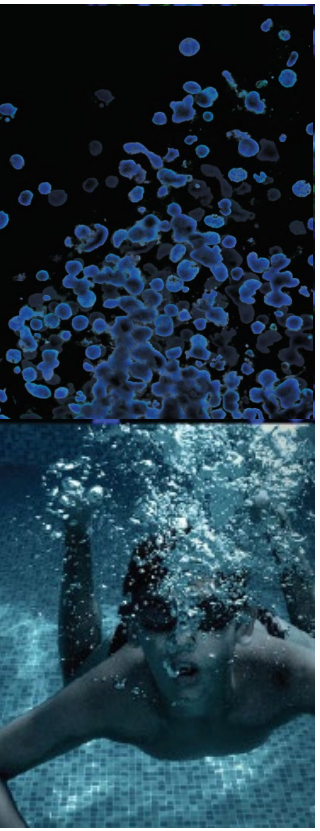
Advanced drug-resistant cell models for cancer therapeutic resistance studies



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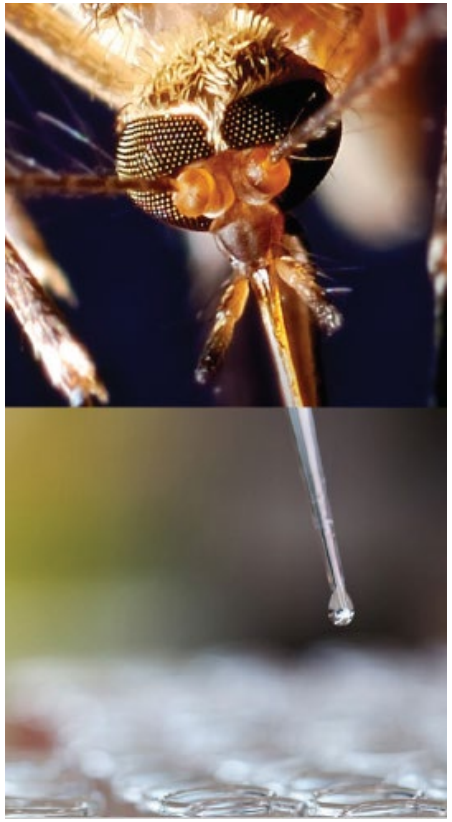
Principal Scientist

