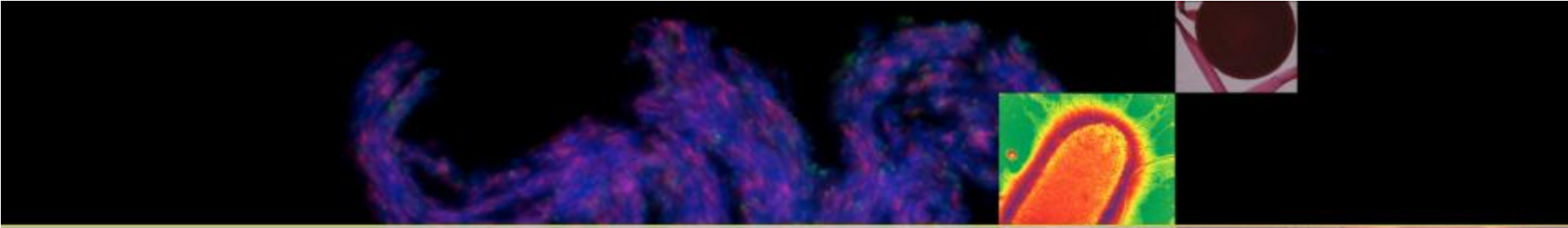
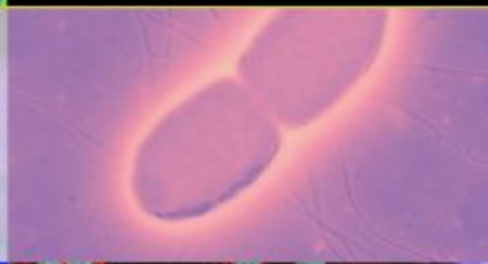
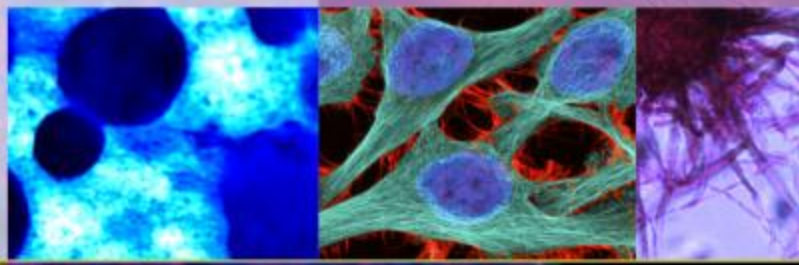
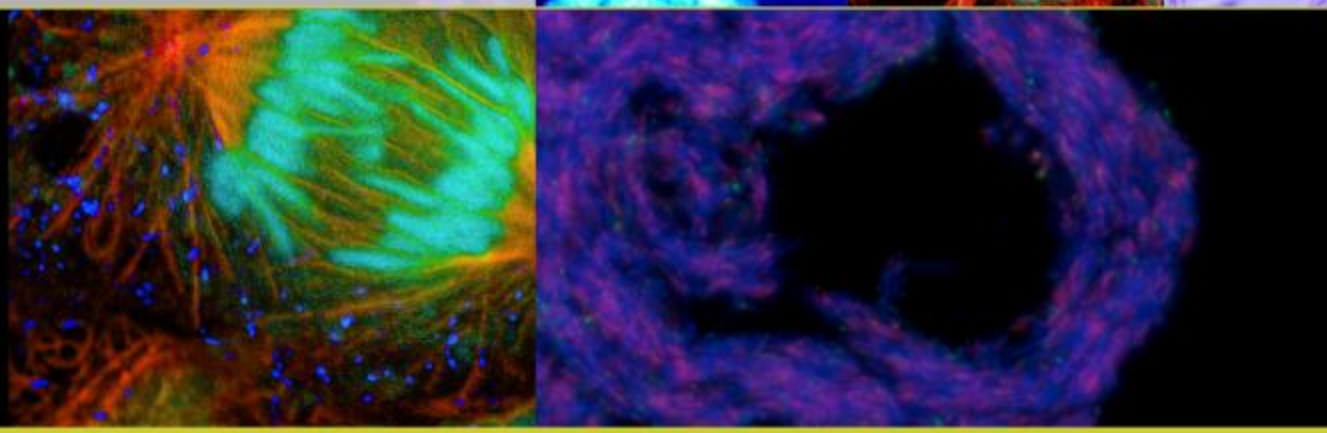
GENETIC ALTERATION CELL PANELS: EFFECTIVE TOOLS FOR HIGH THROUGHPUT *IN VITRO* SCREENING

Fang Tian, Ph.D., *ATCC*
David H. Randle, Ph.D., *Corning*
September 18, 2014

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THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard products and innovative solutions
- Broad range of biomaterials
  - Cell lines
  - Microorganisms
  - Native & synthetic nucleic acids
  - Reagents

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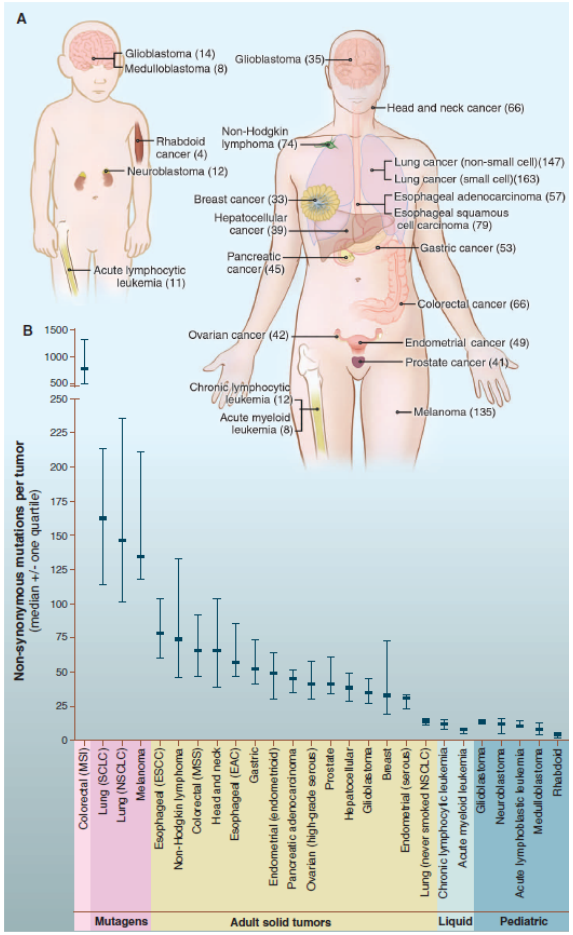
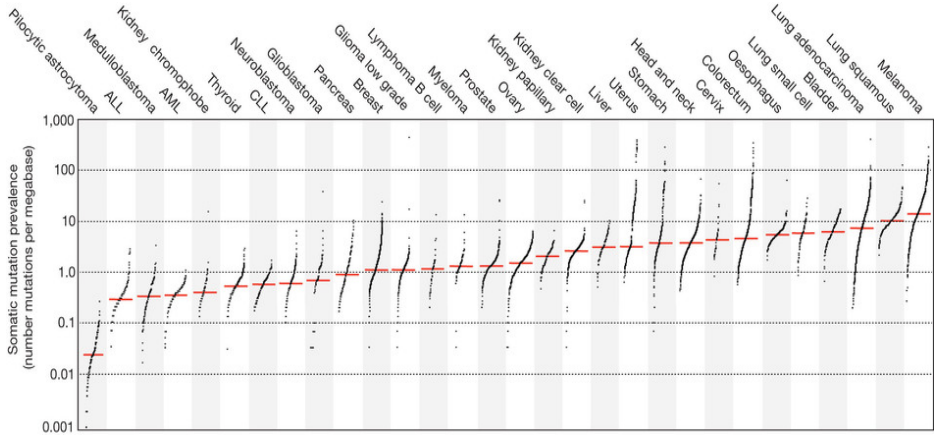
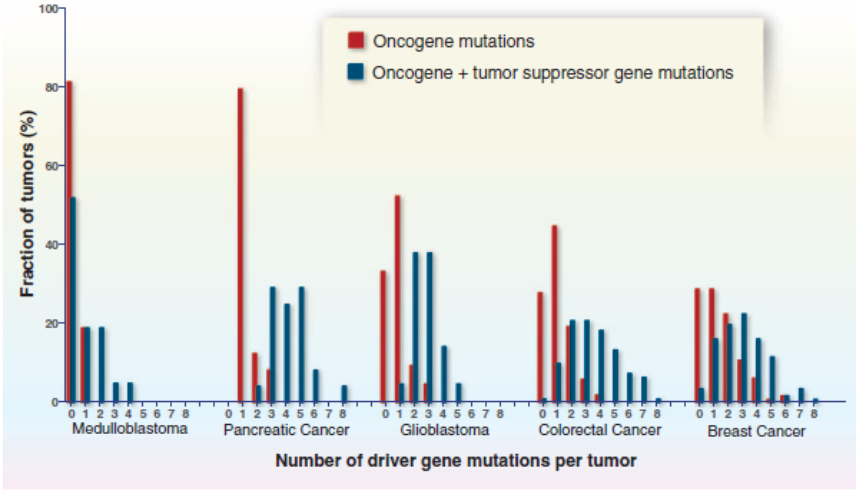
Somatic mutations in cancer



