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
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
Somatic mutations in cancer

The prevalence of somatic mutations across human cancer types


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.
EGFR introduction

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

Family members
- 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
EGFR mutation in cancer

Mutations associated with drug resistance

- **D761Y**
  - (1%)

- **T790M (50%)**
  - D770_N771 (ins NPG)
  - D770_N771 (ins SVQ)
  - D770_N771 (ins G), N771T
  - V769L
  - S768I

Mutations associated with drug sensitivity

- **G719C**
  - G719S
  - G719A
  - V689M
  - N700D
  - E709K/Q
  - S720P
  - (5%)

- **ΔE746-A750**
  - ΔE746-T751
  - ΔE746-A750 (ins RP)
  - ΔE746-T751 (ins A/I)
  - ΔE746-T751 (ins VA)
  - ΔE746-S752 (ins A/V)
  - ΔL747-E749 (A750P)
  - ΔL747-A750 (ins P)
  - ΔL747-T751
  - ΔL747-T751 (ins P/S)
  - ΔL747-S752
  - ΔL747-752 (E746V)
  - ΔL747-752 (P753S)
  - ΔL747-S752 (ins Q)
  - ΔL747-P753
  - ΔL747-P753 (ins S)
  - ΔS752-I759
  - (45%)

- **V765A**
  - T783A
  - (<1%)
# EGFR Genetic Alteration Panel composition and characterization

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Cell line name</th>
<th>Gene</th>
<th>cDNA change</th>
<th>Zygosity</th>
<th>Amino acid change</th>
<th>EGFR copy number variation</th>
<th>ERBB2 copy number variation</th>
<th>Tumor source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL-2868™</td>
<td>HCC827</td>
<td>EGFR</td>
<td>c.2236_2250delGAATTAA GAGAAGCA</td>
<td>Heterozygous</td>
<td>p.ELREA746del</td>
<td>Amplification</td>
<td>-</td>
<td>Lung</td>
</tr>
<tr>
<td>CRL-2871™</td>
<td>HCC4006</td>
<td>EGFR</td>
<td>c.2236_2244delGAATTAA GA</td>
<td>Heterozygous</td>
<td>p.ELR746del</td>
<td>-</td>
<td>-</td>
<td>Lung</td>
</tr>
<tr>
<td>CCL-231™</td>
<td>SW48</td>
<td>EGFR</td>
<td>c.2155G&gt;A</td>
<td>Heterozygous</td>
<td>p.G719S</td>
<td>-</td>
<td>-</td>
<td>Colon</td>
</tr>
<tr>
<td>CRL-5908™</td>
<td>NCI-H1975</td>
<td>EGFR</td>
<td>c.2369C&gt;T</td>
<td>Heterozygous</td>
<td>p.T790M</td>
<td>-</td>
<td>-</td>
<td>Lung</td>
</tr>
<tr>
<td>CRL-5908™</td>
<td>NCI-H1975</td>
<td>EGFR</td>
<td>c.2573T&gt;G</td>
<td>Heterozygous</td>
<td>p.L858R</td>
<td>-</td>
<td>-</td>
<td>Lung</td>
</tr>
<tr>
<td>HTB-132™</td>
<td>MDA-MB-468</td>
<td>EGFR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Breast</td>
</tr>
<tr>
<td>HTB-19™</td>
<td>BT-20</td>
<td>EGFR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Breast</td>
</tr>
<tr>
<td>HTB-178™</td>
<td>NCI-H596</td>
<td>EGFR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Lung</td>
</tr>
<tr>
<td>HTB-177™</td>
<td>NCI-H460</td>
<td>EGFR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Lung</td>
</tr>
<tr>
<td>CRL-5928™</td>
<td>NCI-H2170</td>
<td>ERBB2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Lung</td>
</tr>
<tr>
<td>HTB-20™</td>
<td>BT-474</td>
<td>ERBB2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Breast</td>
</tr>
<tr>
<td>HTB-27™</td>
<td>MDA-MB-361</td>
<td>ERBB2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Breast</td>
</tr>
</tbody>
</table>
Panel includes relevant EGFR mutations for drug screening

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Cell line name</th>
<th>Gene</th>
<th>Amino acid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL-2868™</td>
<td>HCC827</td>
<td>EGFR</td>
<td>p.ELREA746del</td>
</tr>
<tr>
<td>CRL-2871™</td>
<td>HCC4006</td>
<td>EGFR</td>
<td>p.ELR746del, Activating mutations</td>
</tr>
<tr>
<td>CCL-231™</td>
<td>SW48</td>
<td>EGFR</td>
<td>p.G719S</td>
</tr>
<tr>
<td>CRL-5908™</td>
<td>NCI-H1975</td>
<td>EGFR</td>
<td>p.T790M, Resistant mutation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p.L858R, Activating mutation</td>
</tr>
</tbody>
</table>
Panel includes different levels of EGFR and ERBB2 gene amplification

