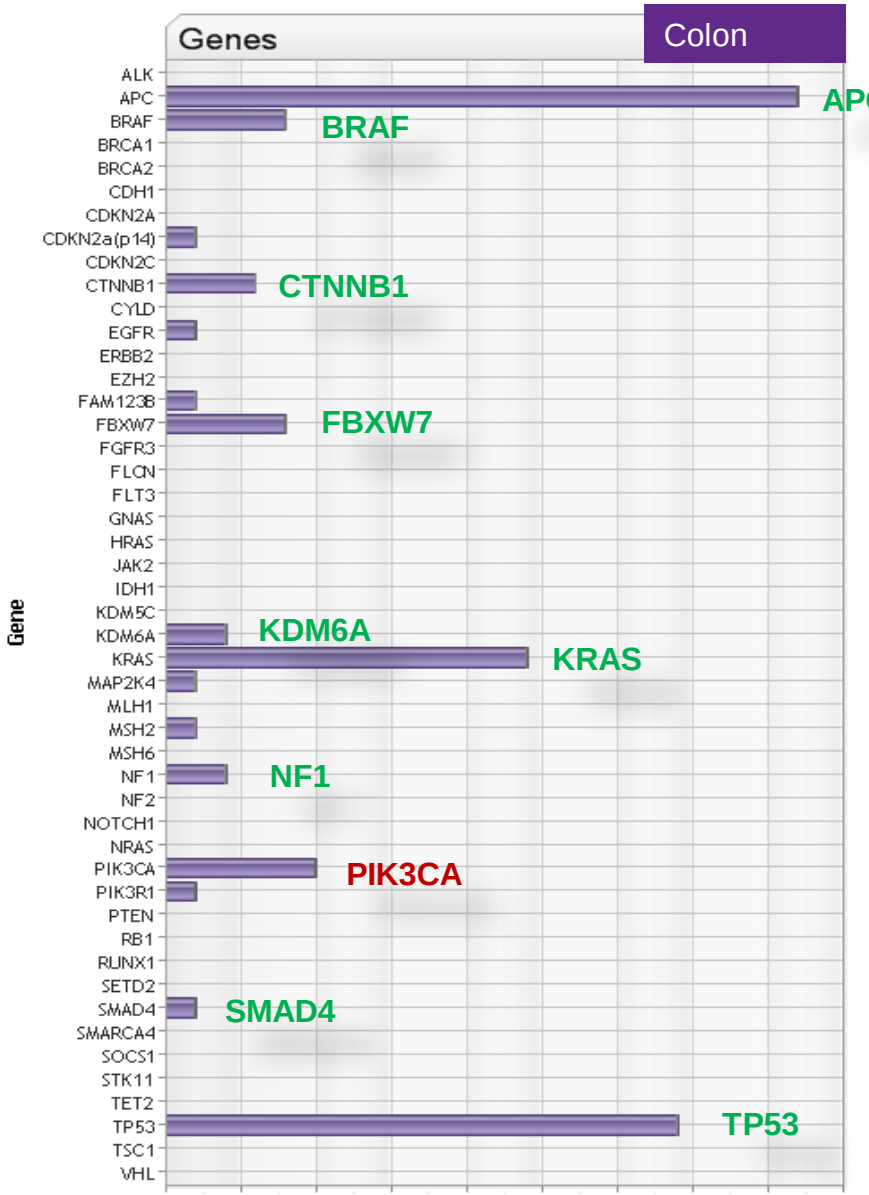
PI3K mutation cell lines



Organization of Cell Lines by Tissue and Mutations





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Gene Mutation Lists for Cells Lines

Gene Mutation List for Cell Lines

ATCC has created gene mutation lists based on the ATCC tumor cell line collection and known mutation information maintained in the Sanger Institute COSMIC database[1]. These references should provide useful information for researchers using cell based models.

ATCC PTEN
Gene of the month

APC

EGFR

RAS

BRAF

PIK3CA

RB1

CDKN2A

PIK3R1

SMAD4

CTNNB1

PTEN

TP53

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PIK3CA Mutation Cells Lines

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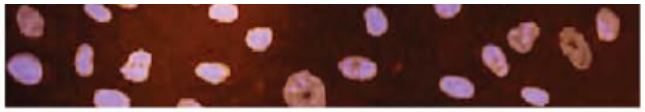

