CRISPR/Cas9 Genome-edited Drug-resistant Cell Models: An Advanced Approach for Overcoming Drug Resistance

Fang Tian, PhD
Director of Biological Content, ATCC
About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World’s largest, most diverse biological materials and standards development organization. Information resource for microbes
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- Multiple accreditations, including ISO 9001 and ISO 13485, cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Global presence. Sales and distribution in 150 countries, 19 international distributors
- Talented team of ~500 employees, over one-third with advanced degrees
Therapy resistance in cancer

- Therapeutic resistance in cancer is multifactorial and heterogeneous.
- Spatial and temporal resistance occurs in tumor cells, within the stroma, or in metastasis.
- The underlying mechanisms of drug resistance are diverse and complex, often driven by:
  - Tumor heterogeneity
  - Genetic and epigenetic alternations
  - Drug transporters
  - Lineage plasticity
  - Adaptive signaling events
  - Tumor microenvironment (TME)
Overcoming resistance: new approaches

- Applying evolutionary principles
  - Competitive release of drug-resistant subclone
  - Intermittent therapy: allow drug-sensitive cells to outcompete drug-resistant subclones

- Modulating the TME
  - Targeting the tumor vasculature, stroma, and immune cells

- Learning from exceptional responders
  - Extending exceptional responses to broader patient populations

- Many of the models used in the early stages of research don’t capture cancer’s mechanisms of resistance to therapeutics, which impede progress in drug development and clinical trials.
- To overcome this roadblock, ATCC is committed to providing the advanced cell models to push the envelope in cancer research.
ATCC drug resistant cell models

Long-term drug selection derived cell lines

- CPT
- FUdR
- doxorubicin
- etoposide
- dactinomycin
- bleomycin
- mitoxantrone
- thioguanine

Resistant to chemotherapy drugs

- Tamoxifen

Resistant to targeted therapies

- Herceptin
- Imatinib

CRISPR engineered cell lines

- BRAF inhibitors
- MEK inhibitors

Resistant to targeted therapies
Establish drug resistance cell model through gene editing

Drug resistance in melanoma

BRAF inhibitor resistance in melanoma patients

Week 1
Week 15
Week 23


Ras/Raf/MEK/ERK MAP kinase signaling regulates BRAF inhibitor drug resistance

Cell survival and proliferation

Develop isogenic lines of drug resistance

Advanced *in vitro* cell models that contain defined genetic drug resistance mechanisms are needed to facilitate the development of next-generation therapeutics that can overcome BRAF drug resistance in melanoma.

**CRISPR gene editing**

**Drug resistant mutations**

**Evaluate host cell lines**

**Edited cell pool test**
- Editing efficiency
- Functional test

**Single clone screening**
- Sequencing
- Bio-functional screening

**Select top clones**
- Genetic verification
- Transcriptional test
- Protein expression
- Off target evaluation
- Stability test
- Morphology and cell growth
- Bio-function verification

Drug resistant isogenic cell models
Characterization of A375 isogenic cell lines

- Genome sequence
- Transcript sequence
- Protein expression
- Off-target screening
- Cell morphology
- Cell growth kinetics
- Drug response
- Cell line stability
- Cell line authentication
- Sterility test

<table>
<thead>
<tr>
<th>Cell Line Name</th>
<th>Engineered Genotype</th>
<th>Target Site Genome Sequence</th>
<th>Transcript Sequence of Target Gene</th>
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<tr>
<td>KRAS Mutant-A375 Isogenic</td>
<td>KRAS G13D heterozygous</td>
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<tr>
<td>NRAS Mutant-A375 Isogenic</td>
<td>NRAS Q61K heterozygous</td>
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<tr>
<td>MEK1 Mutant-A375 Isogenic</td>
<td>MEK1 Q56P homozygous</td>
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A375 isogenic lines for 2D drug screening

<table>
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<tr>
<th>Cell Line Name</th>
<th>ATCC® No.</th>
<th>BRAF V600E</th>
<th>Engineered Mutation</th>
<th>Engineered Genotype</th>
<th>BRAF Inhibitor Resistance</th>
<th>MEK Inhibitor Resistance</th>
<th>3D Functional Validation</th>
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<tbody>
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<td>CRL-1619</td>
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<td>KRAS G13D</td>
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**Dabrafenib Resistance in A375 Isogenic Melanoma Models**