The prevalence of somatic mutations across human cancer types

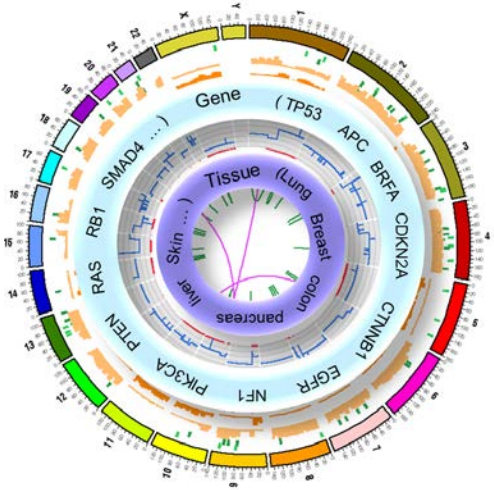


Alexandrov LB, et al., Nature 500:415-421, 2013



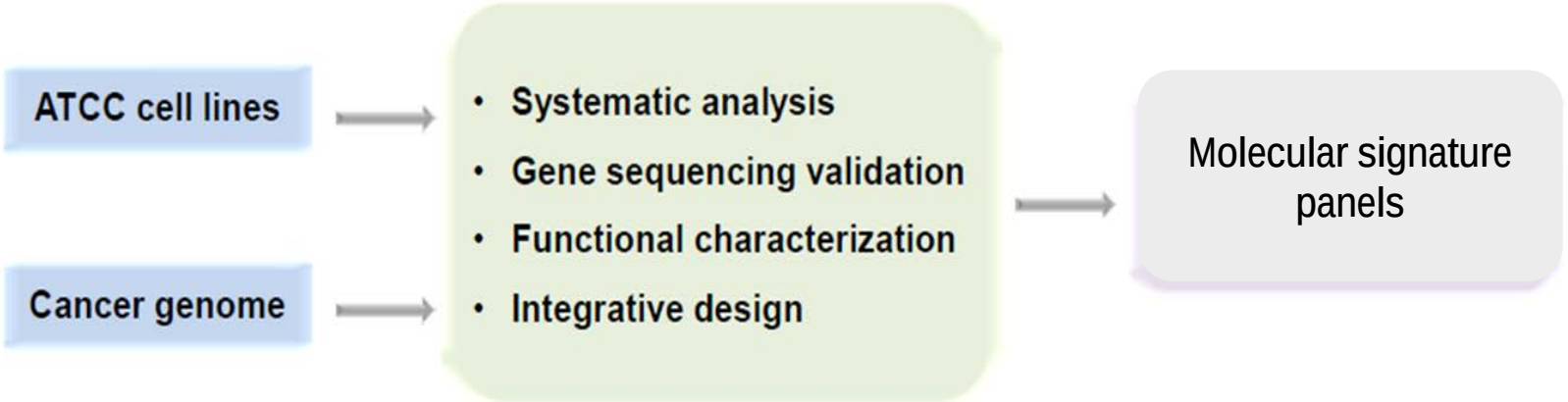
Vogelstein B, et al., Science 339:1546-1558, 2013

Background and definitions



Genetic Alteration Cell Panels

- ATCC has designed a number of panels to facilitate basic and translational cancer research.
- Panels contain key components of cell signaling pathways which have been experimentally validated at ATCC.



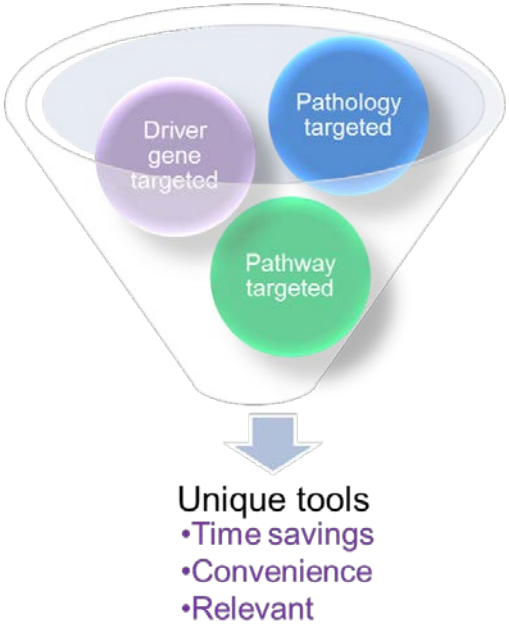
Overview of Genetic Alteration Panels

Oct 15, 2013 released 4 Molecular Signature Panels

- ATCC® TCP-1027™ EGFR Genetic Alteration Panel
- ATCC® TCP-1028™ PI3K Genetic Alteration Panel
- ATCC® TCP-1029™ AKT Genetic Alteration Panel
- ATCC® TCP-1030™ PTEN Genetic Alteration Panel

Dec 15, 2013 released 6 Molecular Signature Panels

- ATCC® TCP-1031™ RAS Genetic Alteration Panel
- ATCC® TCP-1032™ BRAF Genetic Alteration Panel
- ATCC® TCP-1033™ ERK Genetic Alteration Panel
- ATCC® TCP-1034™ FGFR Genetic Alteration Panel
- ATCC® TCP-1035™ MYC Genetic Alteration Panel
- ATCC® TCP-1036™ MET Genetic Alteration Panel



Panels are unique platforms for investigating mutations of molecular pathways implicated in cancer progression.

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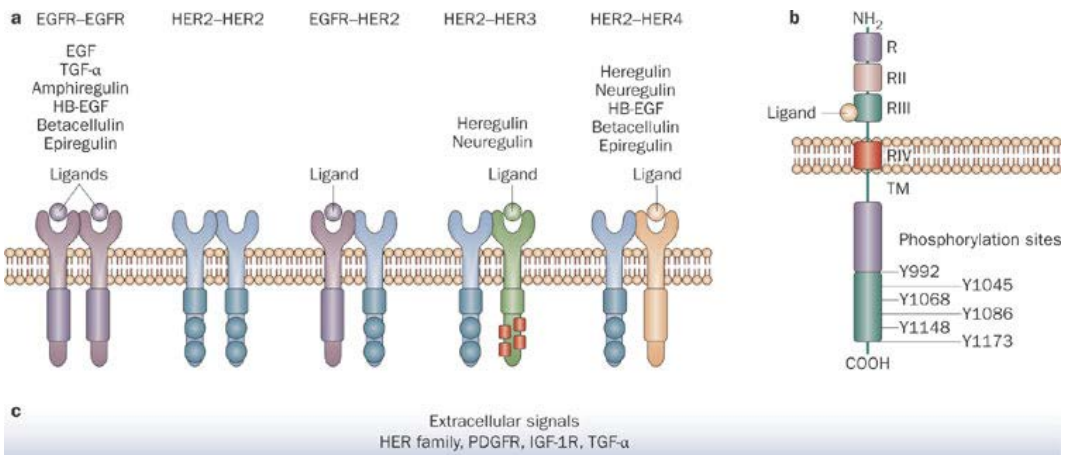
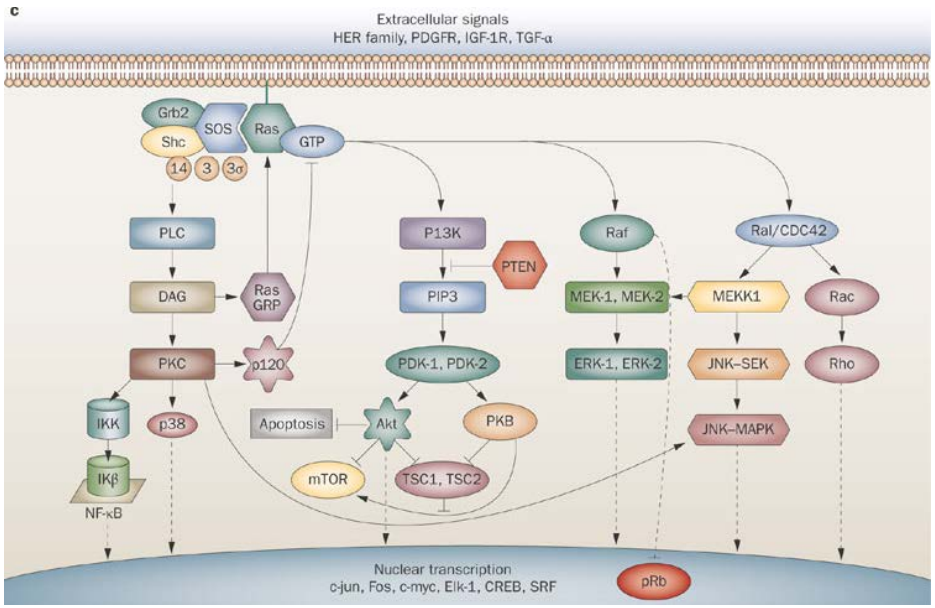
EGFR introduction





Ligand:

EGF, TGF-α, HB-EGF, Amphiregulin, δ cellulin, etc.

Family nembers

- EGFR/ERBB1/HER1
- EGFR2/ERBB2/HER2
- EGFR3/ERBB3/HER3
- EGFR4/ERBB4/HER4

3 domains

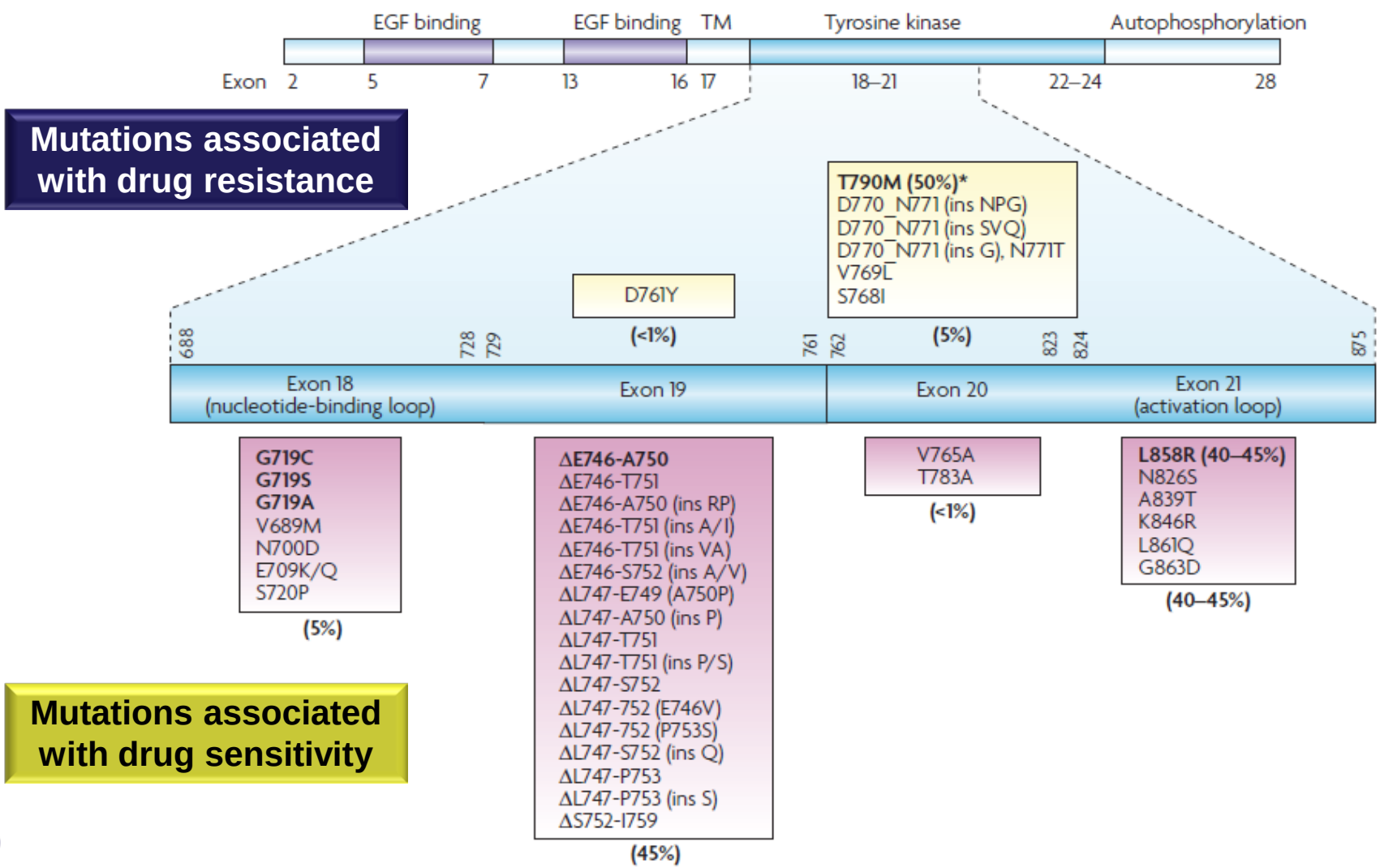
- Extracellular
- Transmembrane
- Intracellular

