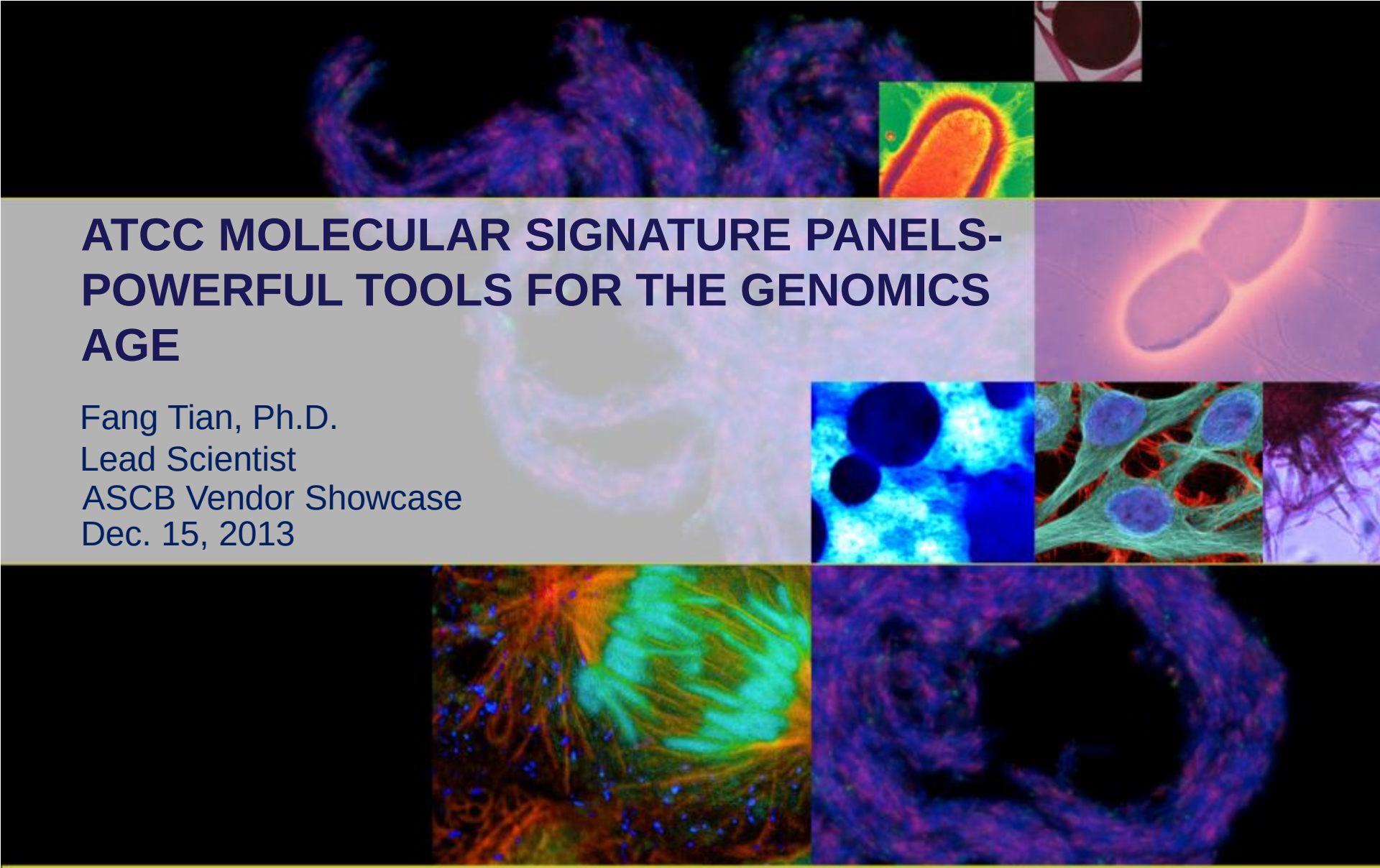
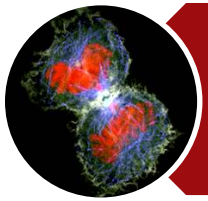
ATCC MOLECULAR SIGNATURE PANELS- POWERFUL TOOLS FOR THE GENOMICS AGE

Fang Tian, Ph.D.
Lead Scientist
ASCB Vendor Showcase
Dec. 15, 2013

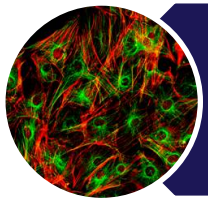


ATCC®
THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

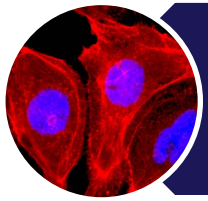
Outline



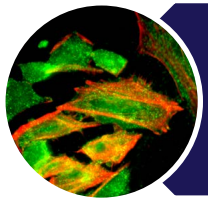
Cancer genome



Tumor Cell Panels



Molecular Signature Tumor Cell Panels



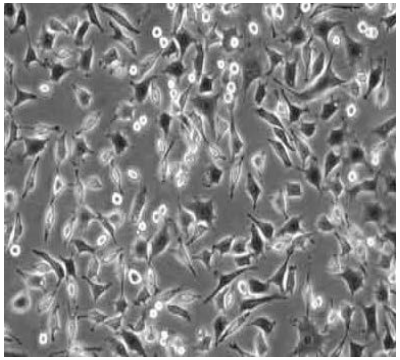
Applications in basic research, drug discovery, and molecular diagnostics

ATCC

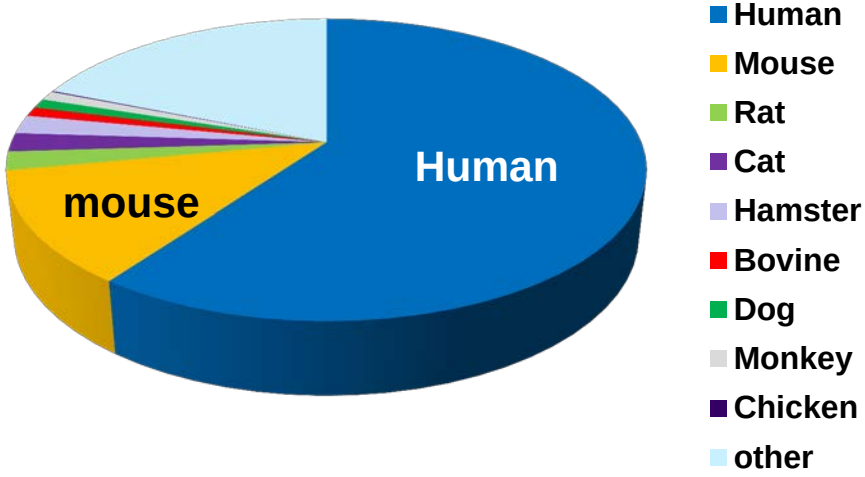
Photo credit: P. Andrews, K Mackenzie

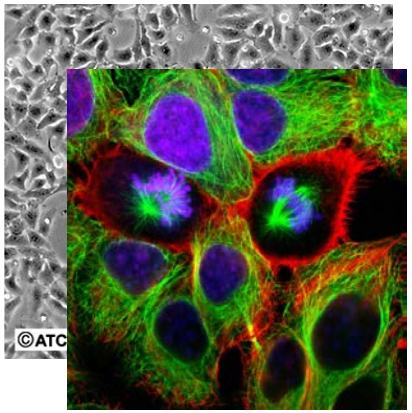
ATCC cell biology general collection

- Established in 1962
- More than 3,000 Animal Cell Lines and Hybridomas
- Over 80 different species
- Most diverse collection of its kind in the world