- A375
- KRAS Mutant A375 Isogenic
- NRAS Mutant A375 Isogenic
- MEK1 Mutant A375 Isogenic

**No Etoposide Resistance in A375 Isogenic Melanoma Models**

- A375
- KRAS Mutant A375 Isogenic
- NRAS Mutant A375 Isogenic
- MEK1 Mutant A375 Isogenic
A375 isogenic lines for 3D culture drug screening
MEK1 isogenic A375 lines for combination therapy study

<table>
<thead>
<tr>
<th>Drug (1h)</th>
<th>BRAF</th>
<th>Combo</th>
<th>MEK</th>
<th>Controls</th>
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<tr>
<td>WT</td>
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<td>IB: pMEK1/2</td>
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<tr>
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<tr>
<td>IB: GAPDH</td>
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</tbody>
</table>

Trametinib (MEK Inhibitor)

Dabrafenib (BRAF Inhibitor)

50/50 MEK/BRAF Combination

2D culture

3D culture

No Drug | Dabrafenib | Trametinib | Combination

A375 WT | MEK1 Q56P
Unique features of KRAS isogenic A375 line

KRAS\textsuperscript{G13D} A375 isogenic line highly expressing EGFR and PD-L1

**Table:**

<table>
<thead>
<tr>
<th>Cell Line Format</th>
<th>A375 WT</th>
<th>NRAS Q61K</th>
<th>KRAS G13D</th>
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<tbody>
<tr>
<td>2D 3D</td>
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</table>

**IB:**
- EGFR
- MEK
- p-MEK
- ERK1/2
- p-ERK1/2
- AKT
- p-AKT
- PD-L1
- GAPDH

**Graph:**

- IFN\gamma +, Isotype Control
- IFN\gamma -, Isotype Control
- IFN\gamma -, PD-L1 staining
- IFN\gamma +, PD-L1 staining

**Figure:**

PD-L1
Isogenic A375 lines for IO and combination therapy study

- KRAS\textsuperscript{G13D} A375 Isogenic line harbors BRAF\textsuperscript{V600E}, KRAS\textsuperscript{G13D} mutations, highly expresses EGFR and IO checkpoint PD-L1
- Together with its parental cell line, KRAS\textsuperscript{G13D} A375 Isogenic line can be an ideal drug resistant melanoma model for the studies of combination therapy using BRAF inhibitor and IO checkpoint blockade
Drug-resistant isogenic A375 lines for in vivo study

**Generation of Luciferase Expressing isogenic A375 Cell Line**

- Lenti-Luc2 plasmid
- Viral transduction
- Pool evaluation and selection
- Expansion and validation of single clones

**In vitro Confirmation of Luciferase Expression and Stability**

**KRAS\textsuperscript{G13D} A375 Isogenic-Luc2 Tumor Model and *in vivo* Bioluminescence Imaging**

Day: 3, 7, 13, 21, 28

**KRAS\textsuperscript{G13D} mutant A-375 Isogenic-Luc2 radiance vs time**

Radiance (p/s/cm²/sr)

Day: 3, 7, 13, 21, 28

Counts

7.0E5

9.3E3
Cell-based models are critical tools for understanding the mechanisms of drug resistance and developing novel therapeutics.

ATCC has been developing state-of-the-art drug-resistant cancer cell models by using CRISPR-Cas9 gene-editing technology to introduce critical mutations into disease-relevant cell lines.

These novel cell lines have several advantages including cell line homogeneity, stability of the relevant genotype, do not require for continued drug pressure to maintain the cell line, and modeling the acquired resistance to newly developed therapeutics.
Thank you and questions?

**Checkpoint Molecule Profiling in Tumor and Immune Cell Lines:**
Applications for Immuno-oncology Drug Screening
April 21 at 12:00 PM EST
Presented by Zhizhan Gu, PhD

**Tips and Techniques for Propagating your Viral Strains**
MAY 12 at 12:00 PM EST
Adria Allen, MS; Alexander Piccirillo, MS; and Megan Yockey, BS

For more ATCC CRISPR-edited cell lines navigate to

[www.atcc.org/isogenic](http://www.atcc.org/isogenic)