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PIK3CA

Phosphatidylinositol 3-kinase (*PI3K*) is a family of enzyme involved in a wide range of cellular functions including proliferation, survival, migration and vesicular trafficking. Many of these functions relate to the ability of class I PI 3-kinases to activate PKB as in the PI3K/AKT/mTOR pathway. The phosphatidylinositol 3-kinase (***PIK3CA)*** gene encodes for the p110α catalytic subunit of the class I PI3K, and the phosphatidylinositol 3-kinase (***PIK3R1***) gene encodes for the regulatory subunit of the protein, p85α. Mutations in PIK3CA and PIK3R1 have been implicated in the pathogenesis of many cancers, such as colon, lung, ovarian and breast cancer.

source | histology | zygosity | gene sequence | protein sequence | name | ATCC#

Breast						
primary	Carcinoma	heterozygous	c.1616C>G	p.P539R	BT-20	HTB-19™
	Carcinoma	heterozygous			BT-20	HTB-19™
primary	Carcinoma, ductal	heterozygous	c.3140A>G	p.H1047R	HCC1954	CRL-2338™
primary	Carcinoma, primary ductal	heterozygous			UACC-893	CRL-1902™
primary	Carcinoma, ductal	hetero-zygous	c.333G>C	p.K111N	BT-474	HTB-20™
	Adenocarcinoma		c.1633G>A	p.E545K	MDA-MB-361	HTB-27™
metastasis brain	Adenocarcinoma	heterozygous			MCF7	HTB-22™
	carcinoma	heterozygous	c.1633G>A	p.E545K	MDA-MB-453	HTB-131™
metastasis, pleural effusion		heterozygous		p.H1047R	T-47D	HTB-133™

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PIK3CA Mutation Cells Lines

PIK3CA mutation	frequency	Tissue source
p.E545K	28%	Breast
p.E545D	3%	Caecum
p.H1047R	28%	Cervix
p.H1047L	3%	Colon
p.E542K	5%	Lung
p.R88Q	5%	Lymphoid
p.K111E	3%	Ovary
p.K111N	3%	Pharynx
p.K111R	3%	Prostate
p.P539R	3%	Stomach
p.Q546R	3%	Urinary bladder
p.D549N	3%	Uterus
p.E453K	3%	
p.G118D	3%	
p.P124L	3%	
p.P449T	3%	
p.G106_R108del	3%	Cell lines: 36

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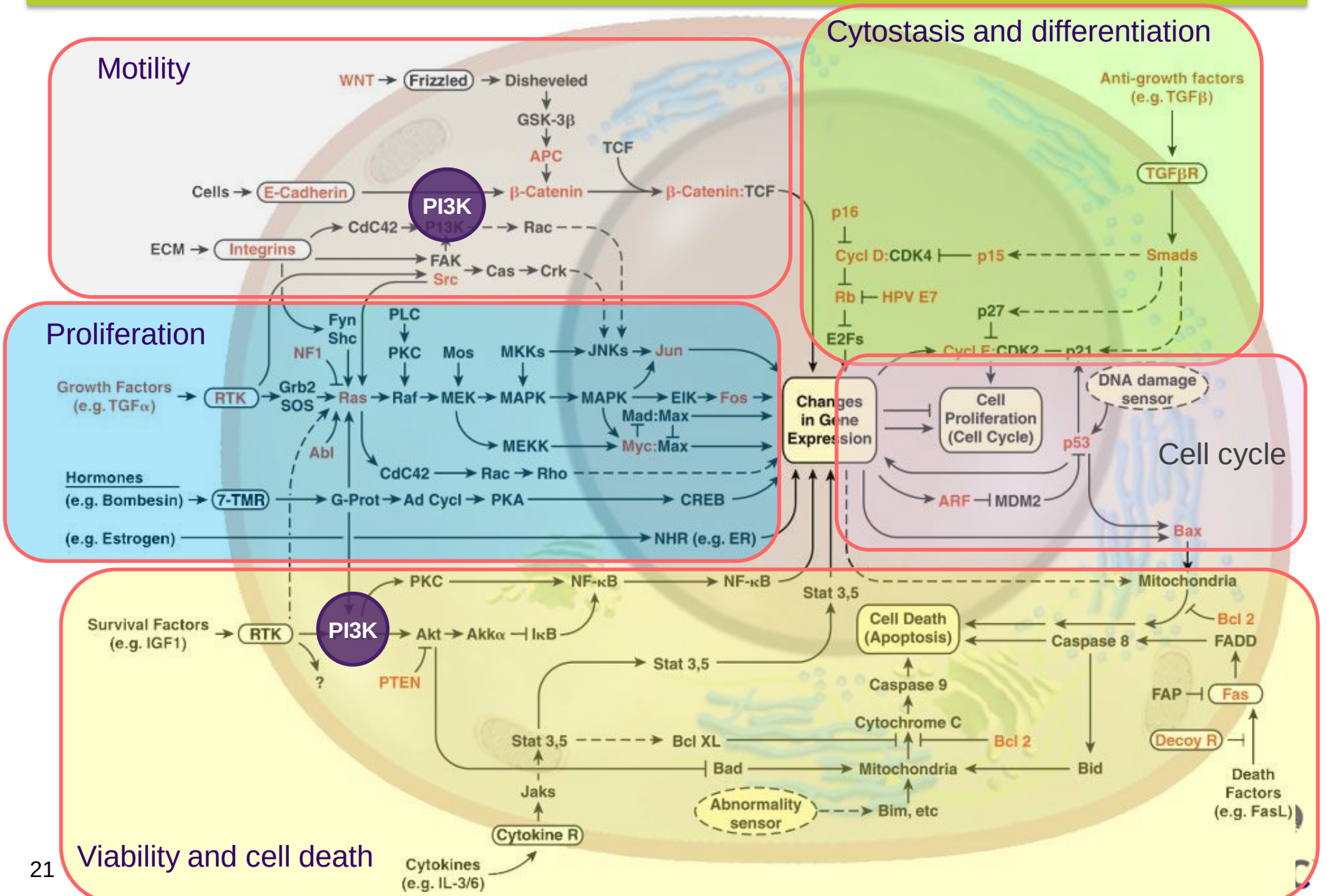
Cell signaling cascades network