CCL-1™: NCTC clone 929

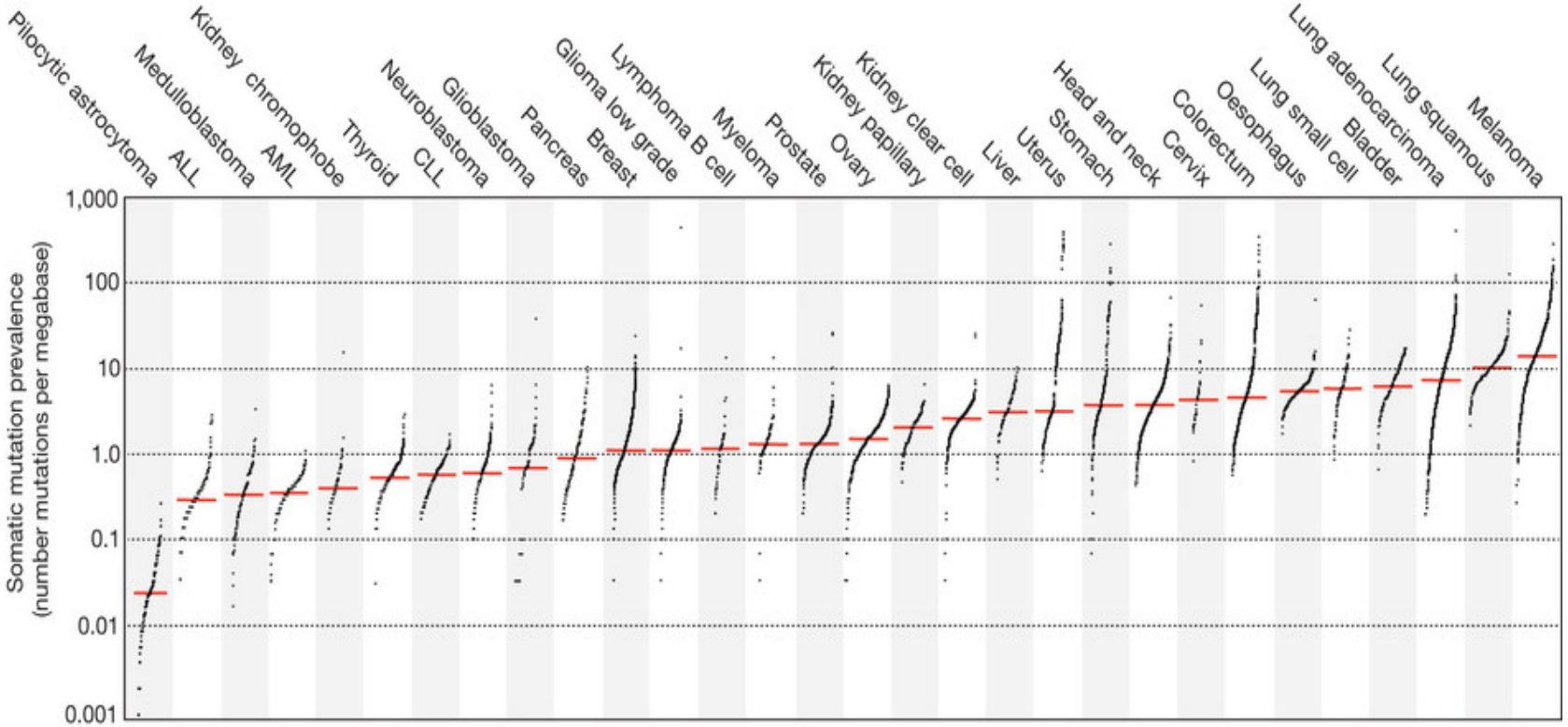




CCL-2™: HeLa

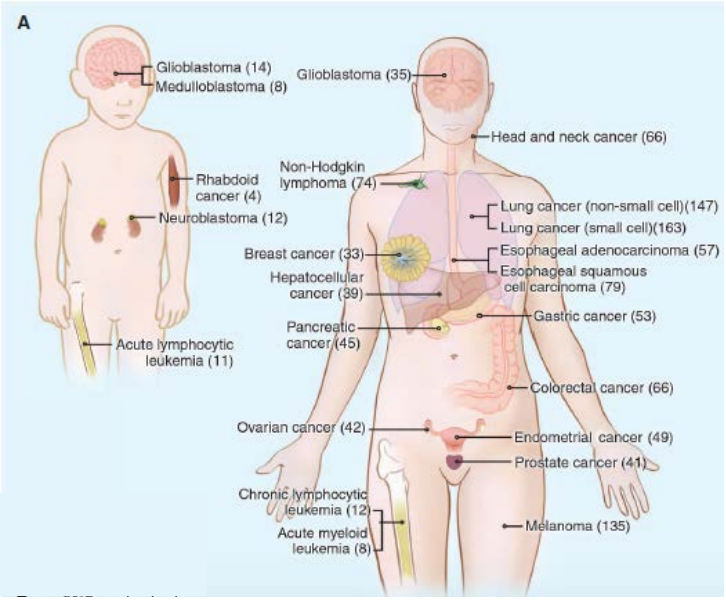
Somatic mutations in cancer

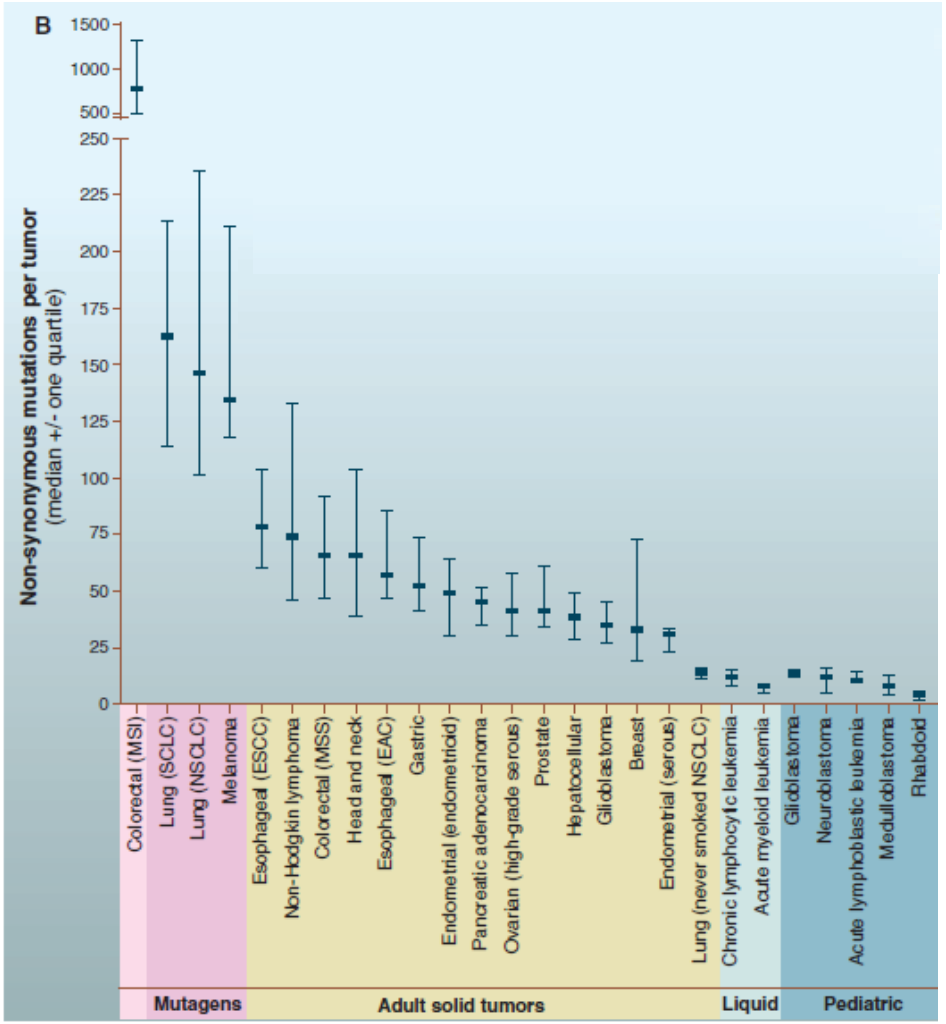
The prevalence of somatic mutations across human cancer types



Alexandrov LB, *et al. Nature* 500: 415-421, 2013

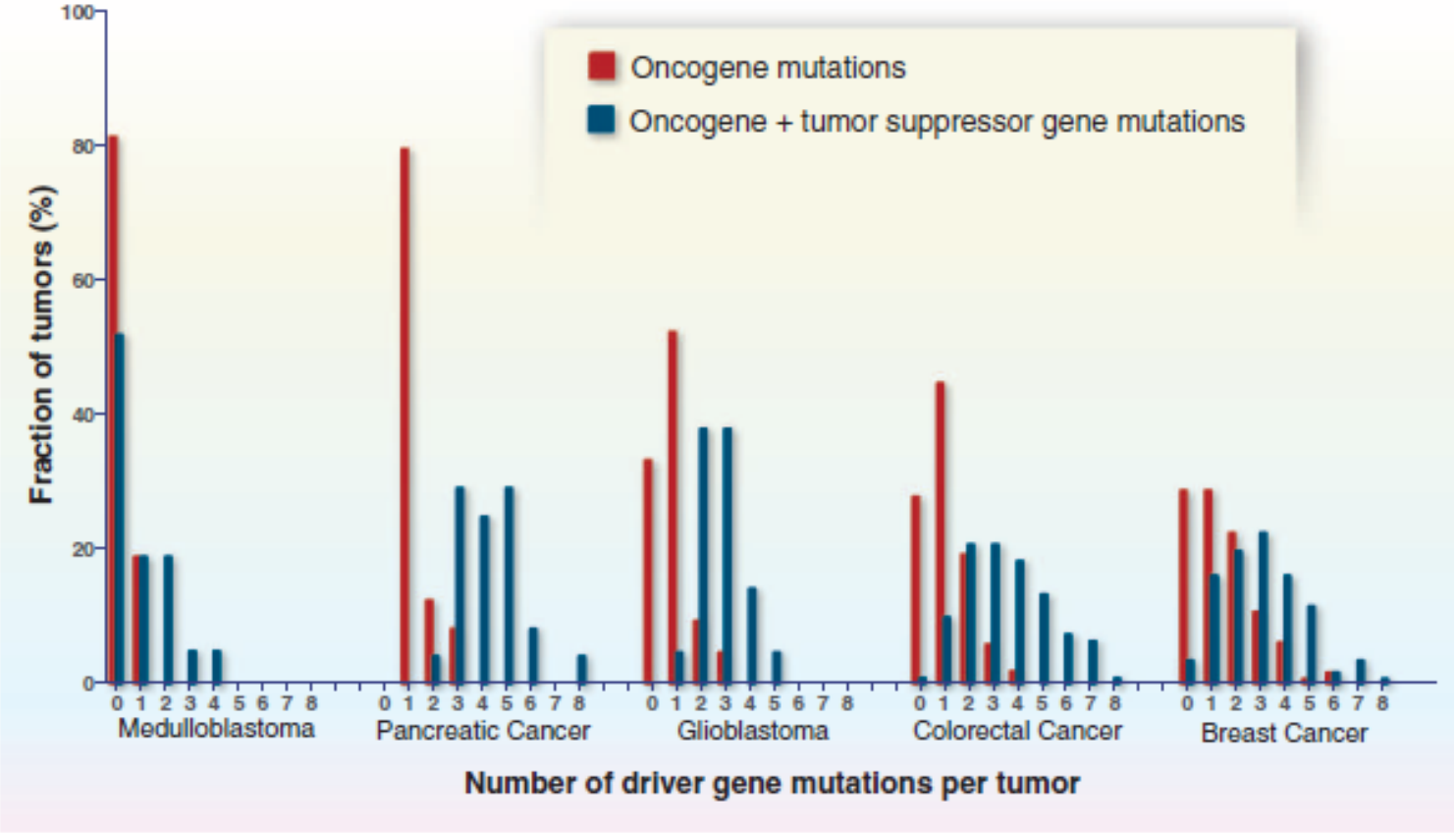
Somatic mutations in cancer





Vogelstein B, *et al. Science* 339: 1546-1558, 2013

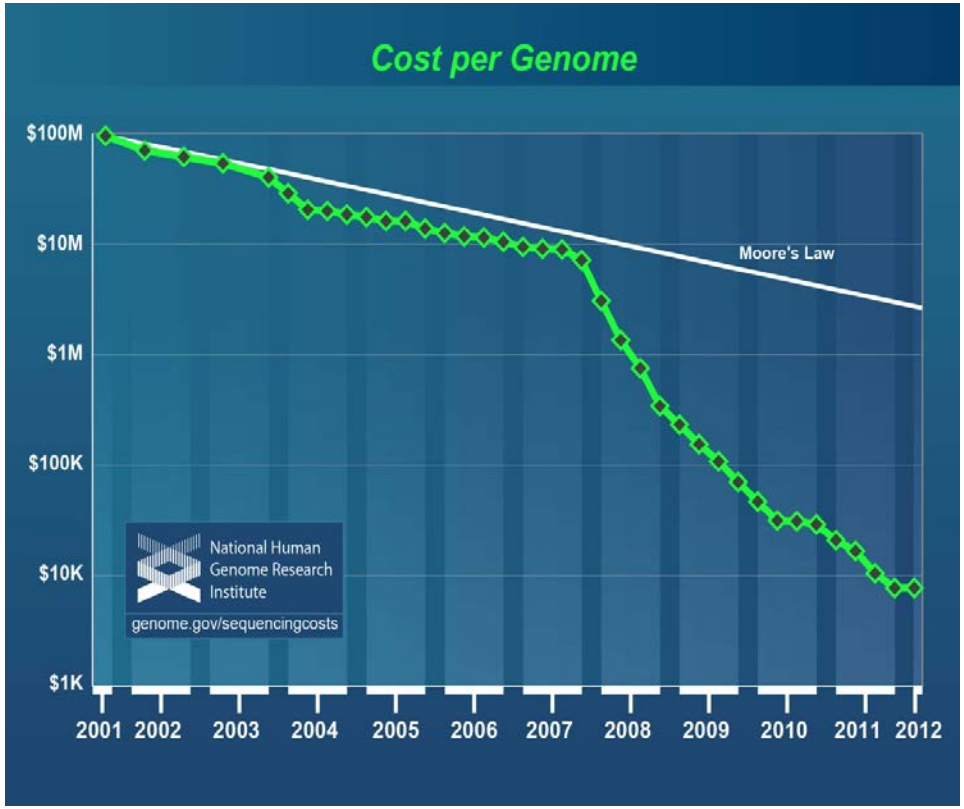
Driver mutations in cancer



Vogelstein B, *et al. Science* 339: 1546-1558, 2013

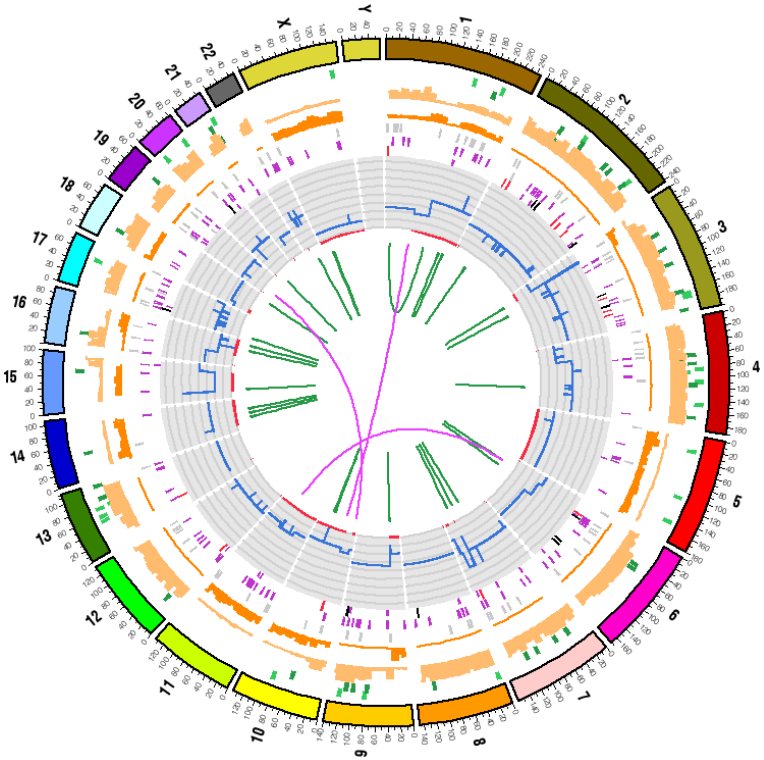
NGS leads to genomic age

Next generation sequencing



National Human Genome Research Institute website (www.genome.gov)

Cancer genome



Circos cancer genome display

circos.ca website (http://circos.ca/)

The changing landscape

What are you working on?

Basic research

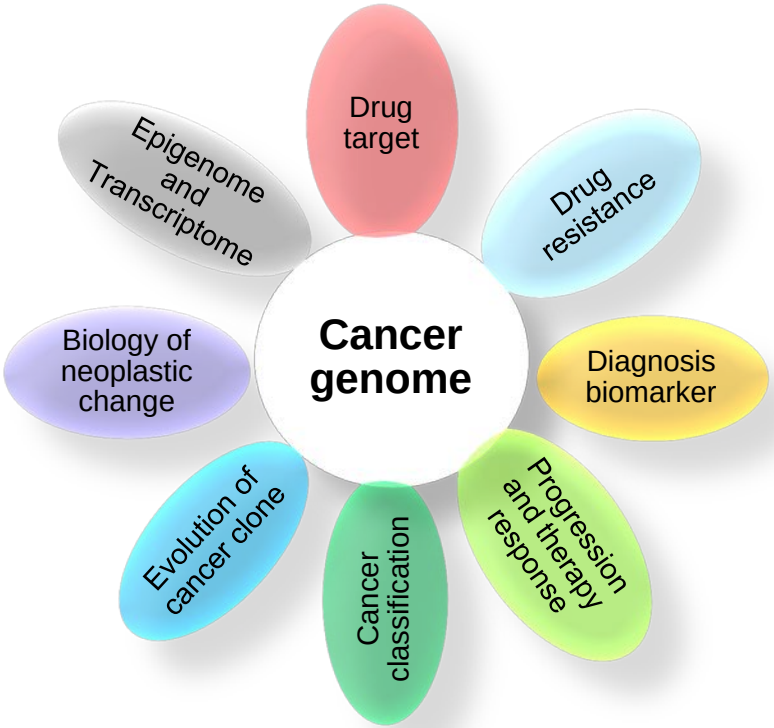
- Discovering new molecular mechanisms
- Identification of novel genes and proteins
- Cell function and biology

Translational research

- Identification of new therapeutic targets
- Novel drug discovery and development
- Drug resistance
- Combination therapeutics