Head of Cell biology R&D group

November 2020

Credible Leads to Incredible™



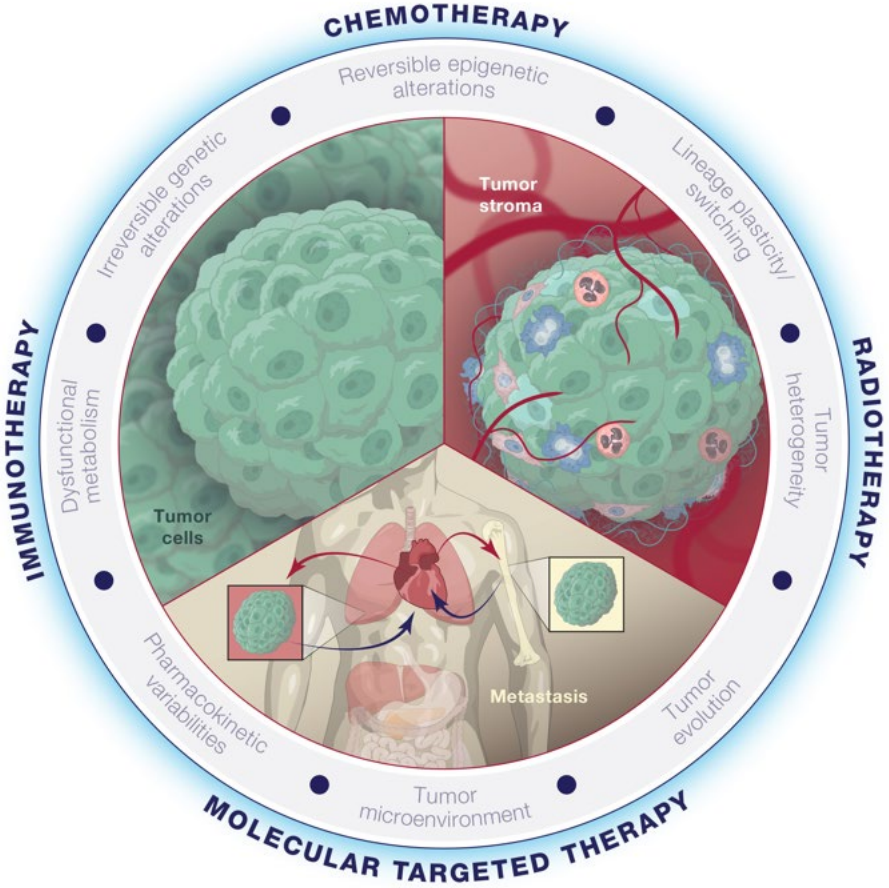
About ATCC

- 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

ATCC®

Therapy resistance in cancer

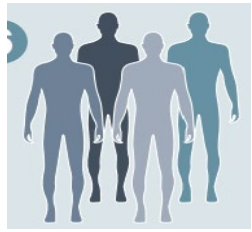
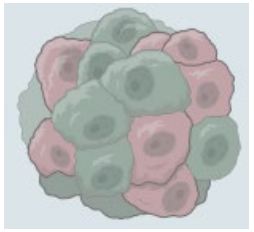
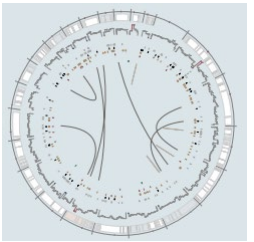
Wheel of Resistance



- 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

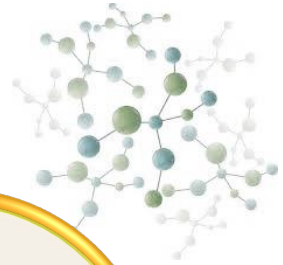
- Many of the models used in the early stages of research don't capture cancer's mechanisms of resistance to therapeutics, which impede progress in drug development and clinical trials.
- To overcome this roadblock, ATCC is committed to providing the advanced cell models to push the envelope in cancer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