Functions:

- Proliferation
- Invasion
- Angiogenesis
- Metastasis
- Inhibition of Apoptosis

Linardou H, et al., *Nat Rev Clin Oncol*, 2009

EGFR mutation in cancer



Sharma S., *et al., Nat Rev Cancer,* 2007

EGFR Genetic Alteration Panel composition and characterization

EGFR Genetic Alteration Cell Panel (ATCC® TCP-1027™)								
ATCC® No.	Cell line name	Gene	cDNA change	Zygosity	Amino acid change	EGFR copy number variation	ERBB2 copy number variation	Tumor source
CRL-2868™	HCC827	EGFR	c.2236_2250delGAATTAAGAGAAGCA	Heterozygous	p.ELREA746del	Amplification	–	Lung
CRL-2871™	HCC4006	EGFR	c.2236_2244delGAATTAAGA	Heterozygous	p.ELR746del		–	Lung
CCL-231™	SW48	EGFR	c.2155G>A	Heterozygous	p.G719S		–	Colon
CRL-5908™	NCI-H1975	EGFR	c.2369C>T	Heterozygous	p.T790M		–	Lung
			c.2573T>G	Heterozygous	p.L858R		–	
HTB-132™	MDA-MB-468	EGFR	–	–	–	Amplification		Breast
HTB-19™	BT-20	EGFR	–	–	–	Amplification		Breast
HTB-178™	NCI-H596	EGFR	–	–	–	Amplification		Lung
HTB-177™	NCI-H460	EGFR	–	–	–	–	–	Lung
CRL-5928™	NCI-H2170	ERBB2	–	–	–		Amplification	Lung
HTB-20™	BT-474	ERBB2	–	–	–		Amplification	Breast
HTB-27™	MDA-MB-361	ERBB2	–	–	–		Amplification	Breast

EGFR SNPs

EGFR amplify

WT

ERBB2 amplify

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Panel includes relevant EGFR mutations for drug screening

ATCC® No.	Cell line name	Gene	Amino acid change	
CRL-2868™	HCC827	EGFR	p.ELREA746del	EGFR inhibitor sensitive cell lines
CRL-2871™	HCC4006	EGFR	p.ELR746del Activating mutations	
CCL-231™	SW48	EGFR	p.G719S	
CRL-5908™	NCI-H1975	EGFR	p.T790M Resistant mutation	EGFR inhibitor resistance
			p.L858R Activating mutation	

Panel includes different levels of EGFR and ERBB2 gene amplification

ATCC® No.	Cell line name	Gene	EGFR copy number variation	Measured CNV of EGFR	ERBB2 copy number variation	Measured CNV of ERBB2	Tumor source
CRL-2868™	HCC827	EGFR	Amplification	63.01	–	–	Lung
HTB-132™	MDA-MB-468	EGFR	Amplification	25.02	–	–	Breast
HTB-19™	BT-20	EGFR	Amplification	15.73	–	–	Breast
HTB-178™	NCI-H596	EGFR	Amplification	0.06	–	–	Lung
HTB-177™	NCI-H460	EGFR	–	–	–	–	Lung
CRL-5928™	NCI-H2170	ERBB2	–	–	Amplification	128.89	Lung
HTB-20™	BT-474	ERBB2	–	–	Amplification	29.70	Breast
HTB-27™	MDA-MB-361	ERBB2	–	–	Amplification	16.85	Breast

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Additional mutations represent the genetic complexity observed in clinical patients

EGFR genetic alteration cell panel (ATCC® TCP-1027™)							
ATCC® No.	**Cell line name**	**Gene**	**Amino acid change**	**EGFR copy number variation**	**ERBB2 copy number variation**	**Tumor source**	**Other mutations observed (COSMIC database)**
CRL-2868™	HCC827	EGFR	p.ELREA746del	Amplification	–	Lung	/
CRL-2871™	HCC4006	EGFR	p.ELR746del	–	–	Lung	/
CCL-231™	SW48	EGFR	p.G719S	–	–	Colon	CTNNB1 p.S33Y; FBXW7 p.S668fs*39
CRL-5908™	NCI-H1975	EGFR	p.T790M	–	–	Lung	TP53 p.R273H; PIK3CA p.G118D; CDKN2A p.E69*
			p.L858R		–		
HTB-132™	MDA-MB-468	EGFR	–	Amplification	–	Breast	TP53 p.R273H; PTEN p.?; RB1 p.?; SMAD4 p.0?
HTB-19™	BT-20	EGFR	–	Amplification	–	Breast	PIK3CA p.H1047R p.539R; RB1 p.515L p.I388S; CDKN2A p.0?
HTB-178™	NCI-H596	EGFR	–	Amplification	–	Lung	TP53 p.G245C; RB1 p.S182fs*3; PIK3CA p.E545K
HTB-177™	NCI-H460	EGFR	–	–	–	Lung	PIK3CA p.E545K; KRAS p.Q61H; STK11 p.Q37*; CDKN2A p.?
CRL-5928™	NCI-H2170	ERBB2	–	–	Amplification	Lung	TP53 p.P158G; CDKN2A p.0?
HTB-20™	BT-474	ERBB2	–	–	Amplification	Breast	TP53 p.E285K; PIK3CA p.K111N
HTB-27™	MDA-MB-361	ERBB2	–	–	Amplification	Breast	PIK3CA p.E545K p.K567R; CDKN2A p.M52I;

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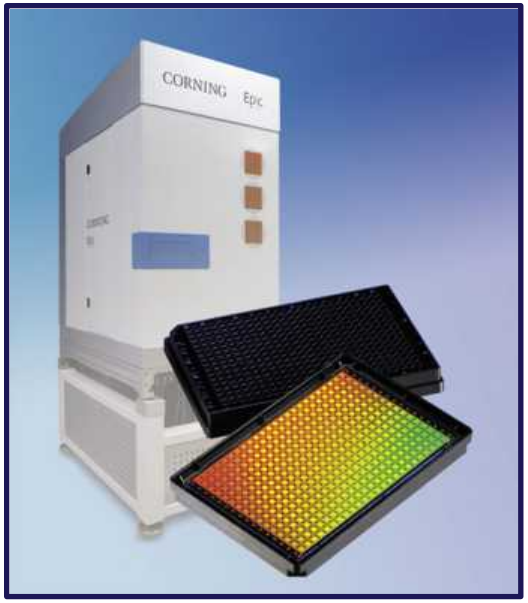
Corning® Epic® Technology

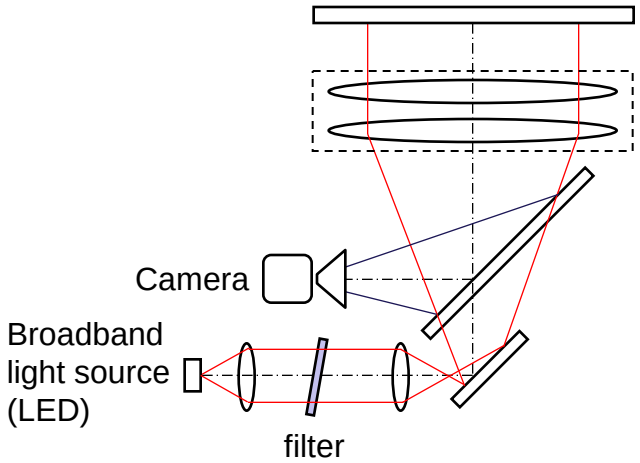


- 2 key components:
  - Reader (Gen 1, BT, or EnSpire®)
  - Microplates (96, 384, and 1536 well)
- Used for biochemical, cell-based and aggregation assays
- Uses optical biosensor technology

ATCC®

Corning® Epic® BT System: Swept wavelength imaging scheme and CCD detection

- CCD detection reduces instrument complexity and footprint
- Lower cost and ease of use attracts larger customer base
- >3 second kinetic interval broadens scope for applications
- Incubator compatibility is beneficial to some customers



ATCC®

Label-free, cell-based assays measure dynamic mass redistribution (DMR) within cells

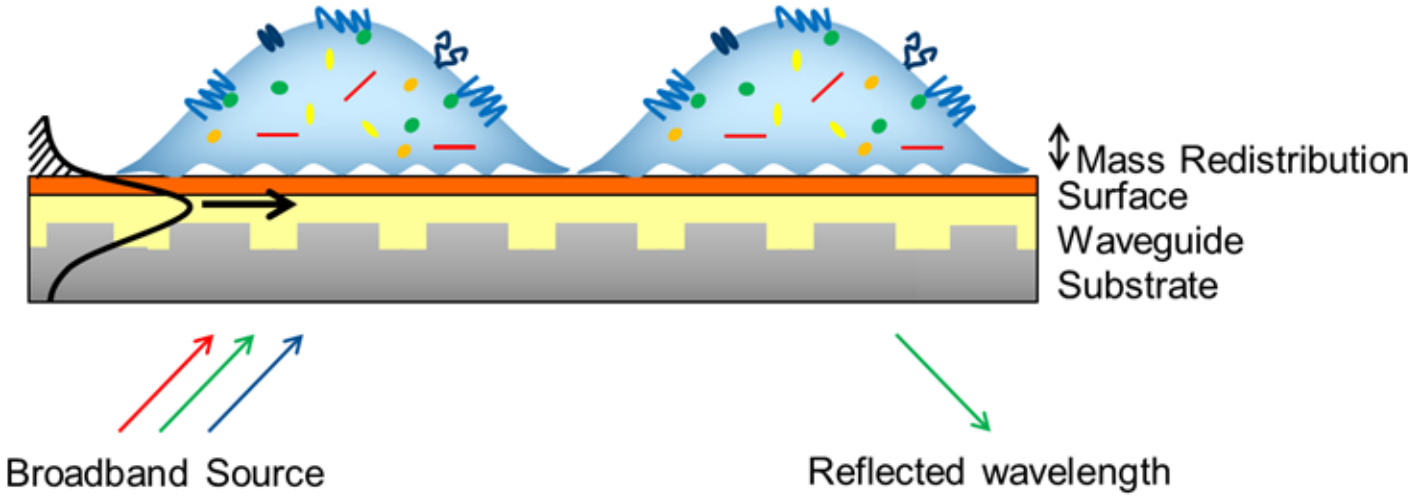
- Cell surface integrins bind to ligands and transduce signals through their intracellular signaling domains, regulating diverse functions within the cell.
- DMR assays can delineate receptor biology, ligand pharmacology and cell biology, and can provide data which is reflective of down-stream signaling pathways.
- DMR agonism assays measure the signal of a ligands.
- DMR antagonism measures the response of antagonism to the receptor agonism induced signal (antagonist is applied before the agonist).