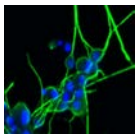
Clinical pathology

- Molecular diagnostics
- Proficiency tests

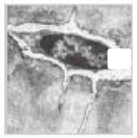


ATCC

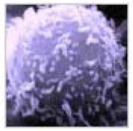
ATCC Tumor Cell Panels



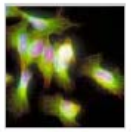
Brain cancer cell panel



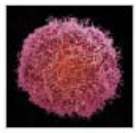
Bone cancer cell panel



Lymphoma cell panel

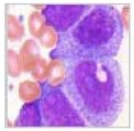


Soft-Tissue Sarcoma cell panel

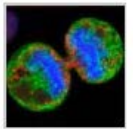


Head/Neck cancer cell panel

Lung cancer cell panel

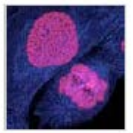


Leukemia cell panel

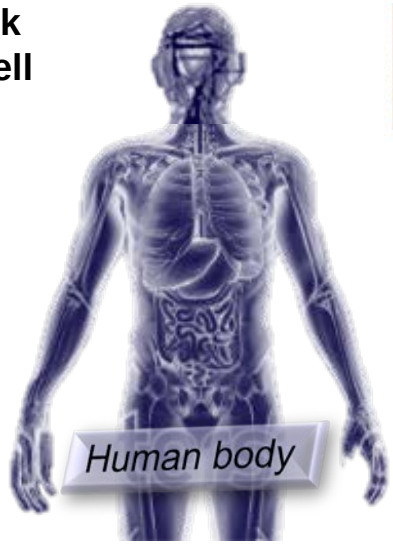


Melanoma cancer cell panel

Pancreatic cancer cell panel



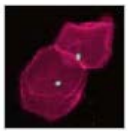
Breast cancer cell panel



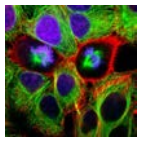
Liver cancer cell panel

Stomach cancer cell panel

Colon cancer cell panel



Bladder cancer cell panel



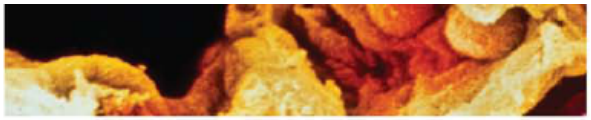
Gynecologic cancer cell panel

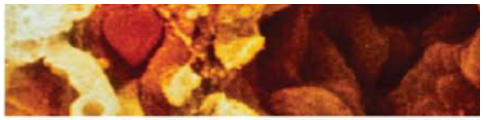
Annotated with genetic alterations

ATCC

ATCC Tumor Cell Panels

THE ESSENTIALS OF
LIFE SCIENCE RESEARCH
GLOBALLY DELIVERED™





ATCC™

COLON CANCER PANELS 1 AND 2

Colon Cancer Panel 1, KRAS (ATCC® No. TCP-1006™) is comprised of eight colon cancer cell lines. Seven of the eight cell lines carry a KRAS mutation as well as other mutations with varying degrees of genetic complexity.

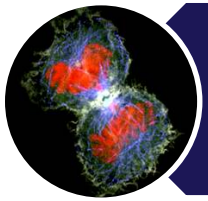
Colon Cancer Panel 2, BRAF (ATCC® No. TCP-1007™) is comprised of eight colon cancer cell lines. Six of the eight cell lines carry a BRAF mutation in addition to mutations in other genes. The table below provides more information for the cell lines included in each panel.

Colon Cancer Panel 1, KRAS (ATCC® No. TCP-1006™)

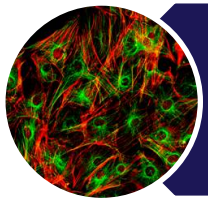
Catalog #	Name	Tissue	Histology	Source	Mutation	Zygosity	Gene sequence	Protein sequence
CRL-5972™	SNU-C1	Colon	Adenocarcinoma	metastasis, peritoneum	TP53	homozygous	c.497C>A	p.S166*
HTB-39™	SK-CO-1	Colon	Adenocarcinoma	metastasis, ascites	APC	heterozygous	c.3266delT	p.F1089fs*37
					APC	heterozygous	c.4328delC	p.P1443fs*30
					KRAS	homozygous	c.35G>T	p.G12V
CCL-233™	SW1116	Colon	Adenocarcinoma	primary	APC	heterozygous	c.4287_4296delAACCATGCCA	p.Q1429fs*41
					APC	heterozygous	c.35G>C	p.G12A
					KRAS			
					TP53		c.476C>A	p.A159D
CCL-237™	SW948	Colon	Adeno-carcinoma	Metastasis lung	APC	Homo-zygous	c.3340C>T	p.R1114*
					APC	heterozygous	c.4285C>T	p.Q1429*
					KRAS		c.182A>T	p.Q61L
					PIK3CA	Hetero-zygous	c.1624G>A	p.E542K
CCL-248™	T84	Colon	Carcinoma	Primary	APC		c.4464delA	p.L1488fs*19
					KRAS	heterozygous	c.38G>A	p.G13D
					APC	heterozygous	c.1624G>A	p.E542K
					FAM123B	homozygous	c.376-1G>T	p.?
CCL-255™	LS123	Rectum	Adenocarcinoma	primary	FBXW7	heterozygous	c.1873C>T	p.Q625*
					KRAS	heterozygous	c.4348C>T	p.R1450*
					TP53	heterozygous	c.34G>A	p.G12S
					SMAD4	homozygous	c.988G>T	p.E330*
					TP53	heterozygous	c.524G>A	p.R175H

ATCC®

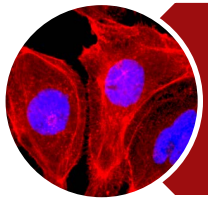
Outline



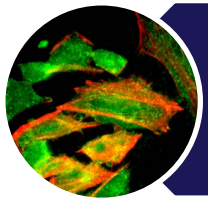
Cancer genome



Tumor Cell Panels



Molecular Signature Tumor Cell Panels

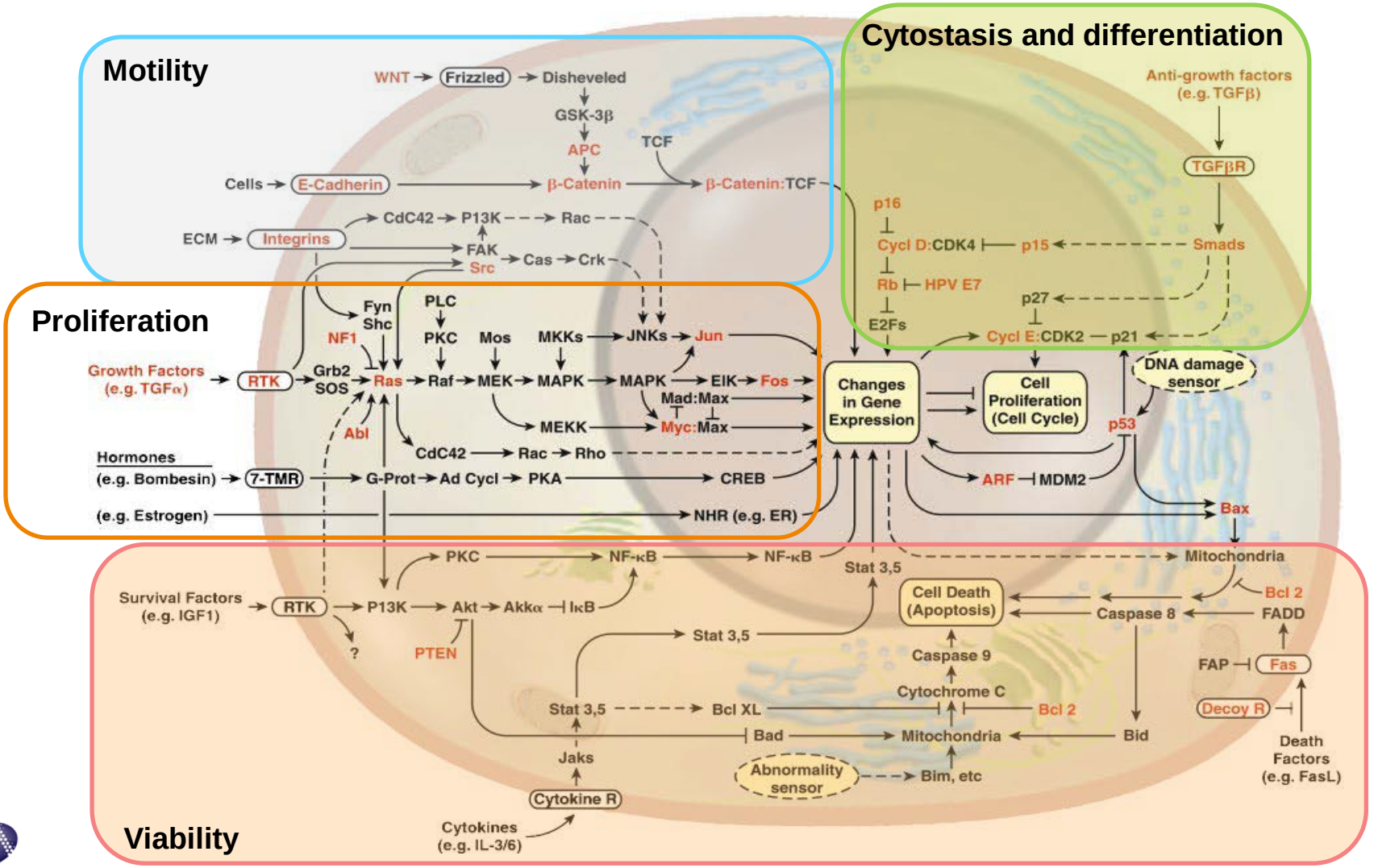


Applications in basic research, drug discovery, and molecular diagnostics

ATCC

Photo credit: P. Andrews, K Mackenzie

Molecular nature of cells



Hanahan D, Weinberg RA. *Cell* 144(5): 646-674, 2011

ATCC Molecular Signature Cell Panels

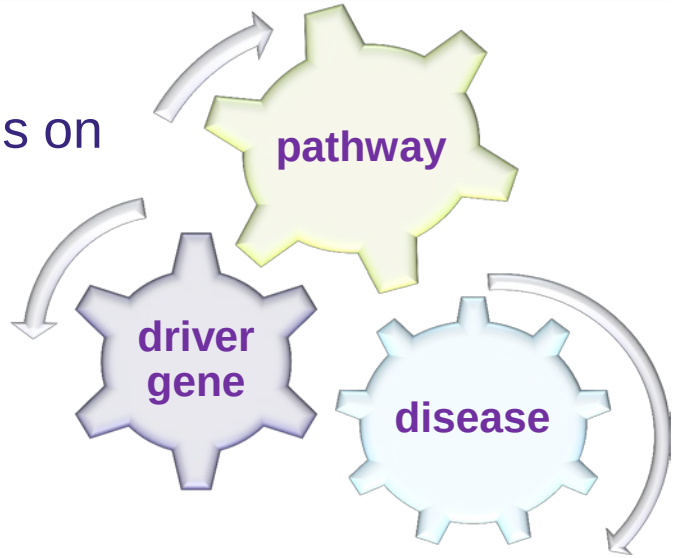
Integrative designed cell panels

ATCC cell lines

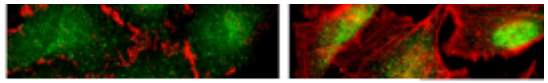
Cancer genome

- Systematic analysis
- Gene sequencing validation
- Functional characterization
- Integrative design