Choose suitable breast cancer cell lines

Targeting PI3K pathway overcomes resistance to HER2-directed therapy

Cell line	HER2 expression	Response to herceptin	Response to GDC-0941
MCF-7	low	Insensitive	Sensitive
SKBR-3	high	Sensitive	Sensitive
BT474	high	Sensitive	Sensitive
AU-565	high	Sensitive	Sensitive
HCC-1419	high	Sensitive	Sensitive
ZR75-30	high	Sensitive	Sensitive
HCC-1954	high	Insensitive	Sensitive
KPL-4	high	Insensitive	Sensitive
JIMT-1	high	Insensitive	Sensitive

Teemu T. Junttila *et al*., *Cancer Cell*, 2012

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Choose suitable breast cancer cell lines

Consider the complexity of genetic alteration

Cell line	Receptor expression	PIK3CA	PTEN	Other mutations
MDA-361	HER2 high, ER +	p.E545K	WT	CDKN2A
UACC-893	HER2 +, ER -	p.H1047R	WT	TP53
T47D	HER2 low, ER high	p.H1047R	WT	TP53
BT-20	triple- negative	p.P539R; p.H1047R	WT	CDKN2A, TP53
Hs-578-T	triple- negative	WT	WT	CDKN2A, HRAS, PIK3R1, TP53
MDA-231	triple- negative	WT	WT	BRAF, CDKN2A, KRAS, NF2, TP53
MDA-453	triple- negative	p.H1047R	WT	CDH1
MDA-468	triple- negative	WT	lost	RB1, SMAD4, TP53

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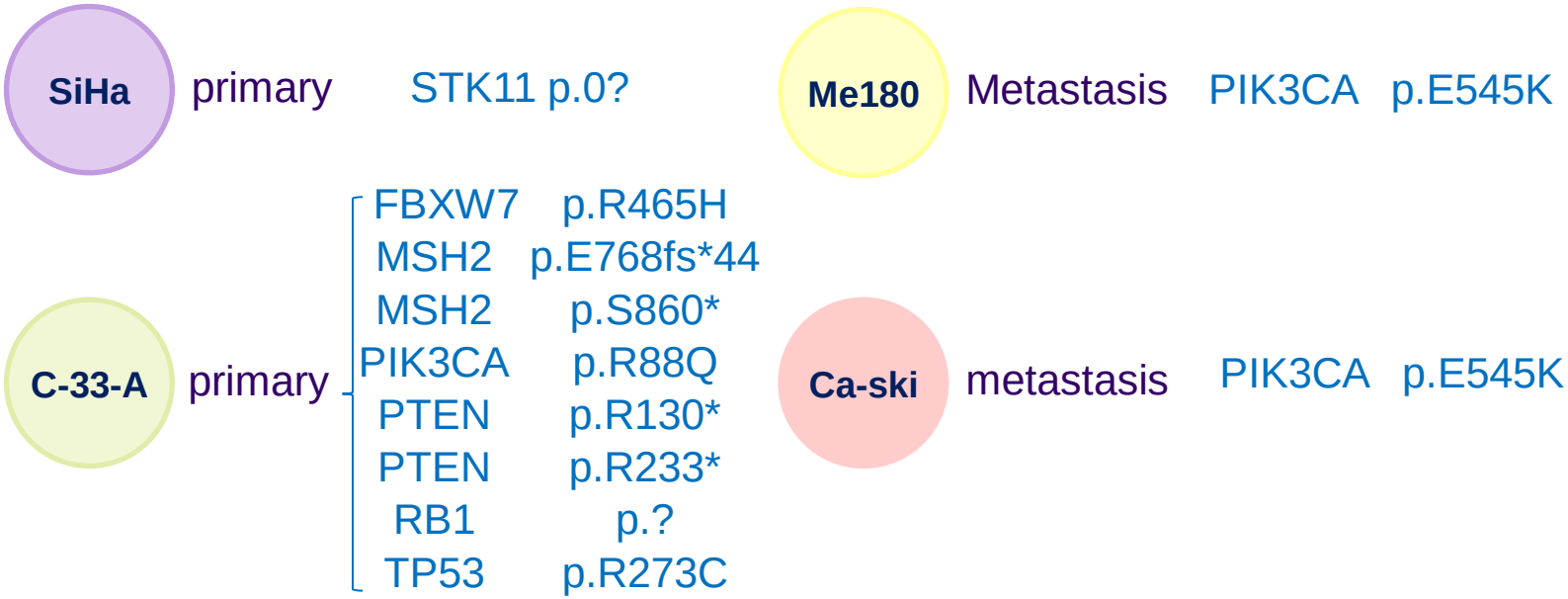
Choose suitable cell lines from other tissue types