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Cell line name</th>
<th>Gene</th>
<th>EGFR copy number variation</th>
<th>Measured CNV of EGFR</th>
<th>ERBB2 copy number variation</th>
<th>Measured CNV of ERBB2</th>
<th>Tumor source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL-2868™</td>
<td>HCC827</td>
<td>EGFR</td>
<td>Amplification</td>
<td>63.01</td>
<td>−</td>
<td>−</td>
<td>Lung</td>
</tr>
<tr>
<td>HTB-132™</td>
<td>MDA-MB-468</td>
<td>EGFR</td>
<td>Amplification</td>
<td>25.02</td>
<td>−</td>
<td>−</td>
<td>Breast</td>
</tr>
<tr>
<td>HTB-19™</td>
<td>BT-20</td>
<td>EGFR</td>
<td>Amplification</td>
<td>15.73</td>
<td>−</td>
<td>−</td>
<td>Breast</td>
</tr>
<tr>
<td>HTB-178™</td>
<td>NCI-H596</td>
<td>EGFR</td>
<td>Amplification</td>
<td>0.06</td>
<td>−</td>
<td>−</td>
<td>Lung</td>
</tr>
<tr>
<td>HTB-177™</td>
<td>NCI-H460</td>
<td>EGFR</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Lung</td>
</tr>
<tr>
<td>CRL-5928™</td>
<td>NCI-H2170</td>
<td>ERBB2</td>
<td>−</td>
<td>−</td>
<td>Amplification</td>
<td>128.89</td>
<td>Lung</td>
</tr>
<tr>
<td>HTB-20™</td>
<td>BT-474</td>
<td>ERBB2</td>
<td>−</td>
<td>−</td>
<td>Amplification</td>
<td>29.70</td>
<td>Breast</td>
</tr>
<tr>
<td>HTB-27™</td>
<td>MDA-MB-361</td>
<td>ERBB2</td>
<td>−</td>
<td>−</td>
<td>Amplification</td>
<td>16.85</td>
<td>Breast</td>
</tr>
</tbody>
</table>
Additional mutations represent the genetic complexity observed in clinical patients

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Cell line name</th>
<th>Gene</th>
<th>Amino acid change</th>
<th>EGFR copy number variation</th>
<th>ERBB2 copy number variation</th>
<th>Tumor source</th>
<th>Other mutations observed (COSMIC database)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL-2868™</td>
<td>HCC827</td>
<td>EGFR</td>
<td>p.ELREA746del</td>
<td>Amplification</td>
<td>−</td>
<td>Lung</td>
<td>/</td>
</tr>
<tr>
<td>CRL-2871™</td>
<td>HCC4006</td>
<td>EGFR</td>
<td>p.ELR746del</td>
<td>−</td>
<td>−</td>
<td>Lung</td>
<td>/</td>
</tr>
<tr>
<td>CCL-231™</td>
<td>SW48</td>
<td>EGFR</td>
<td>p.G719S</td>
<td>−</td>
<td>−</td>
<td>Colon</td>
<td>CTNNB1 p.S33Y; FBXW7 p.S668fs*39</td>
</tr>
<tr>
<td>HTB-132™</td>
<td>MDA-MB-468</td>
<td>EGFR</td>
<td>−</td>
<td>Amplification</td>
<td>−</td>
<td>Breast</td>
<td>TP53 p.R273H; PTEN p.?: RB1 p.?: SMAD4 p.0?</td>
</tr>
<tr>
<td>HTB-178™</td>
<td>NCI-H596</td>
<td>EGFR</td>
<td>−</td>
<td>Amplification</td>
<td>−</td>
<td>Lung</td>
<td>TP53 p.G245C; RB1 p.S182fs*3; PIK3CA p.E545K</td>
</tr>
<tr>
<td>CRL-5928™</td>
<td>NCI-H2170</td>
<td>ERBB2</td>
<td>−</td>
<td>−</td>
<td>Amplification</td>
<td>Lung</td>
<td>TP53 p.P158G; CDKN2A p.0?</td>
</tr>
</tbody>
</table>
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
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
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 downstream signaling pathways.

- DMR agonism assays measure the signal of a ligand.