Focus on

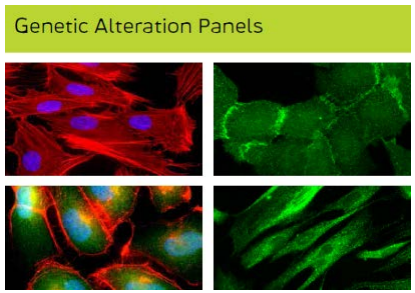


p53 hotspot mutation panels

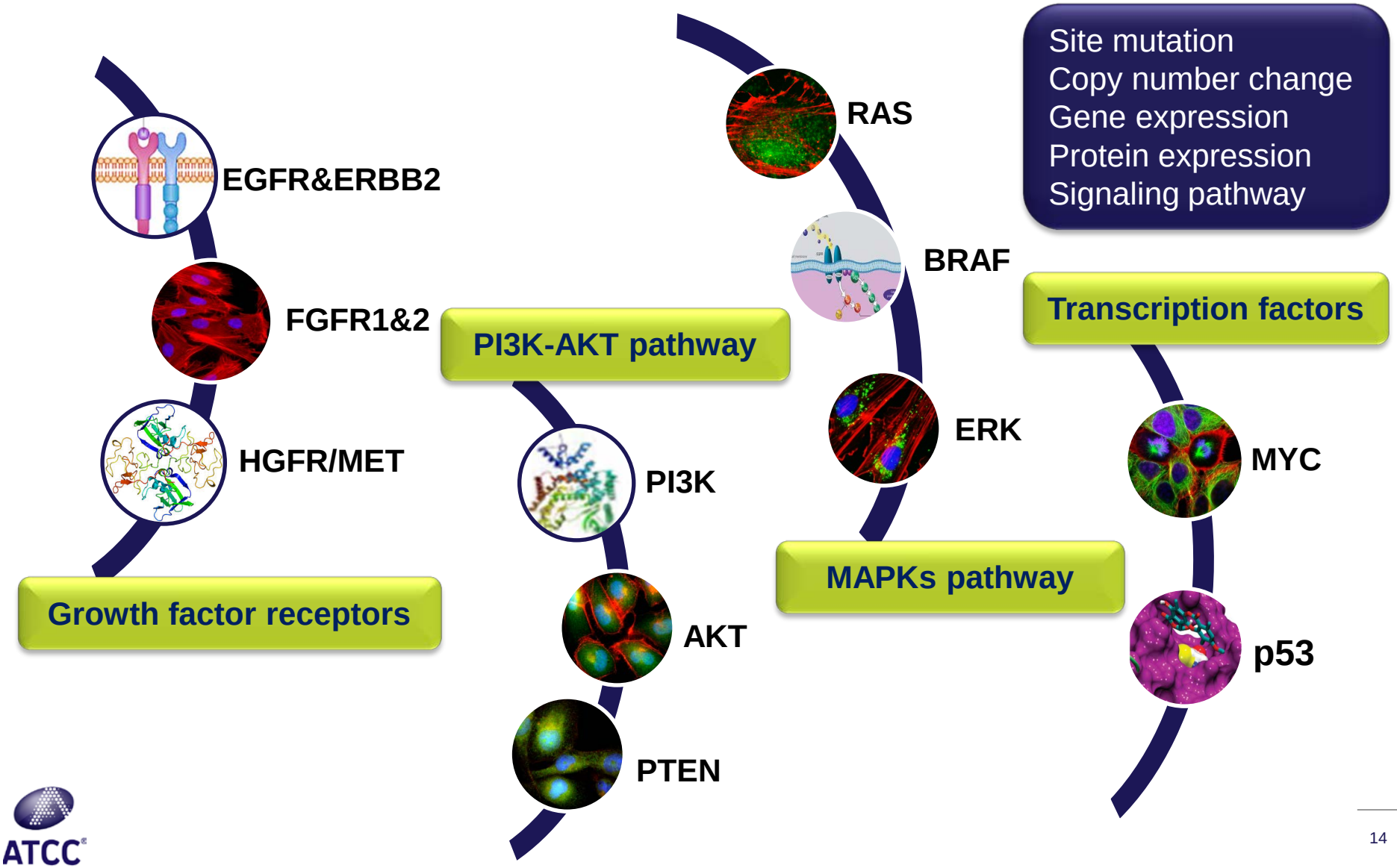


NON-SMALL CELL LUNG CANCER P53 HOTSPOT MUTATION CELL PANEL

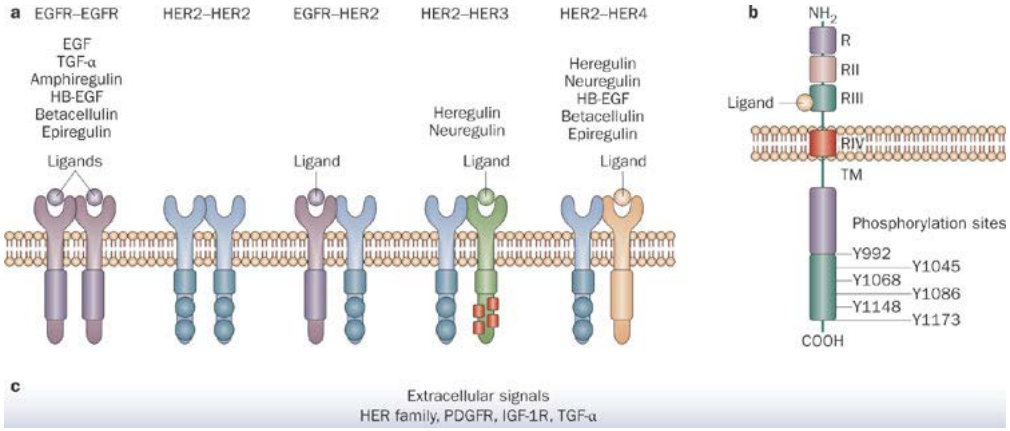
Genetic alteration cell panels

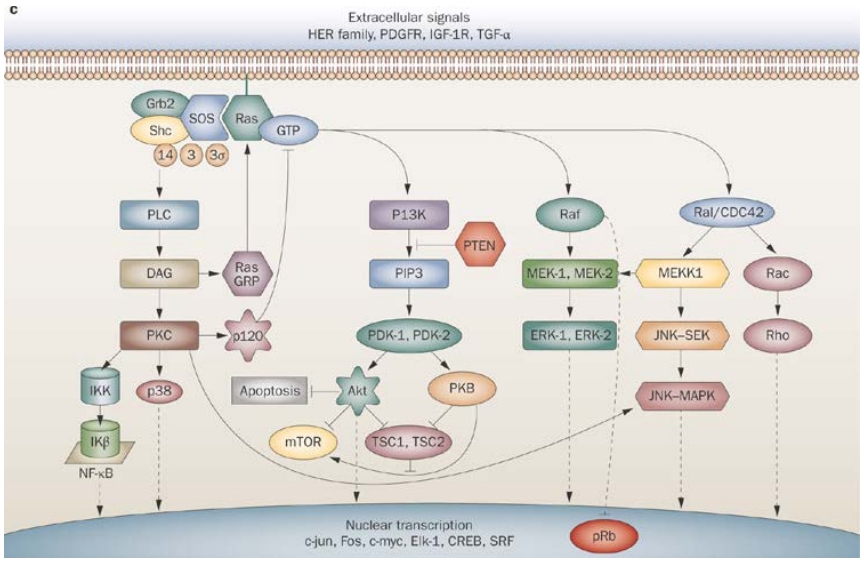


Molecular Signature Cell Panels



EGFR introduction





Domains

- Extracellular
- Transmembrane
- Intracellular

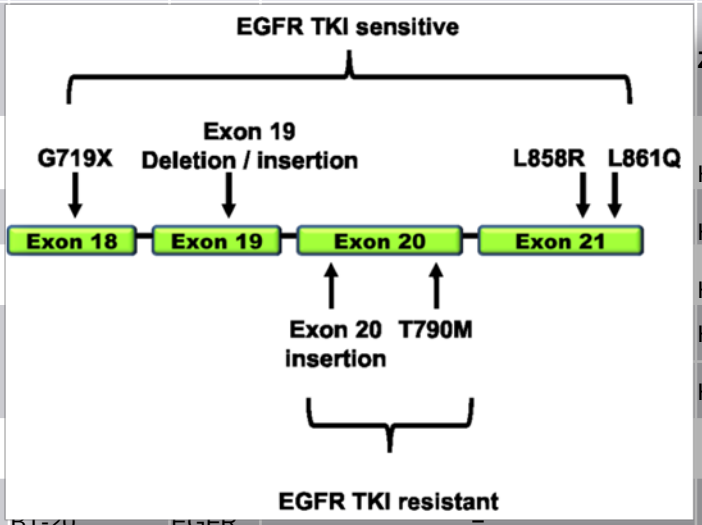
Functions

- Cell proliferation
- Cell viability
- Invasion
- Angiogenesis
- Metastasis

Linardou H, *et al., Nat Rev Clin Oncol* 6: 352-366, 2009

EGFR cell panel characteristics

EGFR Genetic Alteration Cell Panel (ATCC® TCP-1027™)



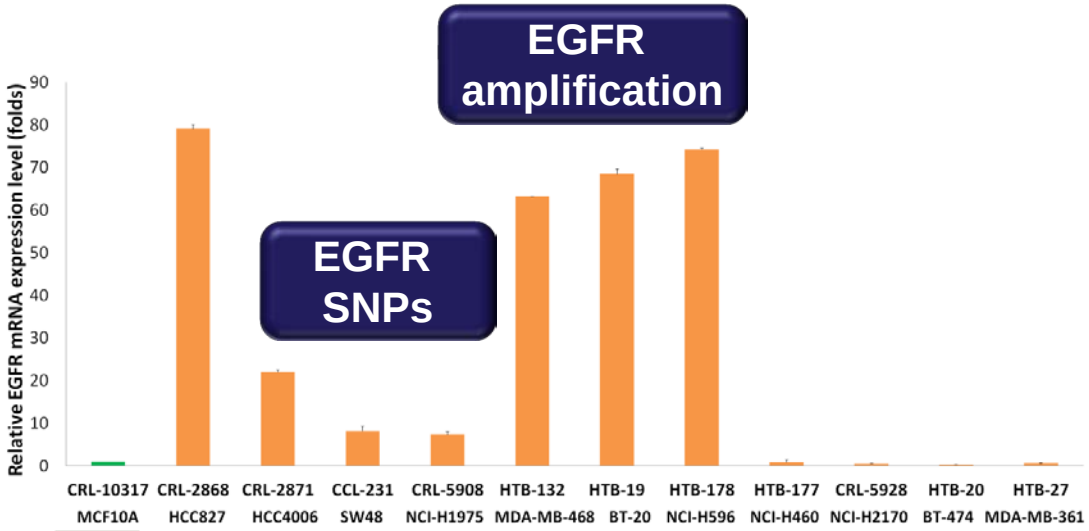
ATCC® number				Zygosity	Amino acid Change	EGFR copy number variation	ERBB2 copy number variation	Tumor source
CRL-2868™				Heterozygous	p.ELREA746del	Amplification	–	Lung
CRL-2871™				Heterozygous		–	–	Lung
CCL-231™				Heterozygous		–	–	Colon
CRL-5908™				Heterozygous		–	–	Lung
				Heterozygous			–	
HTB-132™				–	–			Breast
HTB-19™	BT-20	EGFR	–	–	–			Breast
HTB-178™	NCI-H596	EGFR	–	–	–			Lung
HTB-177™	NCI-H460	EGFR	–	–	–	–	–	WT
CRL-5928™	NCI-H2170	ERBB2	–	–	–	–		
HTB-20™	BT-474	ERBB2	–	–	–	–		
HTB-27™	MDA-MB-361	ERBB2	–	–	–	–		