ATCC®

Orgovan *et al*. (2014) Sci Rep. 2014 Feb 7;4:4034. doi: 10.1038/srep04034.

Label-free, cell-based assays measure dynamic mass redistribution (DMR) within cells

- Measures changes in local index of refraction resulting from ligand-induced DMR within the bottom region (~150 nm) of the cell monolayer.
- Change in index is manifested by a shift in resonant wavelength.



Assess EGF stimuli by using ATCC EGFR Genetic Alteration Panel and Corning® Epic® Technology

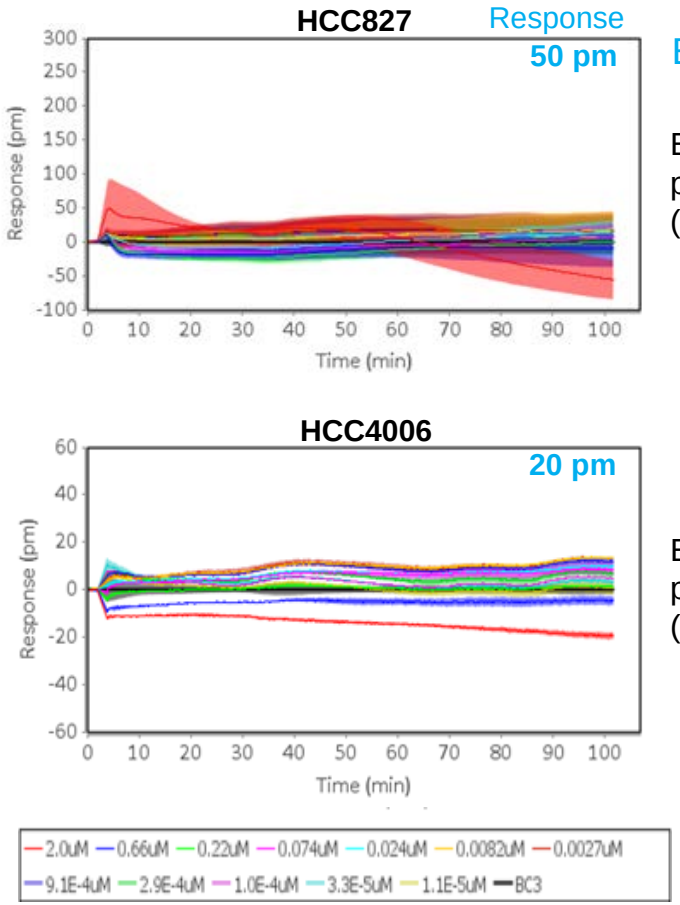
ATCC® No.	Name	Gene	Mutation	EGFR Copy No.	ERBB2 Copy No.	Source	Response (pm)	EGF EC_{50}
CRL-2868™	**HCC827**	EGFR	p.ELREA746del	Amplification	-	Lung	**50**	**1.1 µM**
CRL-2871™	**HCC4006**	EGFR	p.ELR746del	-	-	Lung	**20**	**1.3 µM**
CCL-231™	**SW48**	EGFR	p.G719S	-	-	Colon	**200**	**820 pM**
CRL-5908™	**NCI-H1975**	EGFR	p.T790M p.L858R	-	-	Lung	**130**	**880 pM**
HTB-132™	**MDA-MB-468**	EGFR	-	Amplification	-	Breast	**430**	**1.3 nM**
HTB-19™	**BT-20**	EGFR	-	Amplification	-	Breast	**315**	**160 nM**
HTB-178™	**NCI-H596**	EGFR	-	Amplification	-	Lung	**100**	**71 nM**
HTB-177™	**NCI-H460**	EGFR	-	-	-	Lung	**40**	**1.2 nM**
CRL-5928™	**NCI-H2170**	ERBB2	-	-	Amplification	Lung	**230**	**2.6 nM**
HTB-20™	**BT-474**	ERBB2	-	-	Amplification	Breast	**100**	**N/A**
HTB-27™	**MDA-MB-361**	ERBB2	-	-	Amplification	Breast	**60**	**1.1 µM**

Eleven cell lines from ATCC comprising the EGFR Genetic Alteration Panel were cultured according to ATCC recommendations, optimized for seeding density and control compound identification, and evaluated for EGF responsiveness using Corning Epic technology.

ATCC®

Low magnitude of EGF induced DMR reflects EGFR endogenous hyper-activation

DMR Profile: EGF



Cell Line Feature

EGFR activation

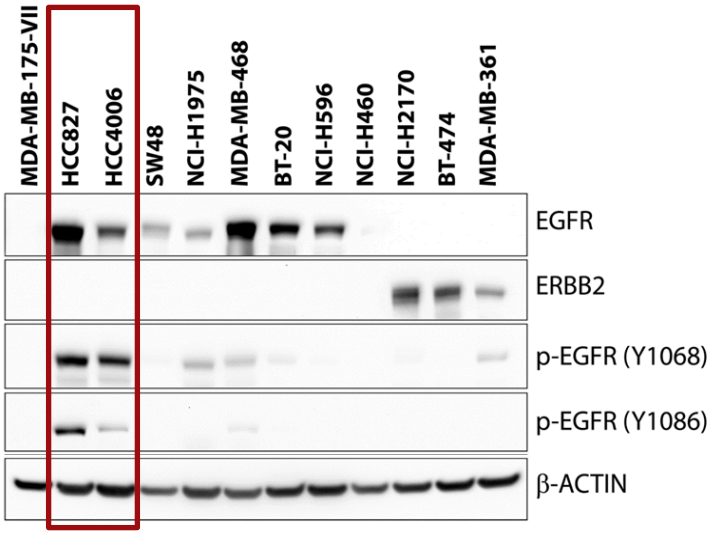
EGFR
p.ELREA746 deletion
(activation mutation)

EGFR
p.ELR746 deletion
(activation mutation)

Mechanism

- EGFR is already hyper-activated and recruits related kinases and adaptor proteins to the cell membrane.
- The cells have limited response to ligand stimulation

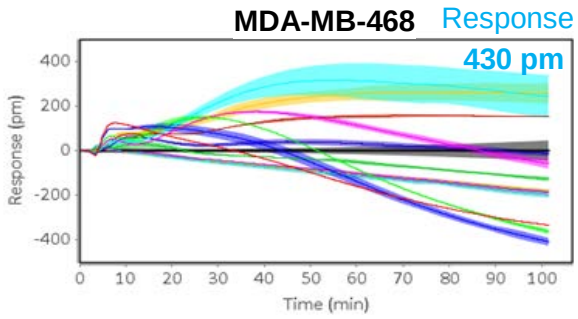
Supported by characterization data



High magnitude of EGF induced DMR correlates with EGFR gene amplification

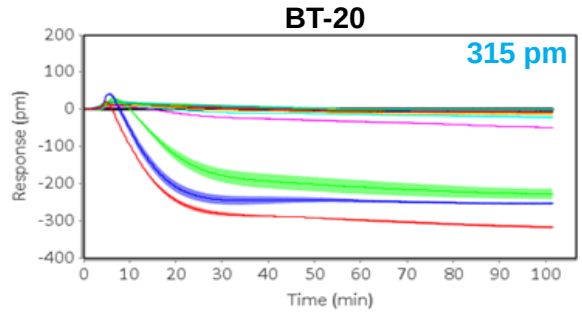
DMR Profile: EGF

Cell Line Feature

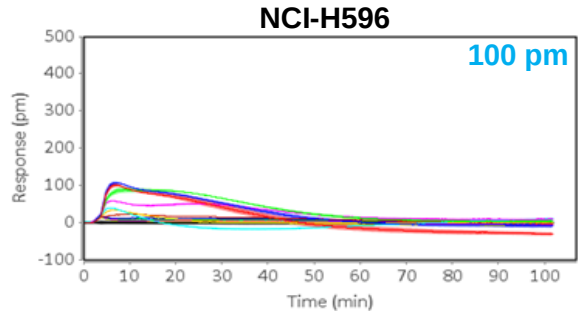


EGFR copy number

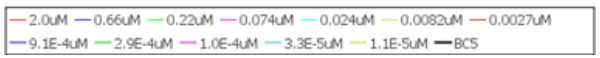
High level amplification



Middle level amplification



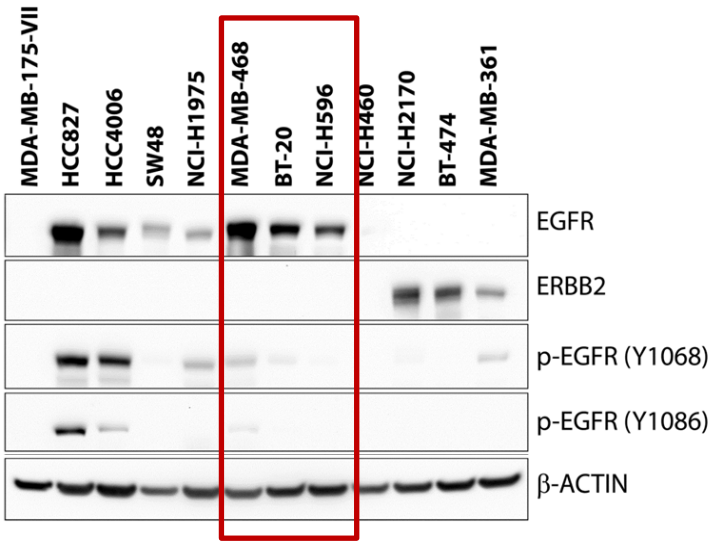
Low level amplification



Mechanism

EGFR gene copy number amplification increases EGFR protein expression on the cell membrane. The cell lines showed enhanced responses to the EGF stimuli.