- DMR antagonism measures the response of antagonism to the receptor agonism induced signal (antagonist is applied before the agonist).
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

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Name</th>
<th>Gene</th>
<th>Mutation</th>
<th>EGFR Copy No.</th>
<th>ERBB2 Copy No.</th>
<th>Source</th>
<th>Response (pm)</th>
<th>EGF EC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRL-2868™</td>
<td>HCC827</td>
<td>EGFR</td>
<td>p.ELREA746del</td>
<td>Amplification</td>
<td>-</td>
<td>Lung</td>
<td>50</td>
<td>1.1 µM</td>
</tr>
<tr>
<td>CRL-2871™</td>
<td>HCC4006</td>
<td>EGFR</td>
<td>p.ELR746del</td>
<td>-</td>
<td>-</td>
<td>Lung</td>
<td>20</td>
<td>1.3 µM</td>
</tr>
<tr>
<td>CCL-231™</td>
<td>SW48</td>
<td>EGFR</td>
<td>p.G719S</td>
<td>-</td>
<td>-</td>
<td>Colon</td>
<td>200</td>
<td>820 pM</td>
</tr>
<tr>
<td>HTB-132™</td>
<td>MDA-MB-468</td>
<td>EGFR</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Breast</td>
<td>430</td>
<td>1.3 nM</td>
</tr>
<tr>
<td>HTB-19™</td>
<td>BT-20</td>
<td>EGFR</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Breast</td>
<td>315</td>
<td>160 nM</td>
</tr>
<tr>
<td>HTB-178™</td>
<td>NCI-H596</td>
<td>EGFR</td>
<td>-</td>
<td>Amplification</td>
<td>-</td>
<td>Lung</td>
<td>100</td>
<td>71 nM</td>
</tr>
<tr>
<td>HTB-177™</td>
<td>NCI-H460</td>
<td>EGFR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Lung</td>
<td>40</td>
<td>1.2 nM</td>
</tr>
<tr>
<td>CRL-5928™</td>
<td>NCI-H2170</td>
<td>ERBB2</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>Lung</td>
<td>230</td>
<td>2.6 nM</td>
</tr>
<tr>
<td>HTB-20™</td>
<td>BT-474</td>
<td>ERBB2</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>Breast</td>
<td>100</td>
<td>N/A</td>
</tr>
<tr>
<td>HTB-27™</td>
<td>MDA-MB-361</td>
<td>ERBB2</td>
<td>-</td>
<td>-</td>
<td>Amplification</td>
<td>Breast</td>
<td>60</td>
<td>1.1 µM</td>
</tr>
</tbody>
</table>

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

DMR Profile: EGF

Cell Line Feature

**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

**HCC827**
- EGFR activation
- EGFR p.ELREA746 deletion (activation mutation)

**HCC4006**
- EGFR p.ELR746 deletion (activation mutation)
High magnitude of EGF induced DMR correlates with EGFR gene amplification

**DMR Profile: EGF**

**MDA-MB-468**
- Response: 430 pm

**BT-20**
- Response: 315 pm

**NCI-H596**
- Response: 100 pm

**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**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>EGFR Copy Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-175-VII</td>
<td>High level amplification</td>
</tr>
<tr>
<td>HCC827</td>
<td>High level amplification</td>
</tr>
<tr>
<td>HCC4006</td>
<td>High level amplification</td>
</tr>
<tr>
<td>SW48</td>
<td>High level amplification</td>
</tr>
<tr>
<td>NCI-H1975</td>
<td>High level amplification</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>High level amplification</td>
</tr>
<tr>
<td>BT-20</td>
<td>Middle level amplification</td>
</tr>
<tr>
<td>NCI-H596</td>
<td>Middle level amplification</td>
</tr>
<tr>
<td>NCI-H480</td>
<td>Middle level amplification</td>
</tr>
<tr>
<td>NCI-H2170</td>
<td>Middle level amplification</td>
</tr>
<tr>
<td>BT-474</td>
<td>Middle level amplification</td>
</tr>
<tr>
<td>MDA-MB-361</td>
<td>Low level amplification</td>
</tr>
</tbody>
</table>

**Protein Expression**

- EGFR
- ERBB2
- p-EGFR (Y1068)
- p-EGFR (Y1086)
- β-ACTIN
High magnitude of EGF induced DMR correlates with EGFR2 gene amplification

**DMR Profile: EGF**

- **NCI-H2170**
  - Response: 230 pm
  - EGFR2/ERBB2 copy number: High level amplification

- **BT-474**
  - Response: 100 pm
  - EGFR2/ERBB2 copy number: Middle level amplification

- **MDA-MB-361**
  - Response: 60 pm
  - EGFR2/ERBB2 copy number: 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**

![Characterization Data](image)
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 blot data are useful resource to facilitate the interpretation of assay results.
Types of agents to target EGFR

**Monoclonal Antibodies:** Cetuximab, Panitumumab
Bind to the extracellular domain of EGFR and compete with endogenous ligands to block the ligand-induced EGFR tyrosine kinase activation by blocking the ligand binding region.