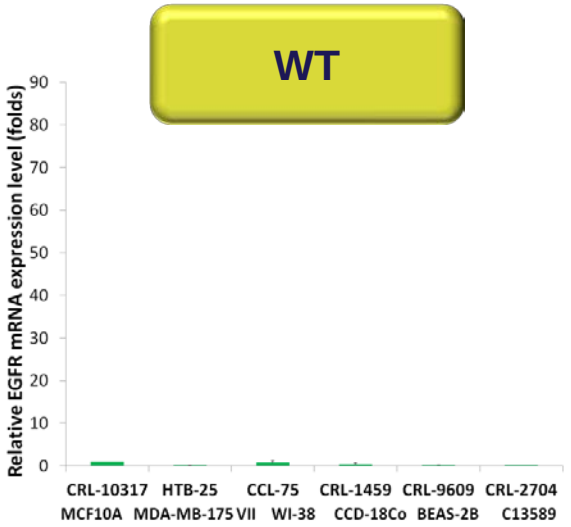
EGFR SNPs

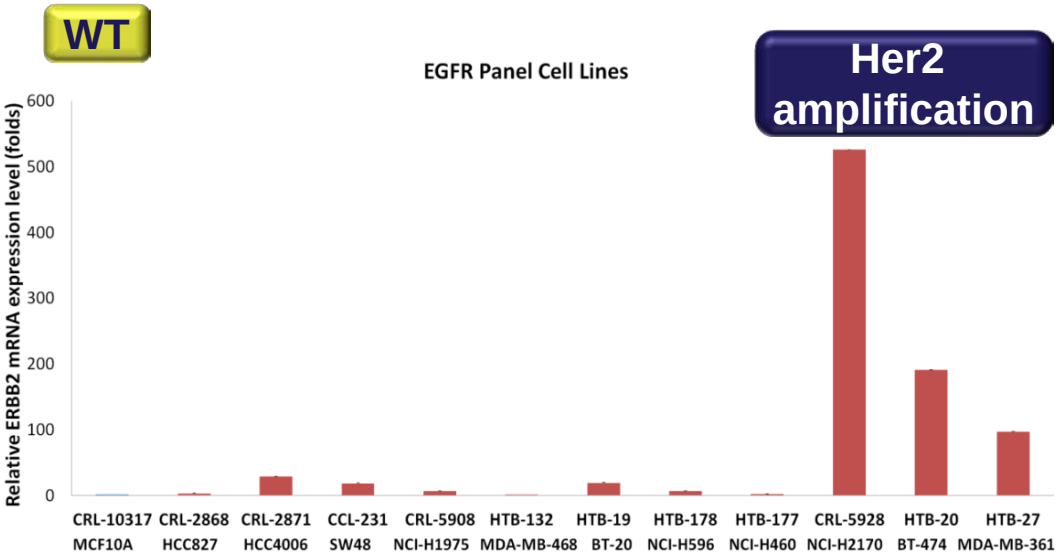
EGFR amplification

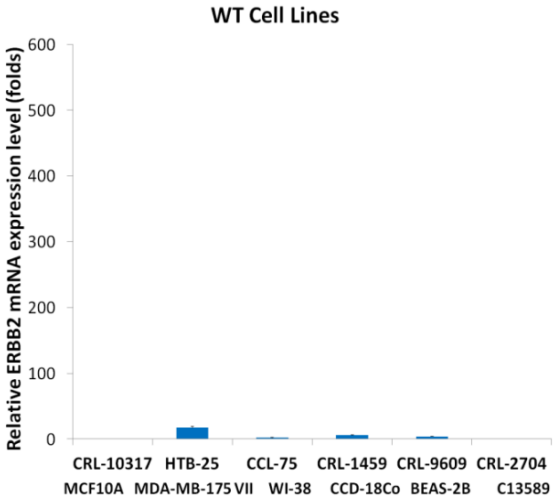
Her2 amplification

EGFR cell panel mRNA expression



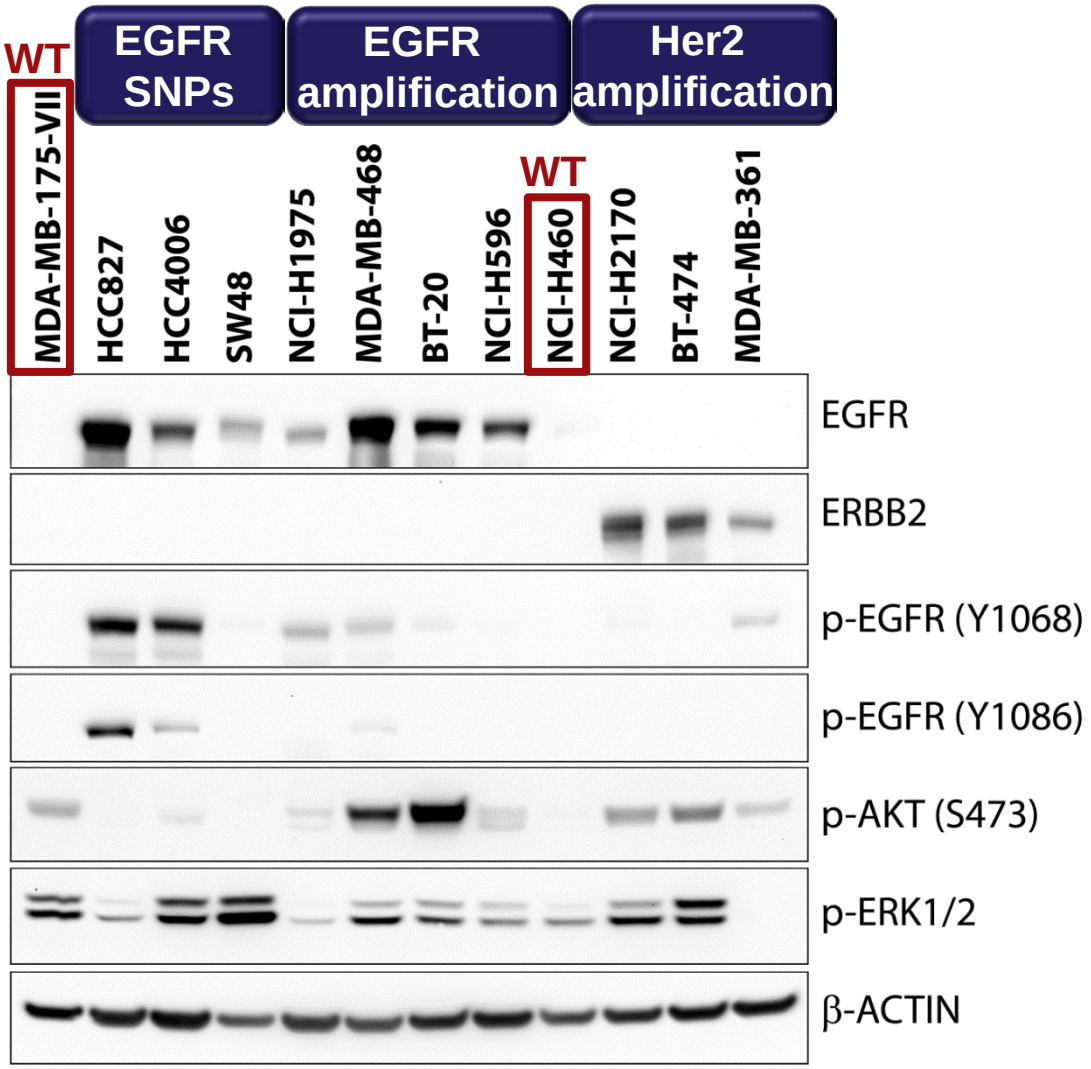




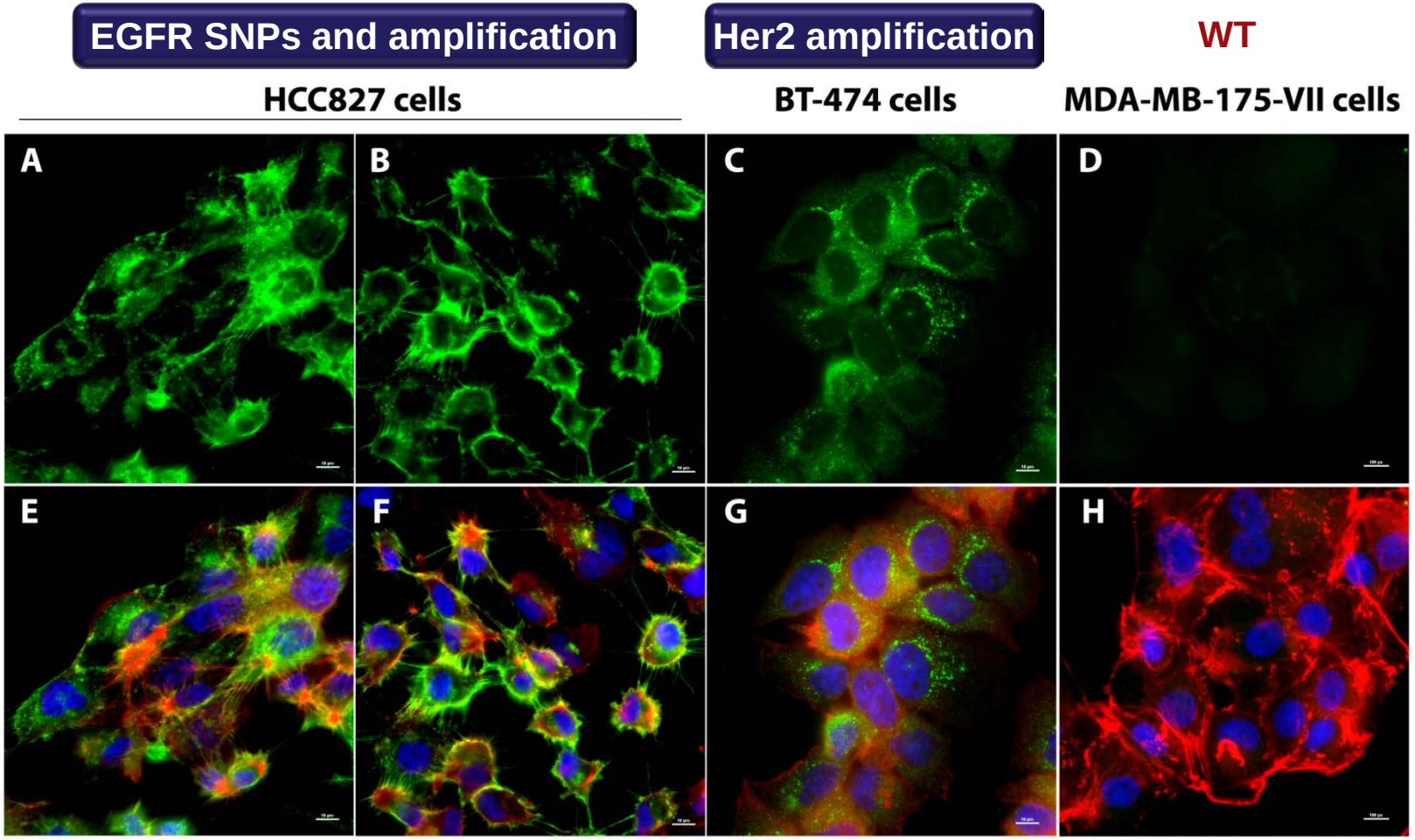


ATCC

EGFR Cell Panel protein expression

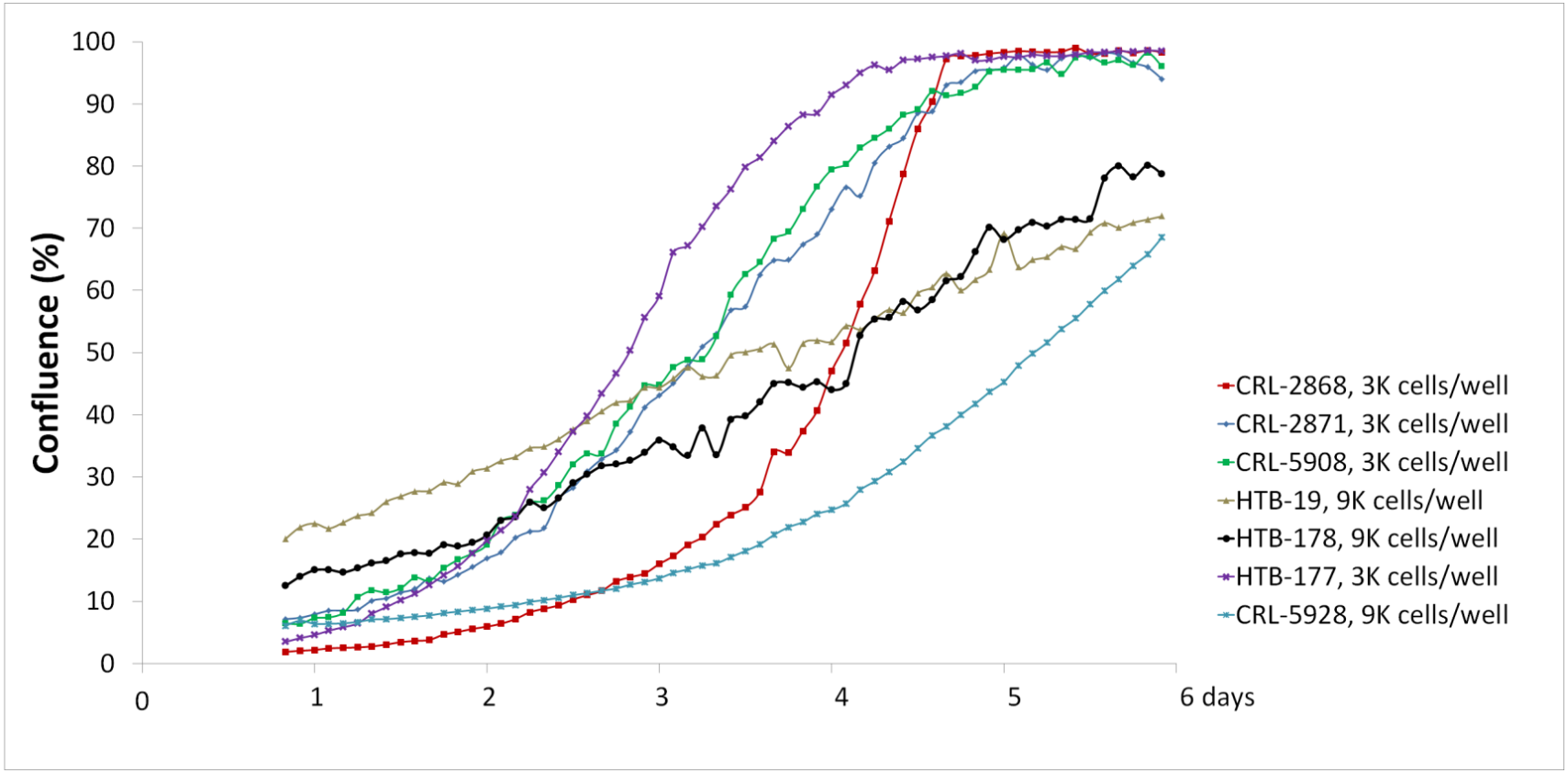


EGFR Cell Panel IF staining



IF Staining: A,D: EGFR , B: phospho-EGFR; C: ERBB2,
E-H, merged with F-actin/ Hoechst