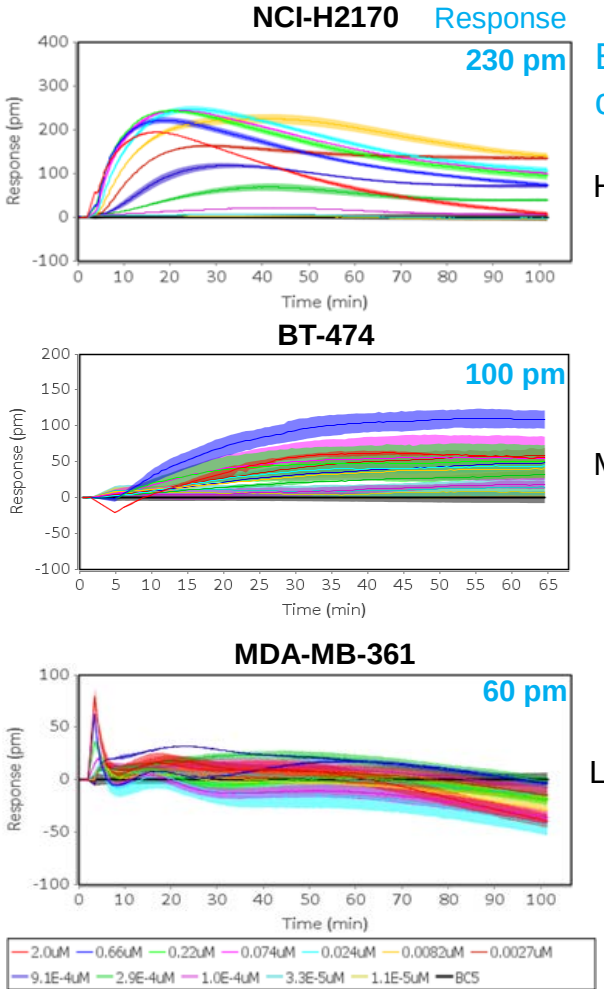
Supported by characterization data



High magnitude of EGF induced DMR correlates with EGFR2 gene amplification

DMR Profile: EGF

Cell Line Feature



EGFR2/ERBB2 copy number

High level amplification

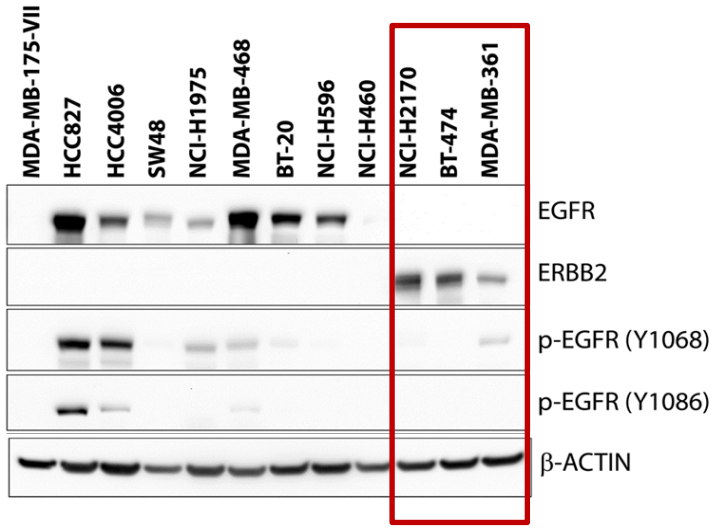
Middle level amplification

Low level amplification

Mechanism

- EGFR2/ERBB2 gene copy number amplification increases ERBB2 protein expression on the cell membrane.
- Overexpression of ERBB2 enhances the stability of EGF and retention of EGFR on cell membrane.
- Therefore, the cell lines showed enhanced responses to the EGF stimuli.

Supported by characterization data



Summary 1

- Corning® Epic® label-free technology allows the measurement of the holistic cellular response upon stimulation with a ligand. Modulation of the DMR signal allows screening for regulators of critical signaling components that are upstream and downstream of receptor activation.
- Eleven cell lines from the EGFR Genetic Alteration Panel encompass activating mutations and various levels of gene copy number amplifications of EGF receptor family members EGFR and ERBB2.
- Combining Corning Epic technology and the EGFR Genetic Alteration Panel provides convenient, high throughput tools to screen ligands or biologics that could directly bind to and affect EGFR receptor biology.
- Cell line characterization data, such as sequencing, qPCR, and western bot data are useful resource to facilitate the interpretation of assay results.

ATCC®

Types of agents to target EGFR

ATCC

C Yewale *et al Biomaterials* 34 (2013) 8690 - 8707

TKI: Iressa/Gefitinib

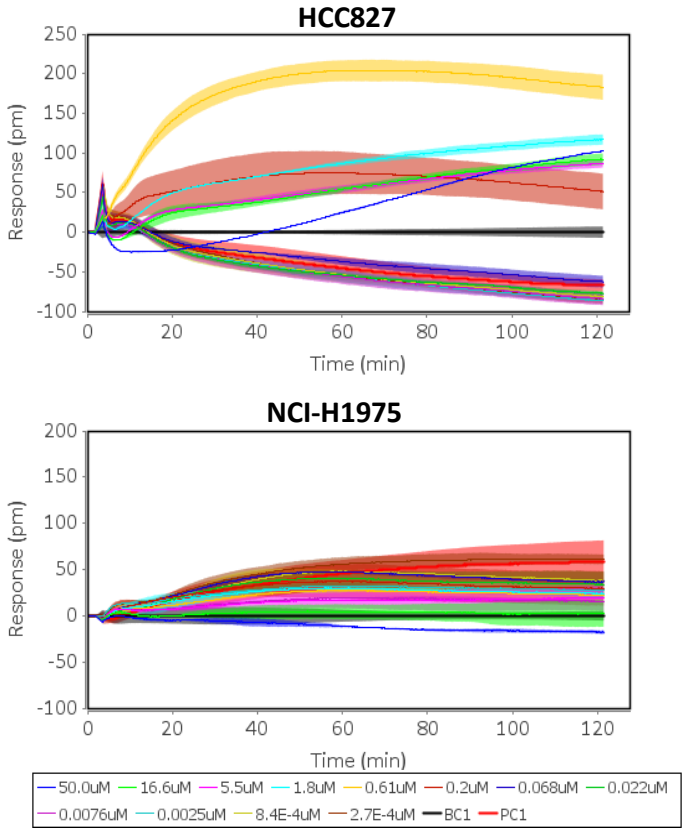
Competes reversibly with ATP to bind to intracellular catalytic domain of EGFR tyrosine kinase, inhibiting EGFR autophosphorylation and downstream signaling

- US FDA approved in 2003 for the treatment of patients with advanced or metastatic:
  - Non-small cell lung cancer
  - Head and neck squamous-cell carcinoma
  - Colorectal cancer
  - Breast cancer
  - Prostate cancer
- Selective EGFR (ErbB1) TKI proposed mechanism: up-regulation of p27 via EGFR kinase inhibition results in inhibition of CDK activity and G1 cell cycle arrest
- Resistance to Gefitinib
  - Mutation in KRAS as primary resistance in lung adenocarcinoma
  - EGFR kinase domain T790M mutation

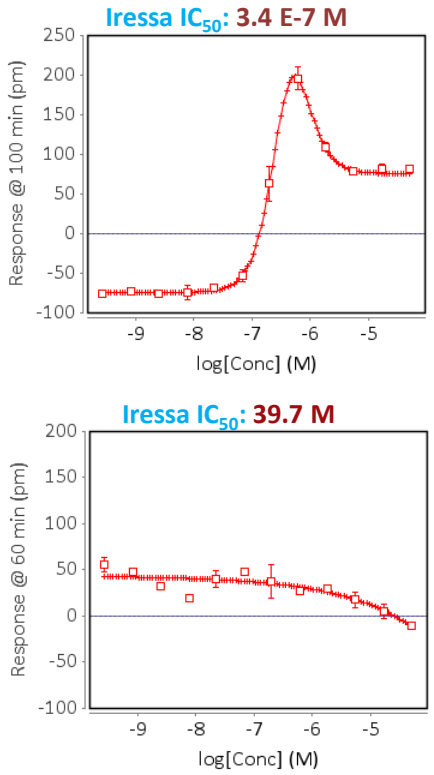
ATCC

DMR profiles demonstrate selective inhibition of EGFR by Iressa based on EGFR mutations

DMR Profile:EGF+Iressa



Inhibition Curve



Cell Line Feature

- EGFR p.ELREA746 deletion (activation mutation)
- EGFR gene amplification
- Verified Iressa-sensitive cell line

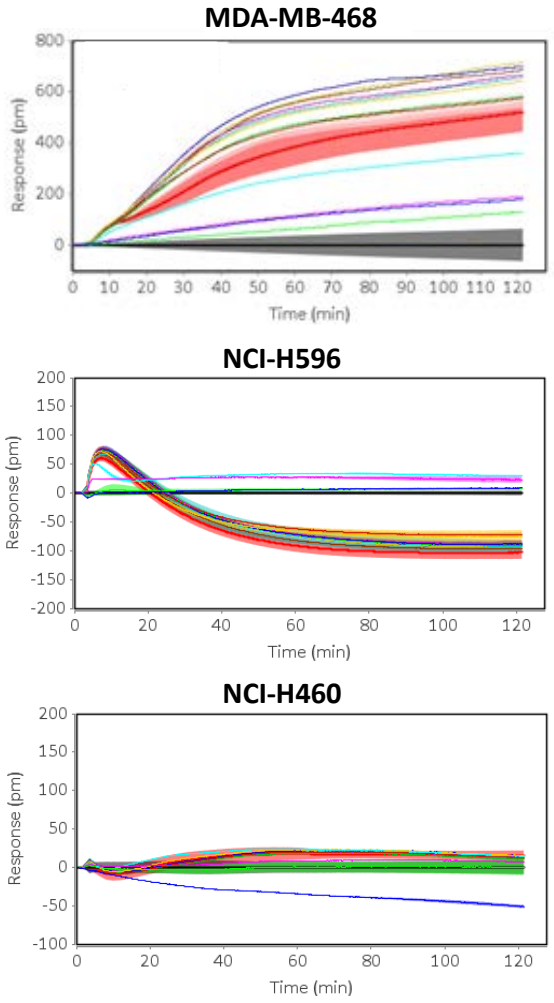
- EGFR T790M (drug-resistant) and L858R (activation) mutations
- Verified Iressa-resistant cell line

- HCC827 represents drug-sensitive tumors, while NCI-H1975 represents drug-sensitive but acquired drug-resistance tumors observed in clinical patients.
- Corning® Epic® DMR profiles and inhibition curves accurately demonstrate drug responses on validated cell models within 2 hours of compound addition.