**Tyrosine Kinase Inhibitors (TKIs):** Gefitinib, Erlotinib, Lapatinib, Canertinib
Compete reversibly with adenosine 5' triphosphate to bind to the intracellular catalytic domain of EGFR tyrosine kinase and inhibit the EGFR autophosphorylation and downstream signaling.

**Antibody Based Immunoconjugates:** Trastuzumab-Emtansine, EQ75-ADR
Improve the therapeutic window of chemotherapeutic agents or render the drug inactive (act as a prodrug) by altering their *in vivo* distribution due to conjugation with tumor-targeting monoclonal antibodies.

**Antisense oligodeoxynucleotides:** GEM 231
Decrease the expression of EGFR and regulates the cell proliferation for potential anti-cancer therapy.

**Other Novel Agents:** FR18, Affibodies, Nanobodies, Peptides
Interfere with the binding mechanism of EGF to its receptor due to structural similarity or have high binding affinity toward EGFR, making them suitable targeting moieties for the delivery of cancer therapeutics.
TKI: Iressa/Gefitinib

- 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

Competes reversibly with ATP to bind to intracellular catalytic domain of EGFR tyrosine kinase, inhibiting EGFR autophosphorylation and downstream signaling
DMR profiles demonstrate selective inhibition of EGFR by Iressa based on EGFR mutations

- **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.

**DMR Profile: EGF+Iressa**

**HCC827**

**NCI-H1975**

**Inhibition Curve**

**Iressa IC<sub>50</sub>: 3.4 E-7 M**

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

**DMR Profile: EGF+Iressa**

**MDA-MB-468**

**Inhibition Curve**

**Iressa IC\(_{50}\): 5.7 E-7 M**

**Cell Line Feature**

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

**NCI-H596**

**Iressa IC\(_{50}\): 2.8 E-6 M**

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

**NCI-H460**

**Iressa IC\(_{50}\): 3.8 E-5 M**

- 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

**BT-20**

**Inhibition Curve**
- *Iressa IC<sub>50</sub>: 2.8 E-6 M*

**NCI-H596**

**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<sub>50</sub> 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.*
## Selected compounds from Tocriscreen™ Kinase Library

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Biological Activity</th>
</tr>
</thead>
</table>
| 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

- Selective inhibitors of IκB 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-κB pathway potential could be a strategy for EGFR inhibitor-resistant tumor/cell lines.
Identification new target in Iressa-sensitive cell line NCI-H2170 by screening Tocriscreen™ Kinase Library

- 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.
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.
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
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.
Thank you!

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

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

---

**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
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 in culture conditions. Cell morphology was observed under Nikon™ microscopy, and images of the indicated cell lines were captured with a digital camera.

IF Staining: A,D: EGFR; B: phospho-EGFR; C: ERBB2; E-H, merged with F-actin/Hoechst
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 3684.

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 signalling pathways such as p-AKT and p-ERK. β-actin protein was included as a loading-control.
# Recommended controls

## Wild-type control cell lines

<table>
<thead>
<tr>
<th>ATCC® number</th>
<th>Cell line name</th>
<th>Tissue source</th>
<th>Cell type</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTB-25™</td>
<td>MDA-MB-175-VII</td>
<td>Breast</td>
<td>Epithelial</td>
<td>Ductal carcinoma</td>
</tr>
<tr>
<td>CRL-10317™</td>
<td>MCF 10A</td>
<td>Breast</td>
<td>Epithelial</td>
<td>Normal</td>
</tr>
<tr>
<td>CCL-75™</td>
<td>WI-38</td>
<td>Lung</td>
<td>Fibroblast</td>
<td>Normal</td>
</tr>
<tr>
<td>CRL-9609™</td>
<td>BEAS-2B</td>
<td>Lung</td>
<td>Epithelial</td>
<td>Normal</td>
</tr>
<tr>
<td>CRL-1459™</td>
<td>CCD-18Co</td>
<td>Colon</td>
<td>Fibroblast</td>
<td>Normal</td>
</tr>
<tr>
<td>CRL-2704™</td>
<td>C13589</td>
<td>Hematopoietic and lymphoid tissue</td>
<td>B lymphoblast</td>
<td>Normal</td>
</tr>
</tbody>
</table>

## 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

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

2-step assay:
1. Herceptin preincubation for 60 min

1-step co-stimulation assay:
1. Herceptin does not compete with EGF for binding