EGFR panel cell growth kinetics



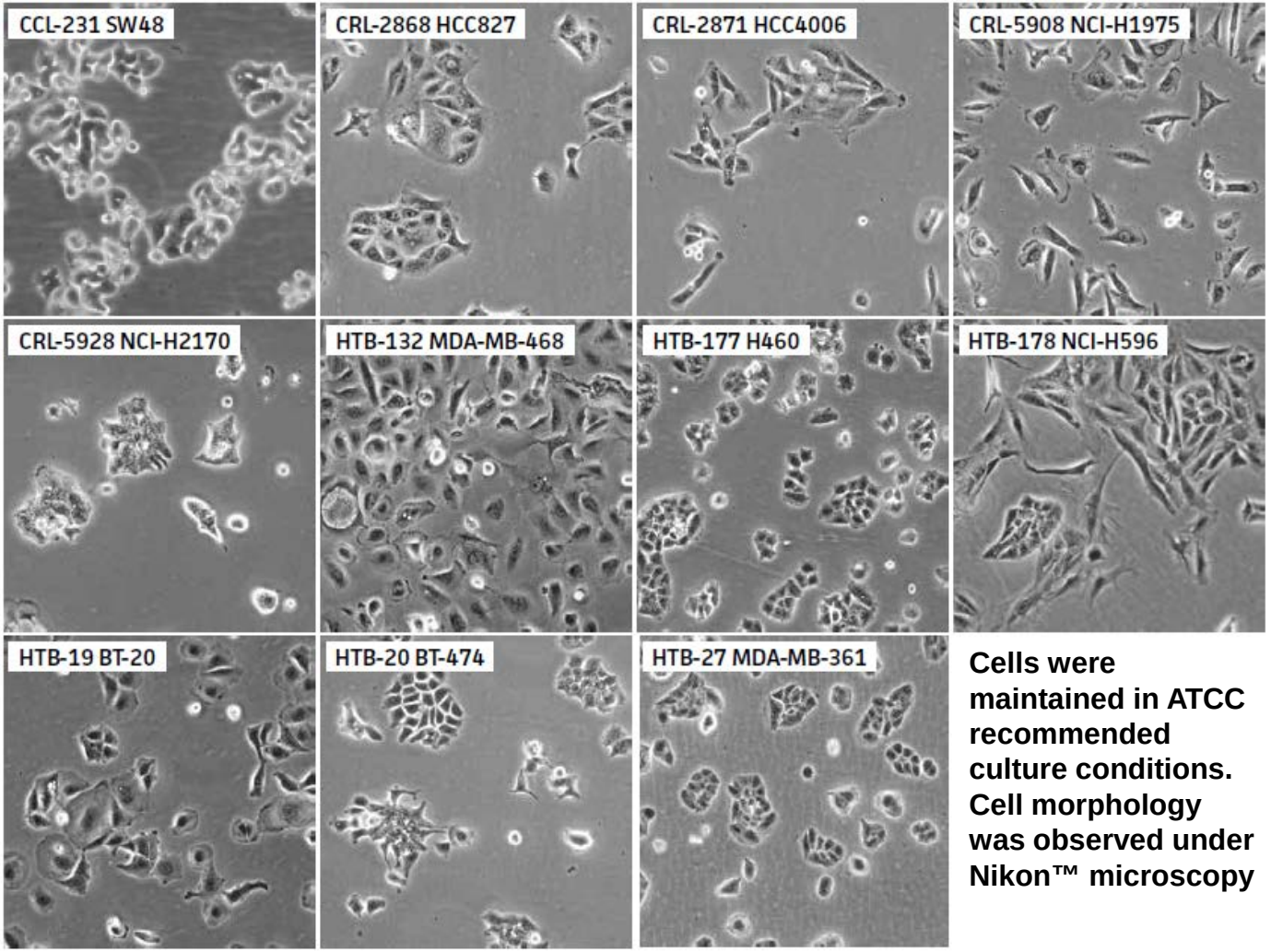
EGFR cell panel culture conditions

EGFR Genetic Alteration Cell Panel (ATCC® TCP-1027™)

ATCC® number	Cell line name	Tumor source	Histology	Media	Seeding Density (cells/cm²)	Time to Subculture	Split Ratio
CRL-2868™	HCC827	lung	adenocarcinoma	RPMI-1640 + 10% FBS	$2x10^4$	3-4 days	1:5
CRL-2871™	HCC4006	lung	adenocarcinoma	RPMI-1640 + 10% FBS	$2x10^4$	4 days	1:5
CCL-231™	SW48	colon	adenocarcinoma	Leibovitz's L-15 + 10% FBS	$5x10^4$ - $1x10^5$	4-5 days	1:5
CRL-5908™	NCI-H1975	lung	non small cell carcinoma	RPMI-1640 + 10% FBS	$4x10^4$	4-5 days	1:5
HTB-132™	MDA-MB-468	breast	adenocarcinoma	Leibovitz's L-15 + 10% FBS	$2x10^4$	4-5 days	1:5
HTB-19™	BT-20	breast	carcinoma	RPMI-1640 + 10% FBS	$2x10^4$ - $4x10^4$	2-5 days	1:2 to 1:5
HTB-178™	NCI-H596	lung	adenosquamous carcinoma	EMEM + 10% FBS	$2x10^4$ - $4x10^4$	5-7 days	1:2 to 1:4
HTB-177™	NCI-H460	lung	large cell carcinoma	RPMI-1640 + 10% FBS	$6x10^4$	3-4 days	1:10
CRL-5928™	NCI-H2170	lung	squamous cell carcinoma	RPMI-1640 + 10% FBS	$6x10^4$	3-4 days	1:5
HTB-20™	BT-474	breast	ductal carcinoma	Hybricare + 10% FBS	$3x10^4$	6-7 days	1:5
HTB-27™	MDA-MB-361	breast	adenocarcinoma	Leibovitz's L-15 + 20% FBS	$6x10^4$	4-5 days	1:5

ATCC®

EGFR panel cell morphology

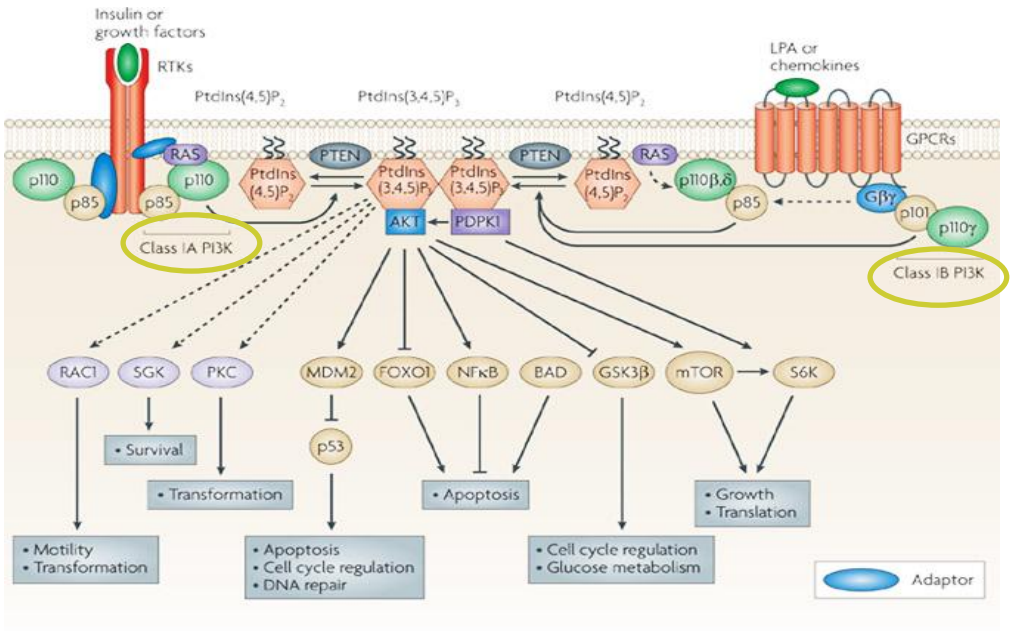


Cells were maintained in ATCC recommended culture conditions. Cell morphology was observed under Nikon™ microscopy

PI3k pathway

Regulates:

- Proliferation
- Survival/apoptosis
- Metabolism
- Angiogenesis
- Transformation

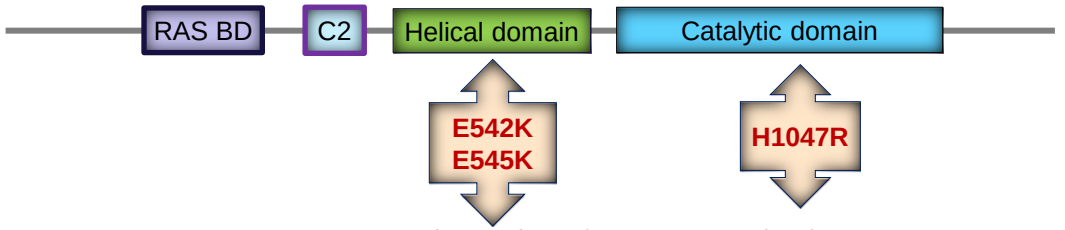


Liu P, et al. *Nature Reviews Drug Discovery,* 8: 627-644, 2009

Nature Reviews | Drug Discovery

Genetic alteration frequently found in cancers

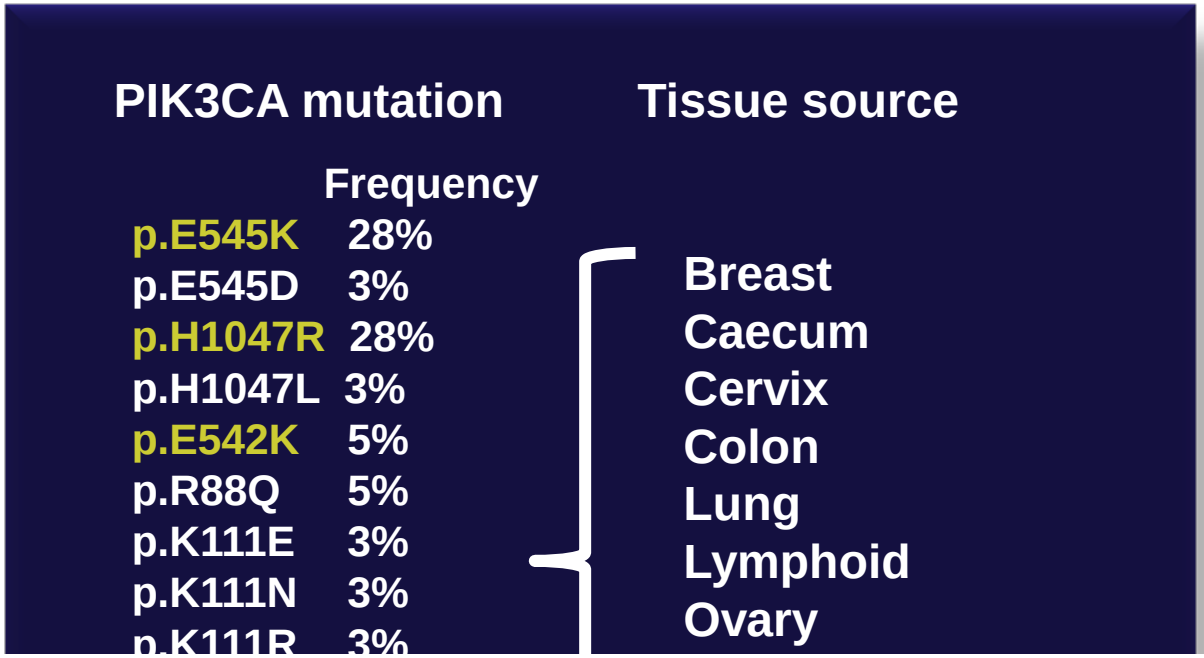
Most frequent mutation p110α (PIK3CA)