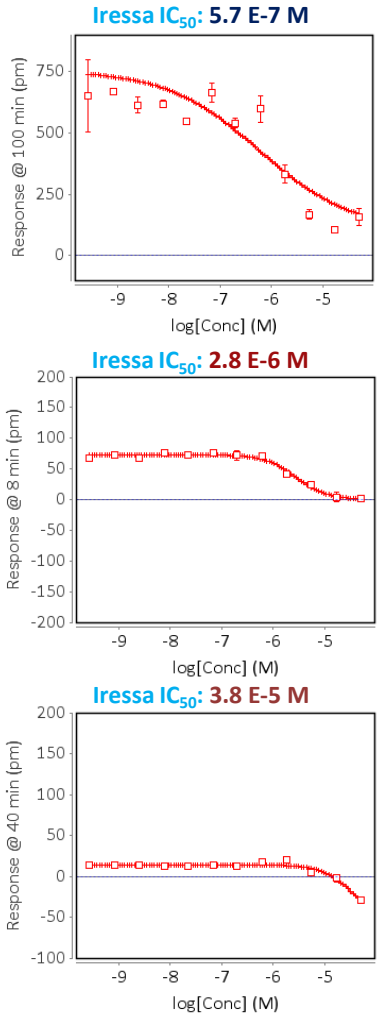
ATCC®

DMR profiles demonstrate selective inhibition of EGFR by Iressa based on EGFR CNV

DMR Profile:EGF+Iressa



Inhibition Curve



Cell Line Feature

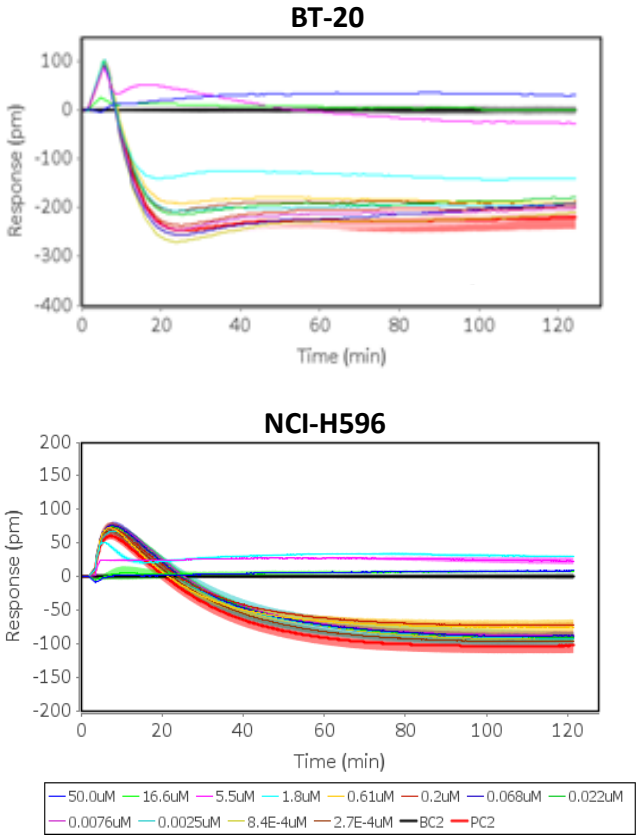
- EGFR copy number: high level amplification
- Sensitive to EGF stimulation and responds well to EGFR inhibitor

- EGFR copy number: low level amplification
- Responds to EGF stimulation and EGFR inhibitor

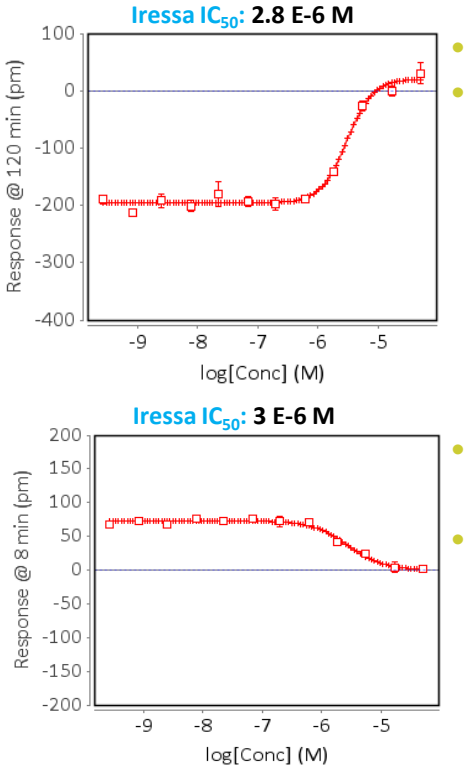
- No EGFR activating mutation or gene copy number variation
- Responds to EGF stimulation and EGFR inhibitor but less effective than drug-sensitive cell lines

DMR profile can indicate involvement of other signal pathways

DMR Profile:EGF+Iressa



Inhibition Curve



Cell Line Feature

- EGFR copy number amplification
- Other Mutations in EGFR downstream pathway
  - PIK3CA p.H1047R p.539R leads to PI3K activation
  - AKT hyper-activated

- EGFR copy number: slight amplification
- Other Mutations in EGFR downstream pathway
  - PIK3CA p.E545K leads to PI3K activation

- Corning® Epic® DMR profiles showed similar IC_{50} value of Iressa on BT-20 and NCI-H596 but the unique profile of BT-20 cell line indicates possible additional mechanism.
- BT-20 cell line contains PIK3CA gain of function mutation as well as AKT constant activation confirms Corning Epic data indication.

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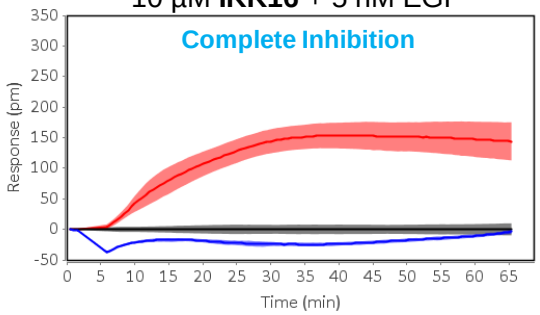
Selected compounds from Tocriscreen™ Kinase Library

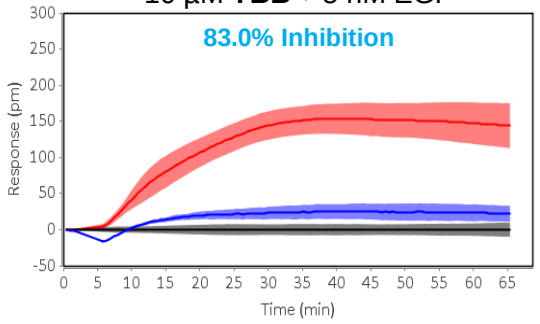
Inhibitor	Biological Activity
TBB	Selective inhibitor of Casein Kinase-2 (CK2) Acts in an ATP/GTP-competitive manner
NSC 693868	Inhibitor of cyclin-dependent kinases (CDKs) and glycogen synthase kinase-3 (GSK-3)
IKK16	Selective inhibitor of IκB kinase (IKK) Inhibits TNF-α-stimulated IκB degradation and expression of adhesion molecules E-selectin, ICAM, and VCAM
Aminopurvalanol A	CDK inhibitor and ERK1/ERK2 inhibitor Arrests cell cycle at G2/M boundary and induces apoptosis

Identification of new target in Iressa-resistant cell line NCI-H1975 by screening Tocriscreen™ Kinase Library

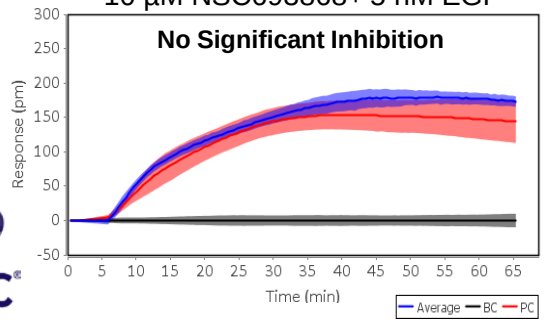
10 µM **IKK16** + 5 nM EGF



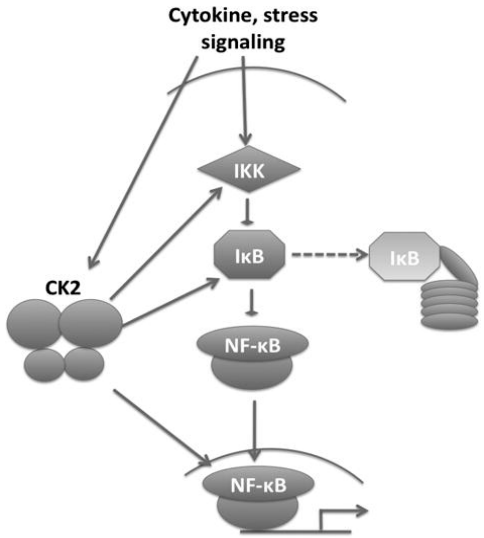
10 µM **TBB** + 5 nM EGF



10 µM NSC693868+ 5 nM EGF



CK2 Action in the NF-κB Pathway



- Selective inhibitors of IkB kinase (IKK16) or CK2 inhibitor (TBB) showed dramatic inhibition on Iressa-resistant cell line NCI-H1975.
- Selective inhibitor (NSC693868) of CDKs and GSK-3 showed little inhibition on NCI-H1975 cells.
- Therefore, targeting NF-kB pathway potential could be a strategy for EGFR inhibitor-resistant tumor/cell lines.

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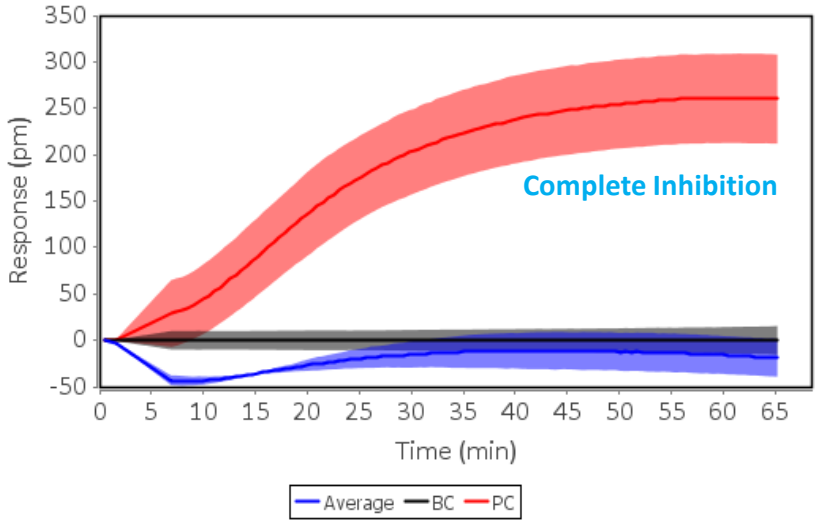
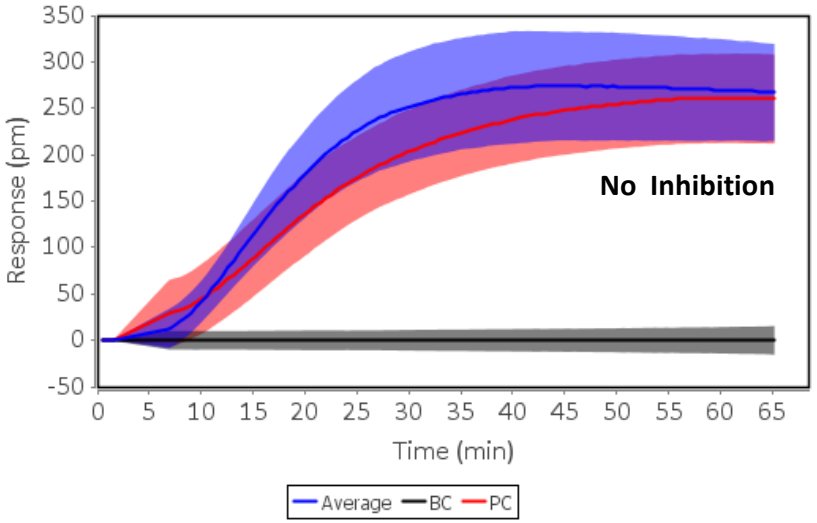
Identification new target in Iressa-sensitive cell line NCI-H2170 by screening Tocriscreen™ Kinase Library