Cervical cancer



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Mutations in PI3K and Ras/Raf pathway

Colon cancer as example

HT-29

PIK3CA	**p.P449T**
BRAF	**p.V600E**
SMAD4	p.Q311*
TP53	p.R273H
APC	p.E853*
APC	p.T1556fs*3

RKO

PIK3CA	**p.H1047R**
BRAF	**p.V600E**
NF1	p.Y628fs*3
NF1	p.N2341fs*5

LS-174T

PIK3CA	**p.H1047R**
KRAS	**p.G12D**
CTNNB1	p.S45F
KDM6A	p.E1316fs*17

HCT116

PIK3CA	**p.H1047R**
KRAS	**p.G13D**
CDKN2A	p.R24fs*20
CTNNB1	p.S45del
MLH1	p.S252*

T84

PIK3CA	**p.E542K**
KRAS	**p.G13D**
TP53	p.?
APC	p.L1488fs*19

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Meet the challenges

- Combination therapy
  - Effect of signaling cascades network
  - Drug resistance and sensitivity
  - Targeting multiple pathways
    - Inhibition of PI3K, mTOR, ERK
    - Melanoma, beyond BRAFV600E
      additional potential drivers in Melanoma: PI3K pathway, NRAS mutations, KIT mutation, etc.
    - P53 activates the transcription of *PTEN* and *TSC2*, and functions as a negative regulator of the entire PI3K signaling pathway
    - Other players compromise PI3K inhibition in breast cancer: HER3, ER, IGFR…

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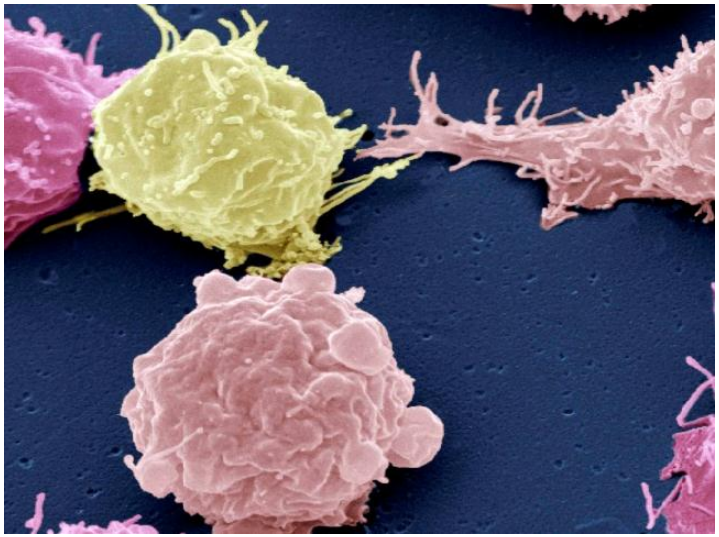
ATCC: Your Trusted Source

Applications for ATCC tumor cell panels

- Biological understanding of top genetic alterations across tumor types
- Validation and characterization of potential cancer driver genes
- Functional profiling and molecular profiling of subtype-specific cancer cell lines
- Testing small molecules or biologics for cancer drug development

For reproducible and reliable results

- Critical culture attributes:
  - Low-passage cell line
  - Cell growth properties and morphology
  - Population doubling level and time
  - Verification and authentication



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Winning the War on Cancer: Collaboration

Thank you
for your attention

Collaboration & Teamwork

ATCC