ATCC drug resistant cell models

Long-term drug selection derived cell lines

Resistant to chemotherapy drugs

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



Resistant to targeted therapies

- Herceptin
- Imatinib

Resistant to hormonal therapies

- Tamoxifen

CRISPR engineered cell lines

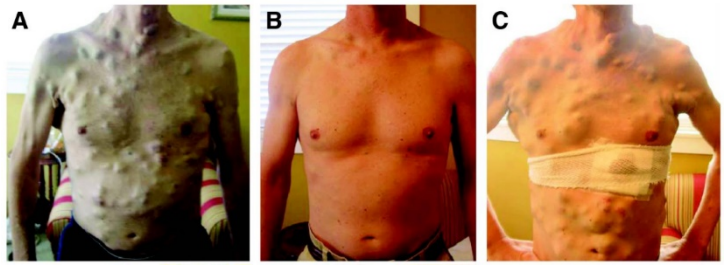
Resistant to targeted therapies

- BRAF inhibitors
- MEK inhibitors

Establish drug resistance cell model through gene editing

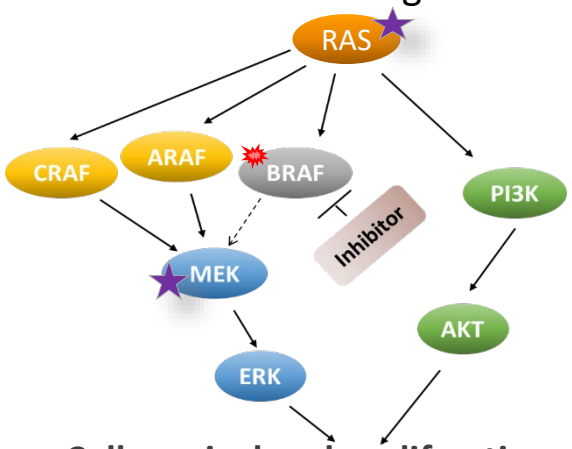
Drug resistance in melanoma

BRAF inhibitor resistance in melanoma patients



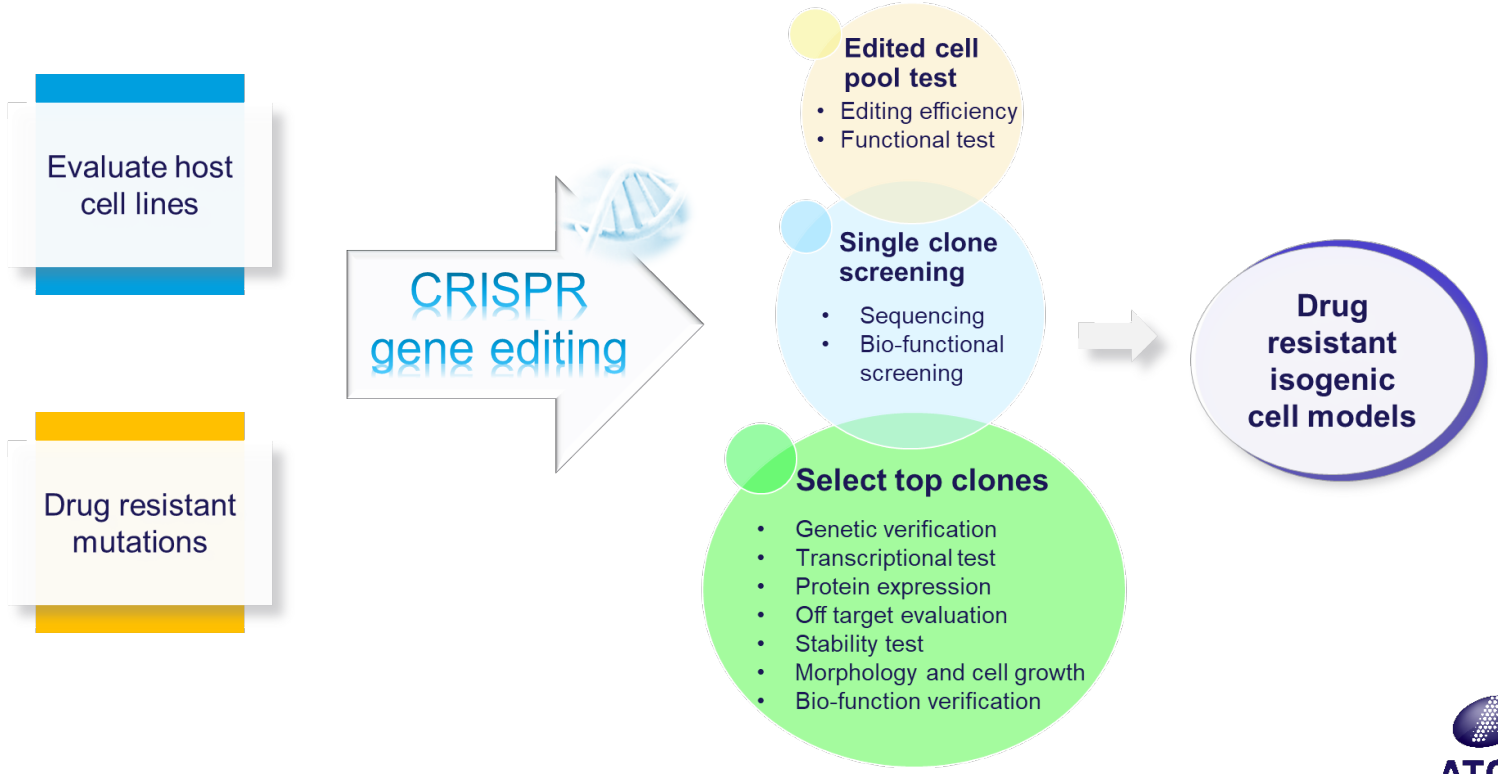
Wagle N, *et al.* J Clin Oncol 29(22): 3085-3096, 2011