10 µM **aminopuralanol A**+ 5 nM EGF



10 µM **IKK16** + 5 nM EGF



- Selective inhibitor of CDKs and ERK1/ERK2 showed complete inhibition on ERBB2-amplified cell line NCI-H2170.
- Selective inhibitor of IkB kinase showed no inhibition on ERBB2 amplified NCI-H2170 cells.
- Data support the current combination strategy of targeting downstream of the same pathway in EGFR targeted therapy. Moreover, demonstrate NCI-H2170 cell line as a screening model for CDKs and ERK1/ERK2 inhibitors.

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Summary 2

- Corning® Epic® label-free technology provides a convenient and fast high throughput cell-based assay. Drug response can be determined within 2 hours.
- EGFR Genetic Alteration Panel encompass EGFR inhibitor-resistant and -sensitive cell lines. The component cell lines faithfully capture the molecular profile of clinical patient tumors and the drug response mechanisms.
- DMR profiles and inhibition curves are able to recapitulate the drug responses of the known EGFR inhibitor Iressa on validated cell models that have been reported by many other studies. Moreover, DMR data can indicate the involvement of other signaling pathways.
- Additional drug targets have been identified within Iressa-resistant cell line NCI-H1975 and Iressa-sensitive cell line NCI-H2170 by screening the Tocriscreen™ Kinase Inhibitor Library.

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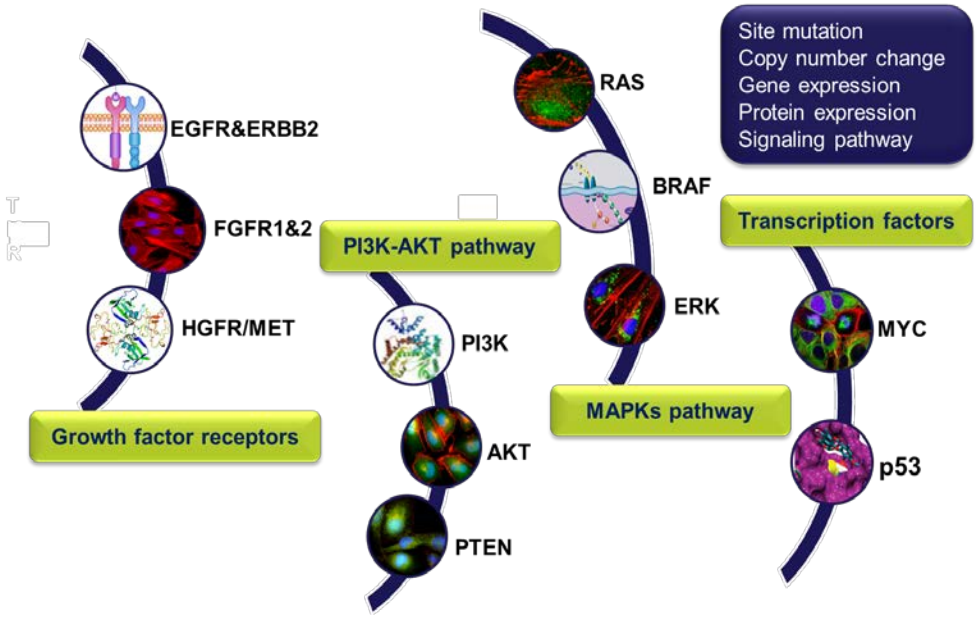
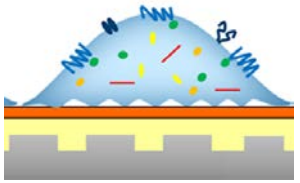
Effective tools for high throughput screening

ATCC Molecular Signature Cell Panels



- Extensive characterization
- Represent patient tumor molecular profiles
- Tools for targeted therapeutic drug discovery

Corning® Epic® Technology



- High throughput screening
- ≤ 2 hours assay
- Signal pathway identification and real time drug response

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Conclusions

- Capturing molecular profile of human disease and the drug response mechanisms, Genetic Alteration Cell Panels utilize cancer genomics supporting a pathway targeted approach for investigation and drug discovery.
- The Corning® Epic® label-free system can be utilized to evaluate the receptor responsiveness to ligands and predict drug response. Combining Corning Epic technology and the EGFR Genetic Alteration Panel provides convenient tools for screening ligands or biologics that direct bind to and affect EGFR receptor biology and reagents that inhibit EGFR.
- The Corning Epic system provides researchers an alternative method for investigating cellular signaling pathways downstream of receptor activation while differentiating between EGFR mutations/amplifications in these human cell lines.
- Alternative mechanisms of action for pathway-targeted cell lines can also be revealed by the responses measured by the Corning Epic system.

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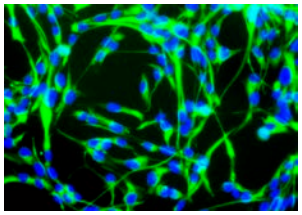
Thank you!

Register for more webinars in the ATCC "*Excellence in Research*" webinar series at www.atcc.org/webinars.



October 16, 2014
10:00 AM, 3:00 PM EST
Dr. Tigwa H. Davis
Using LUHMES cells as a model system to study dopaminergic neuron cell biology

October 30, 2014
10:00 AM, 3:00 PM EST
Dr. Francisco Bizouarn
Precise counting of targeted nucleic acids has never been easier

Thank you for joining today!
Please send additional questions to tech@atcc.org

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Photo credit: Yirui Sun

Supplemental slides

- EGFR Genetic Alteration Panel characterization data
- Recommended WT control cells

Characterization - Morphology, cell growth, and IF assay

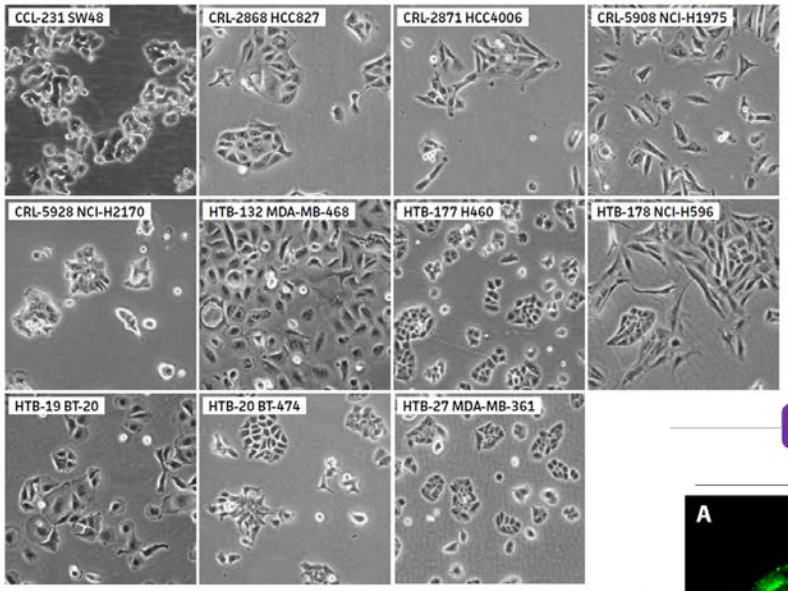
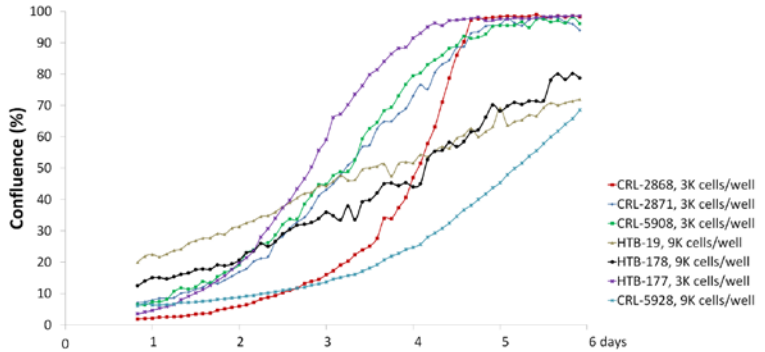
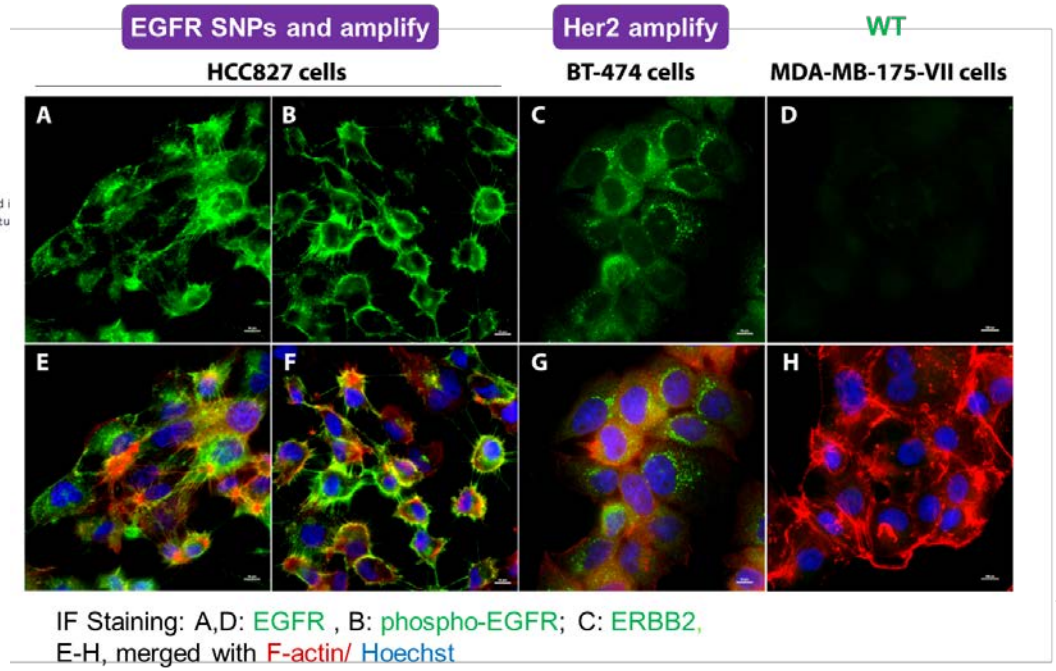


Figure 2. Cell morphology of the eleven tumor cell lines in the EGFR Genetic Alteration Cell Panel. Cells were maintained i culture conditions. Cell morphology was observed under Nikon™ microscopy, and images of the indicated cell lines were captu digital camera.