Abrogation of the inhibitory effect of the p85 subunit

Activation through conformational changes

PIK3CA mutation cells lines and panel

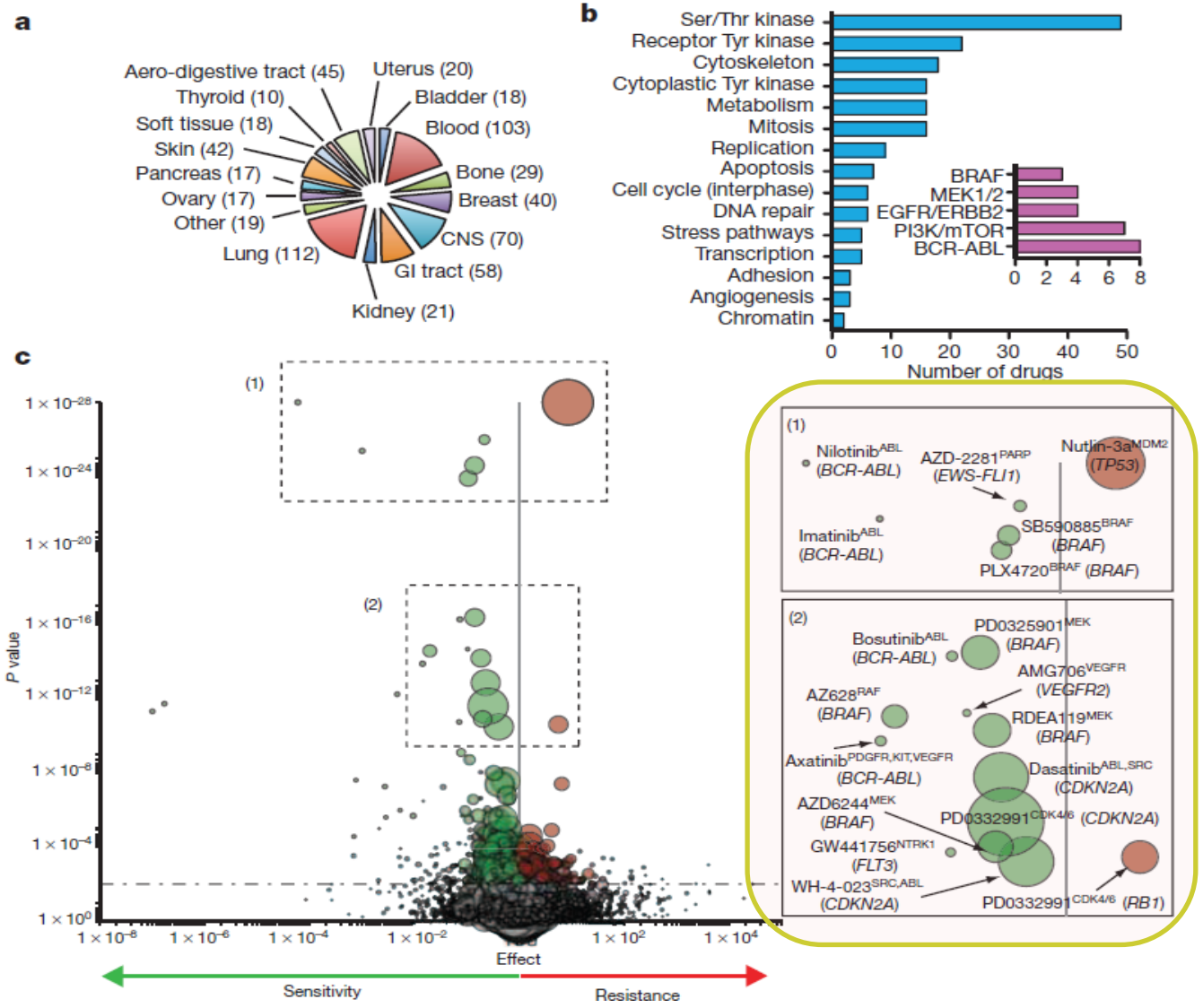


ATCC® No. TCP-1028™ PI3K genetic alteration cell panel

ATCC® No.	Name	Gene	DNA Change	Zygosity	Amino acid Change	Tumor source
CCL-225™	HCT-15	PIK3CA	c.1633G>A c.1645G>A	Heterozygous	p.E545K p.D549N	colon large intestine
CCL-237™	SW948	PIK3CA	c.1624G>A	Heterozygous	p.E542K	colon
CRL-1739™	AGS	PIK3CA	c.1634A>C	Heterozygous	p.E545A	stomach
CRL-2577™	RKO	PIK3CA	c.3140A>G	Heterozygous	p.H1047R	colon
HTB-112™	HEC-1-A	PIK3CA	c.3145G>C	Heterozygous	p.G1049R	endometrium
HTB-121™	BT-483	PIK3CA	c.1624G>A	Heterozygous	p.E542K	breast
HTB-131™	MDA-MB-453	PIK3CA	c.3140A>G	Heterozygous	p.H1047R	breast
HTB-178™	NCI-H596	PIK3CA	c.1633G>A	Heterozygous	p.E545K	lung
HTB-19™	BT-20	PIK3CA	c.3140A>G	Heterozygous	p.H1047R	breast
HTB-27™	MDA-MB-361	PIK3CA	c.1633G>A c.1700A>G	Heterozygous	p.E545K p.K567R	breast

ATCC®

Genetic alterations affect drug sensitivity

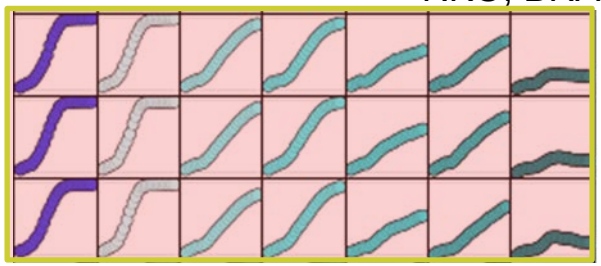


Garnett MG, Edelman EJ, *et al*. *Nature* 483: 570-575, 2012.

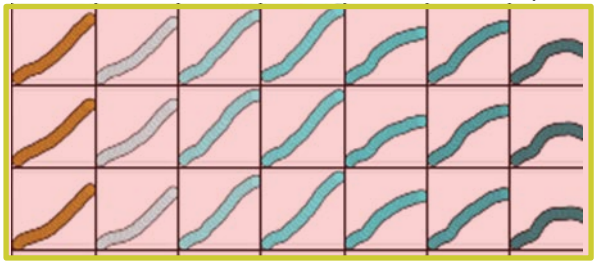
Genetic alterations affect drug sensitivity

MEK inhibitor treatment at various doses, cell growth kinetics were recorded for 6 days

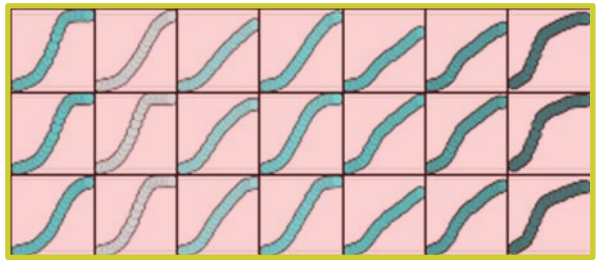
Colon cancer cell line RKO, $BRAF^{V600E}$



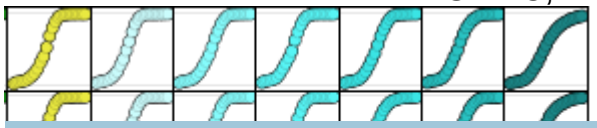
Melanoma cell line MeWo, ERK^{P246S}

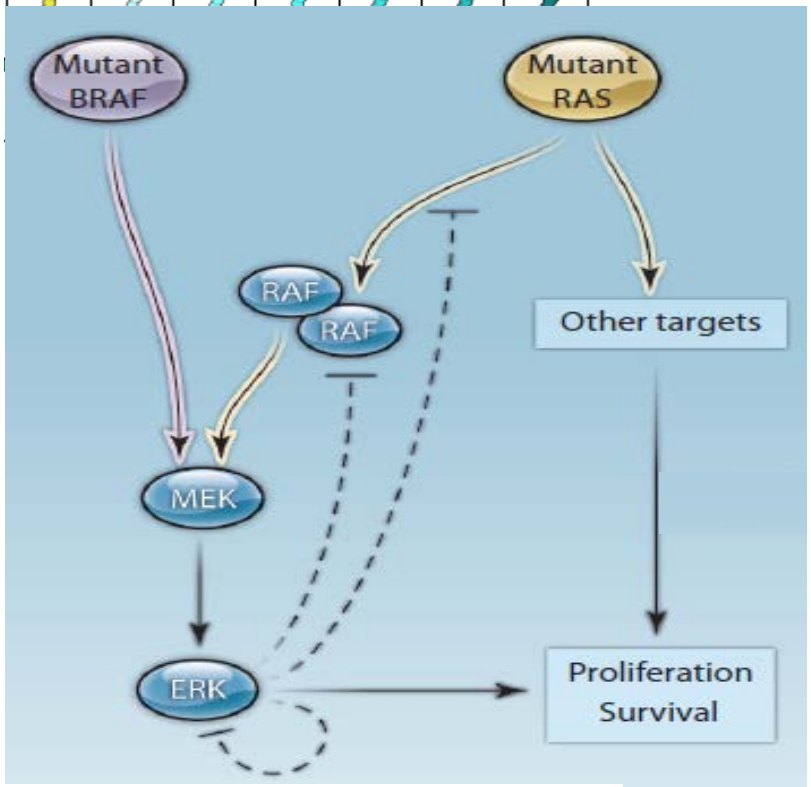


Melanoma cell line A2058 $BRAF^{V600E}$



Colon cancer cell line HCT-15, $KRAS^{G13D}$





Poulikakos P. *et.al*., *Science signaling* 4: 16, 2011.

ERK somatic mutations

MAPK1: Mitogen-activated protein kinase 1

- Encodes for ERK2
- Location: 22q11.21

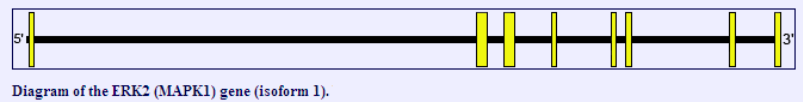


Diagram of the ERK2 (MAPK1) gene (isoform 1).

MAPK3: Mitogen-activated protein kinase 3

- Encodes for ERK1
- Location: 16p11.2

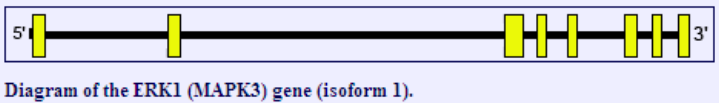
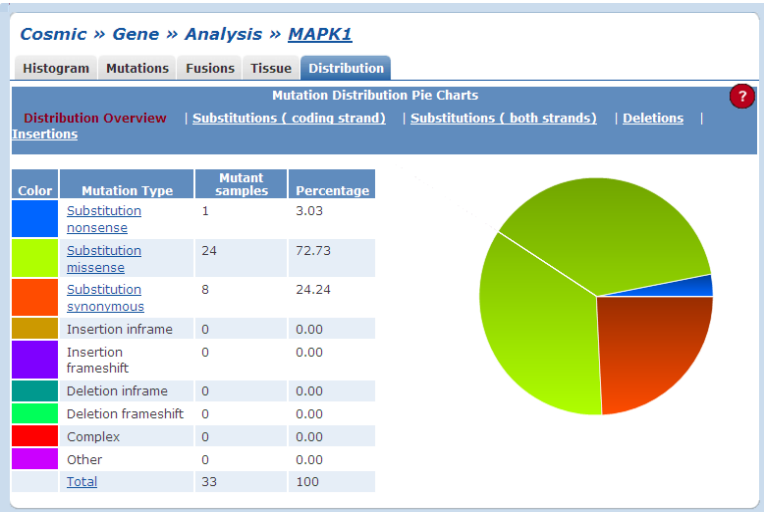


Diagram of the ERK1 (MAPK3) gene (isoform 1).



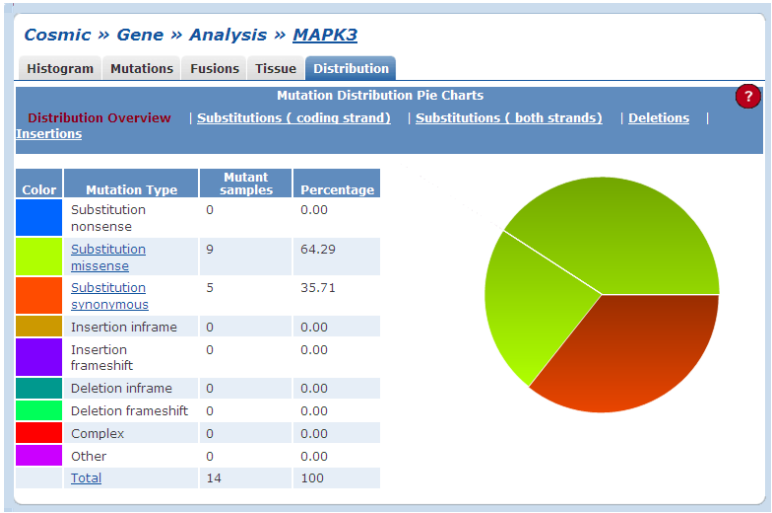
Cosmic » Gene » Analysis » MAPK1

Histogram | Mutations | Fusions | Tissue | Distribution

Mutation Distribution Pie Charts

Distribution Overview | Substitutions (coding strand) | Substitutions (both strands) | Deletions | Insertions

Color	Mutation Type	Mutant samples	Percentage
	Substitution nonsense	1	3.03
	Substitution missense	24	72.73
	Substitution synonymous	8	24.24
	Insertion inframe	0	0.00
	Insertion frameshift	0	0.00
	Deletion inframe	0	0.00
	Deletion frameshift	0	0.00
	Complex	0	0.00
	Other	0	0.00
	Total	33	100



Cosmic » Gene » Analysis » MAPK3

Histogram | Mutations | Fusions | Tissue | Distribution

Mutation Distribution Pie Charts

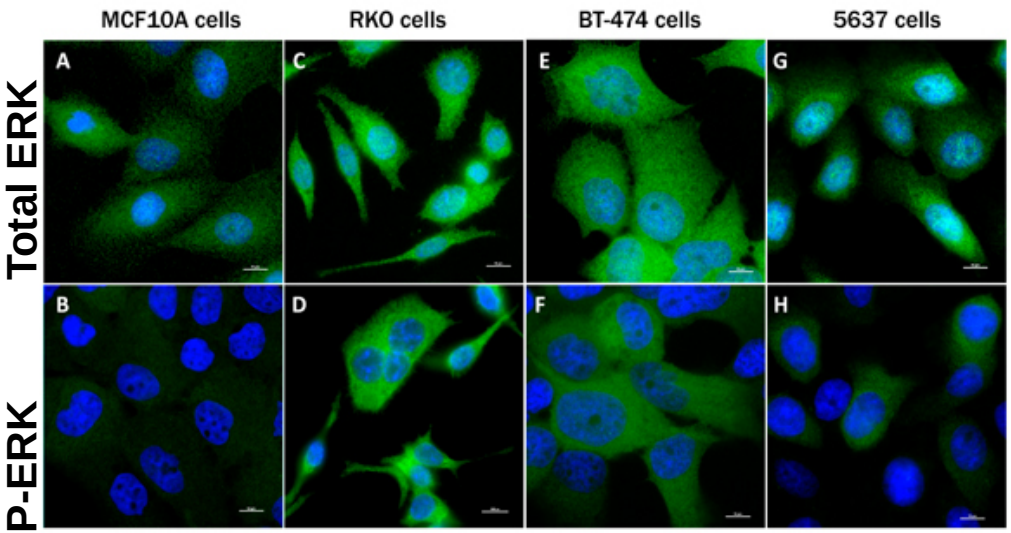
Distribution Overview | Substitutions (coding strand) | Substitutions (both strands) | Deletions | Insertions