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



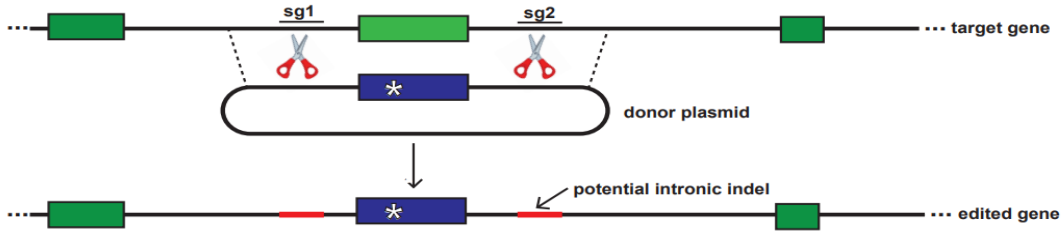
Develop isogenic lines of drug resistance

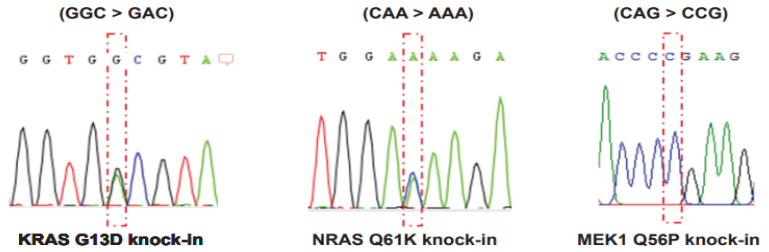
Advanced *in vitro* cell models that contain defined genetic drug resistance mechanisms are needed to facilitate the development of next-generation therapeutics that can overcome BRAF drug resistance in melanoma.

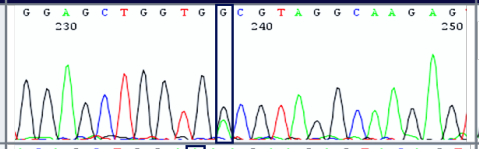
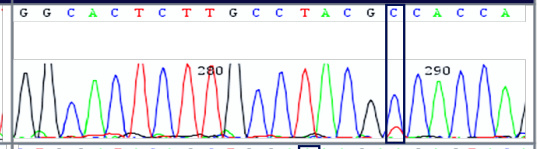
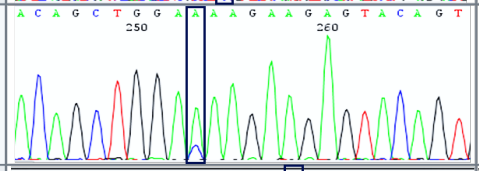
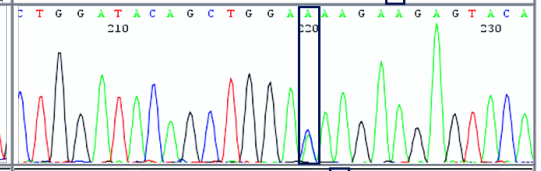
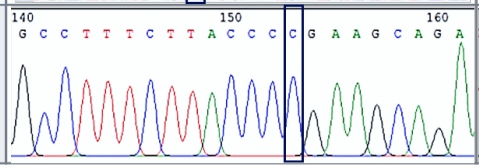
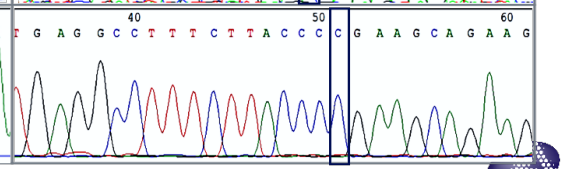