Characterization – gene expression and protein expression

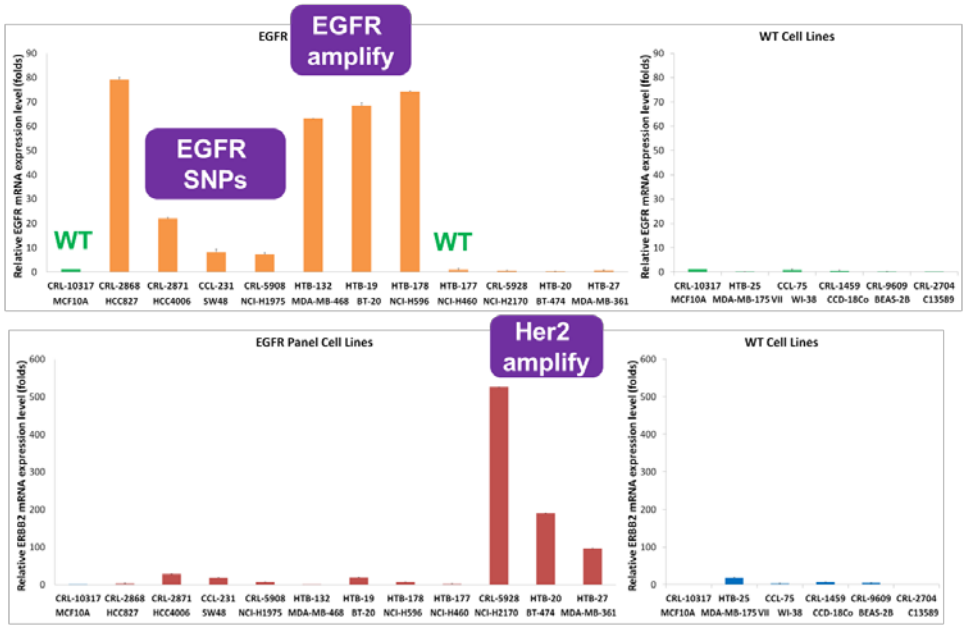


Figure 4. Real-time qPCR analysis of mRNA levels. The mRNA expression level of EGFR and ERBB2 were determined by real time quantitative PCR. Relative EGFR (orange and green bars, upper panel) and ERBB2 (red and blue bars, lower panel). mRNA expression for the indicated cell lines was calculated by normalizing their levels to the wild-type cell line MCF10A (Set to 1) and the housekeeping gene 36B4.

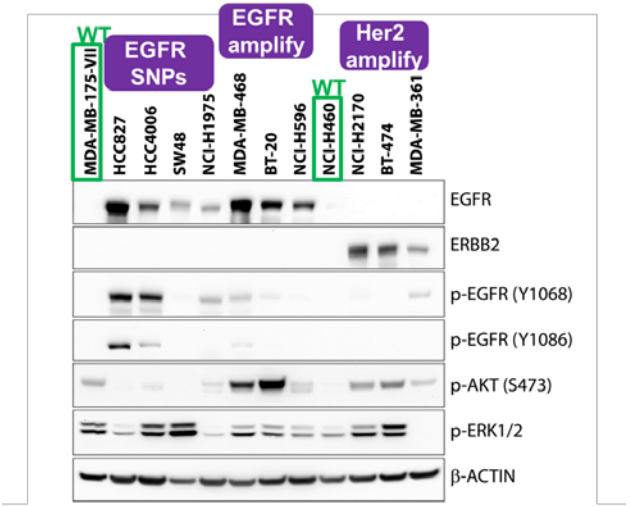


Figure 5. Western blotting analysis of endogenous protein expression. The indicated cell lines were lysed and processed to extract protein. Western blotting was used to examine the total protein level and phosphorylation of EGFR, as well as markers of downstream EGFR signaling pathways such as p-AKT and p-ERK. β-actin protein was included as a loading-control.

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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	Hematopoietic and lymphoid tissue	B lymphoblast	Normal

ATCC primary normal cells

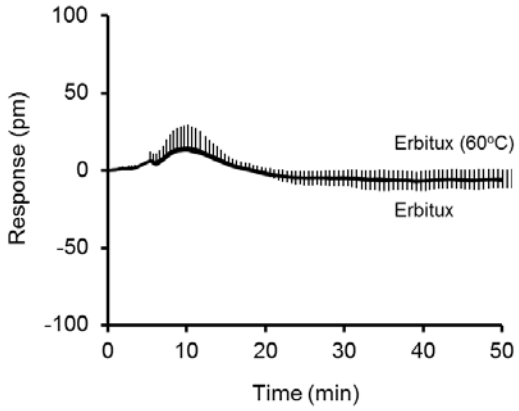
Epithelial cells – bronchial/tracheal; prostate; renal mammary; corneal; keratinocytes; melanocytes

ATCC immortalized cell lines

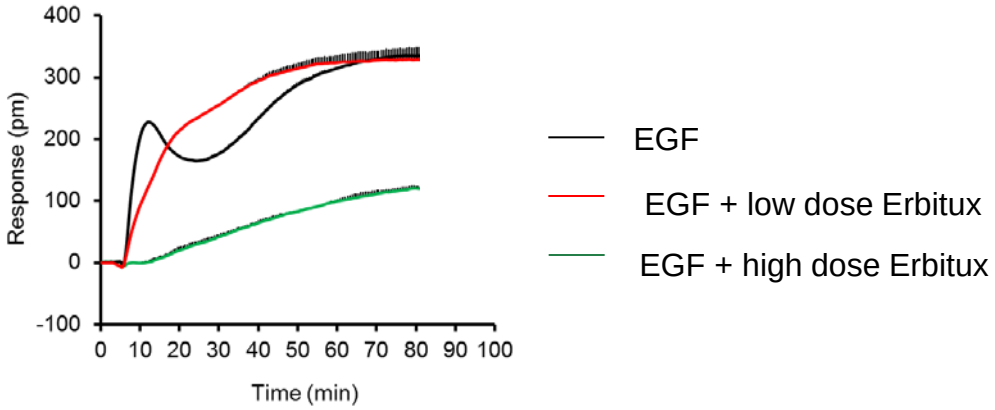
Human telomerase reverse transcriptase (hTERT) immortalized cell lines

Monoclonal Antibody (Erbitux) can Modulate DMR Profiles in Colon Cancer Cells

Erbitux alone



Erbitux impact on EGF



DMR plots upon EGF stimulation of a colon cancer cell line in the absence (L) and presence (R) of Erbitux. Erbitux is an anti-EGFR drug for treatment of metastatic colorectal, head and neck cancer.

- Greater modulation of early phase DMR at low Erbitux concentrations

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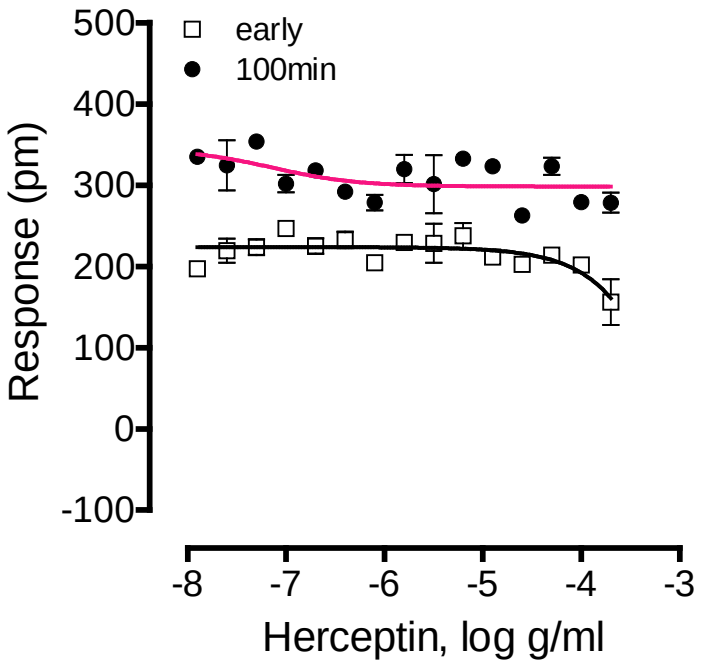
Herceptin Shows No Activity for EGF Pathway in HT-29 Colon Cell Line

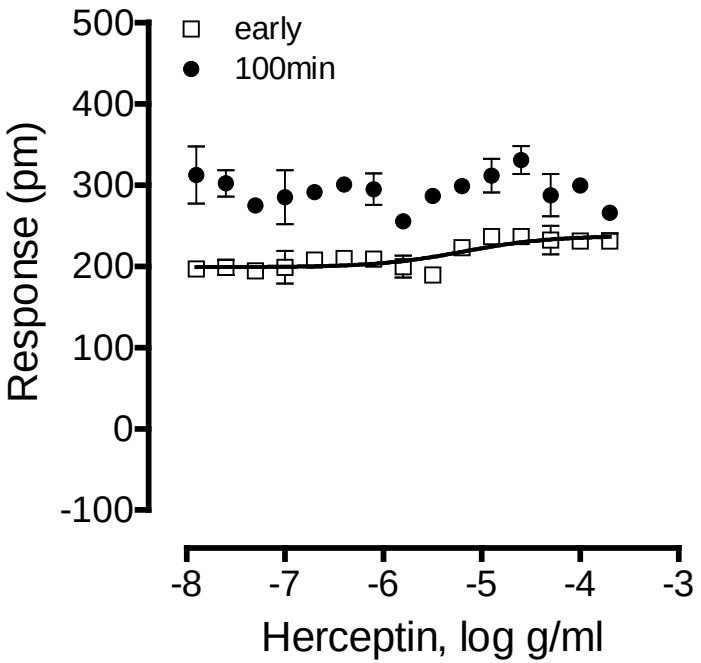
a



2-step assay:

1. Herceptin preincubation for 60 min

b



1-step co-stimulation assay:

1. Herceptin does not compete with EGF for binding