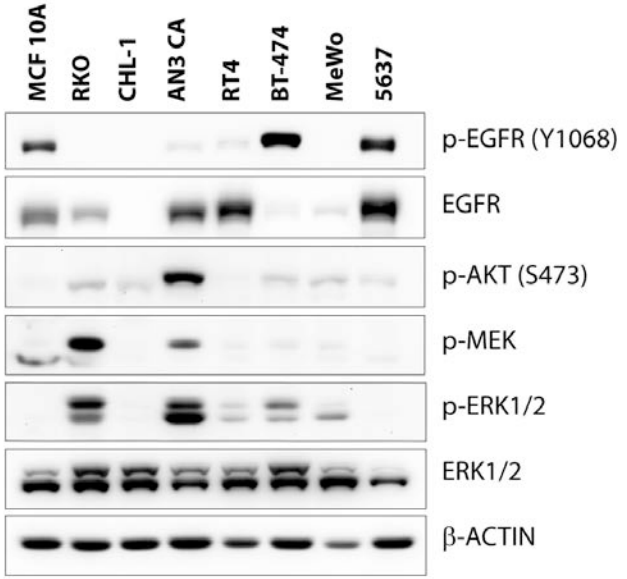
Color	Mutation Type	Mutant samples	Percentage
	Substitution nonsense	0	0.00
	Substitution missense	9	64.29
	Substitution synonymous	5	35.71
	Insertion inframe	0	0.00
	Insertion frameshift	0	0.00
	Deletion inframe	0	0.00
	Deletion frameshift	0	0.00
	Complex	0	0.00
	Other	0	0.00
	Total	14	100

ERK Genetic Alteration Panel

ERK Genetic Alteration Cell Panel (ATCC® TCP-1033™)

ATCC® number	Cell line name	Gene	cDNA Change	Zygosity	Amino acid Change	Tumor source
CRL-2577™	RKO	MAPK3	c.288C>T	Heterozygous	p.R96R	Colon
CRL-9446™	CHL-1	MAPK3	c.682A>G	Homozygous	p.I228V	Skin
HTB-111™	AN3 CA	MAPK3	c.1117C>T	Heterozygous	p.P373S	Endometrium
HTB-2™	RT4	MAPK3	c.327G>A	Heterozygous	p.A109A	Urinary bladder
HTB-65™	MeWo	MAPK3	c.736C>T	Heterozygous	p.P246S	Skin
HTB-20™	BT-474	MAPK1	c.183C>G	Heterozygous	p.H61Q	Breast
HTB-9™	5637	MAPK1	c.236G>A	Heterozygous	p.R79K	Urinary bladder





Recommended controls

Wild-type control cell lines				
ATCC® number	**Cell line name**	**Tissue source**	**Cell type**	**Histology**
HTB-25™™	MDA-MB-175-VII	Breast	Epithelial	Ductal carcinoma
CRL-10317™	MCF 10A	Breast	Epithelial	Normal
CCL-75™	WI-38	Lung	Fibroblast	Normal
CRL-9609™	BEAS-2B	Lung	Epithelial	Normal
CRL-1459™	CCD-18Co	Colon	Fibroblast	Normal
CRL-2704™	C13589	Haematopoietic and lymphoid tissue	B lymphoblast	Normal

ATCC primary normal cells
Epithelial cells – bronchial/tracheal; prostate; renal; mammary; corneal; keratinocytes; melanocytes

ATCC immortalized cell lines
Human telomerase reverse transcriptase (hTERT) immortalized cell lines

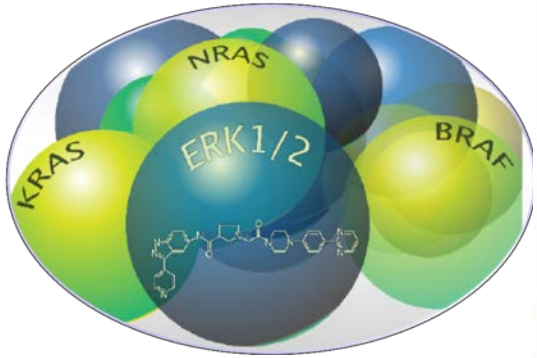
Molecular signature panels to facilitate novel anti-cancer drug discovery

Classical therapeutics

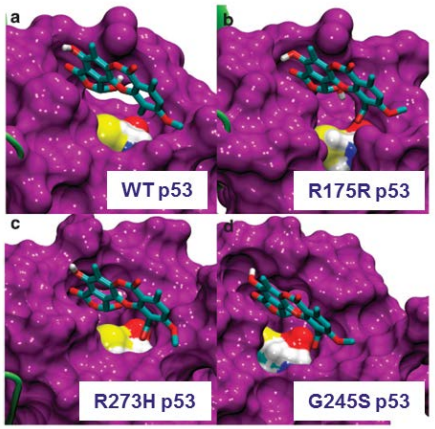
- EGFR inhibitor
- PI3K inhibitor
- AKT inhibitor
- BRAF inhibitor
- FGFR inhibitor
- MET inhibitor
- MEK inhibitor

Novel approach

- Direct target ‘non-druggable’ targets
  - p53 direct activation agent
  - KRAS direct inhibitor
- Emerging target
  - ERK inhibitor

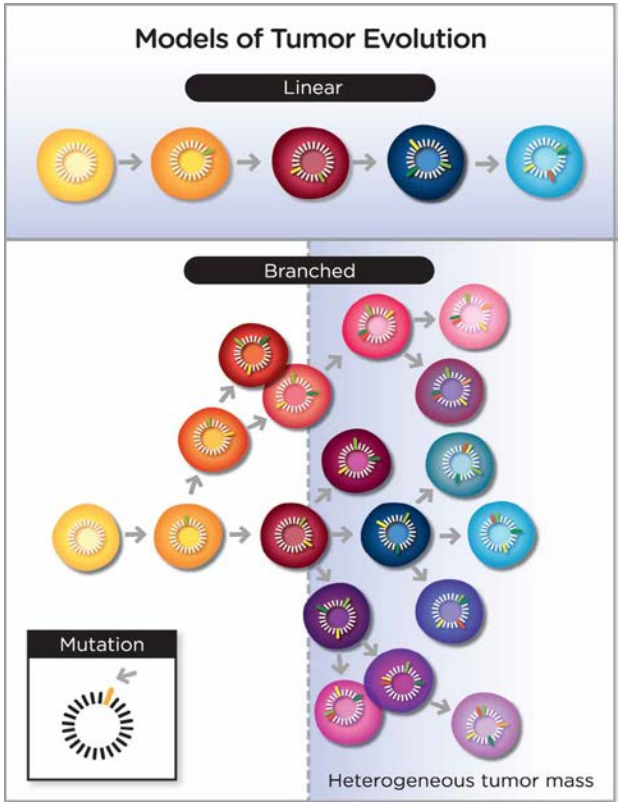


Morris E. *et. a*l., *Cancer Discovery* 3, 2013



Wassman C. *et. al.*, *Nat commu.*, 4, 2013

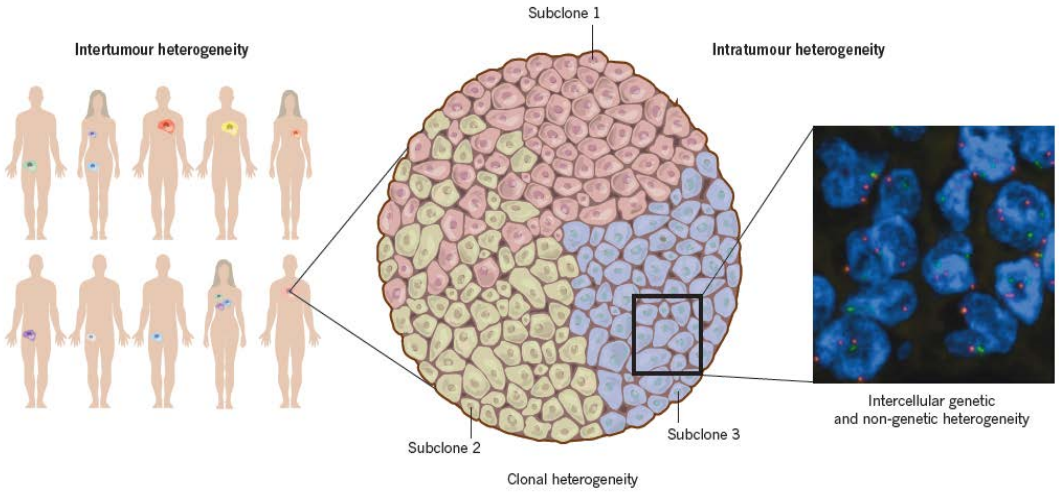
Tumor heterogeneity



Kaiser J. *Science* 339: 1543-1545, 2013

Inter-tumor heterogeneity

Intra-tumor heterogeneity



Burrell R. et. al., *Nature* 501: 338-345, 2013

Reference material needed in molecular diagnostic tests

ATCC

Using authenticated cell lines as controls

- Fully authenticated
- COI and STR testing to avoid inter-species and intra-species contamination or misidentification
- Faithfully capture tumor genetic alterations
- Stable molecular profiles
- Control FFPE process
- Control IF or IHC staining process

Although over 1900 genetic tests are available, the majority of tests still need characterized reference or QC materials

ATCC

KRAS mutation CRM cell lines and DNAs

KRAS mutation analysis is currently used as a predictive marker of therapeutic response

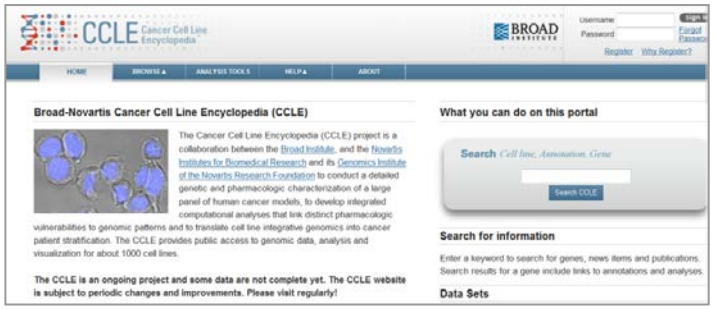
Item no.	Cell line name	Amino acid change	DNA change
CRM-TIB-161™	HuT 78	**WT**	WT
CRM-CCL-119™	CCRF-CEM	**p.G12D**	c.35G>A
CRM-CCL-185™	A549	**p.G12S**	c.34G>A
CRM-CRL-1420 ™	MIA PaCa-2	**p.G12C**	c.34G>T
CRM-HTB-174™	NCI-H441	**p.G12V**	c.35G>A
CRM-CRL-3211™	PSN1	**p.G12R**	c.34G>C
CRM-CCL-155™	RPMI 8226	**p.G12A**	c.35G>C
CRM-HTB-26™	MDA-MB-231	**p.G13D**	c.38G>A

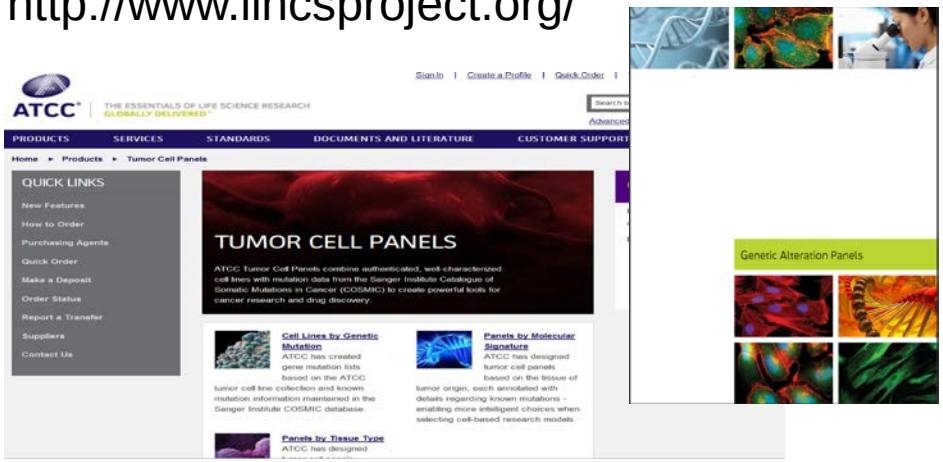
CRM DNAs are available now

ATCC®

Some useful cell line databases

- COSMIC
  http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/
- CCLE
  http://www.broadinstitute.org/ccle/home
- LINCS
  http://www.lincsproject.org/





ATCC

Conclusion

- Next generation sequencing has led to the genomic age.
- The cancer genome is impacting every aspect of cancer research.
- ATCC Molecular Signature Panels
  - Focus on key components of cell signaling pathways
  - Contain critical gene copy number changes and actionable site mutations
  - Useful tools for both basic research and drug discovery
- Authenticated cell lines, validated genetic alteration cell panels, and derived DNAs can be used as reliable controls in molecular diagnostic testing.