Characterization of A375 isogenic cell lines

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

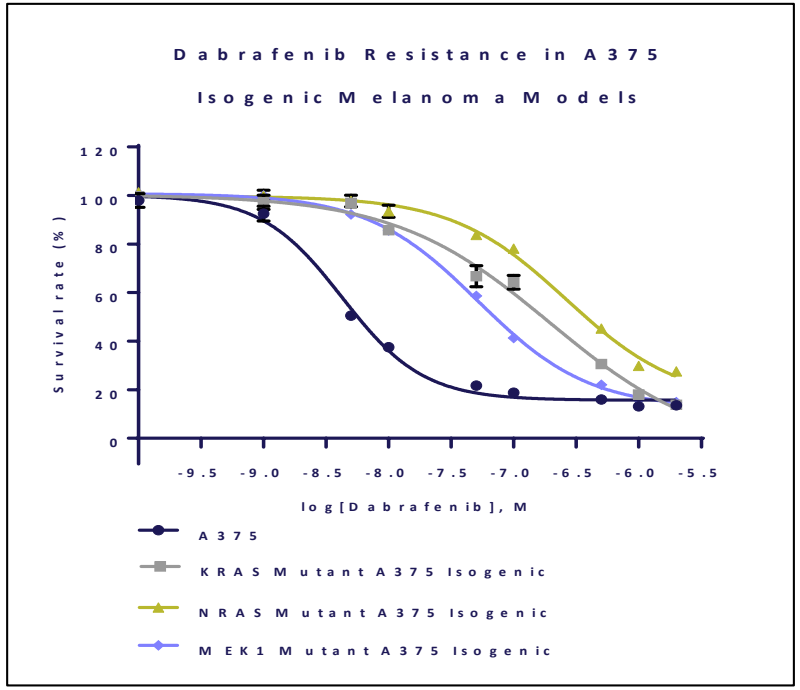


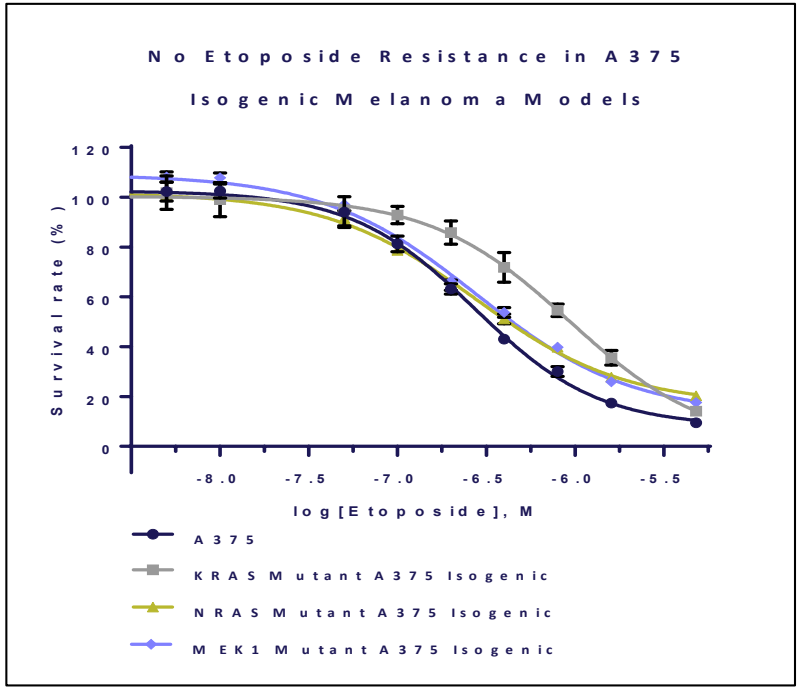


Cell Line Name	Engineered Genotype	Target Site Genome Sequence	Transcript Sequence of Target Gene
KRAS Mutant-A375 Isogenic	KRAS G13D heterozygous		
NRAS Mutant-A375 Isogenic	NRAS Q61K heterozygous		
MEK1 Mutant-A375 Isogenic	MEK1 Q56P homozygous		

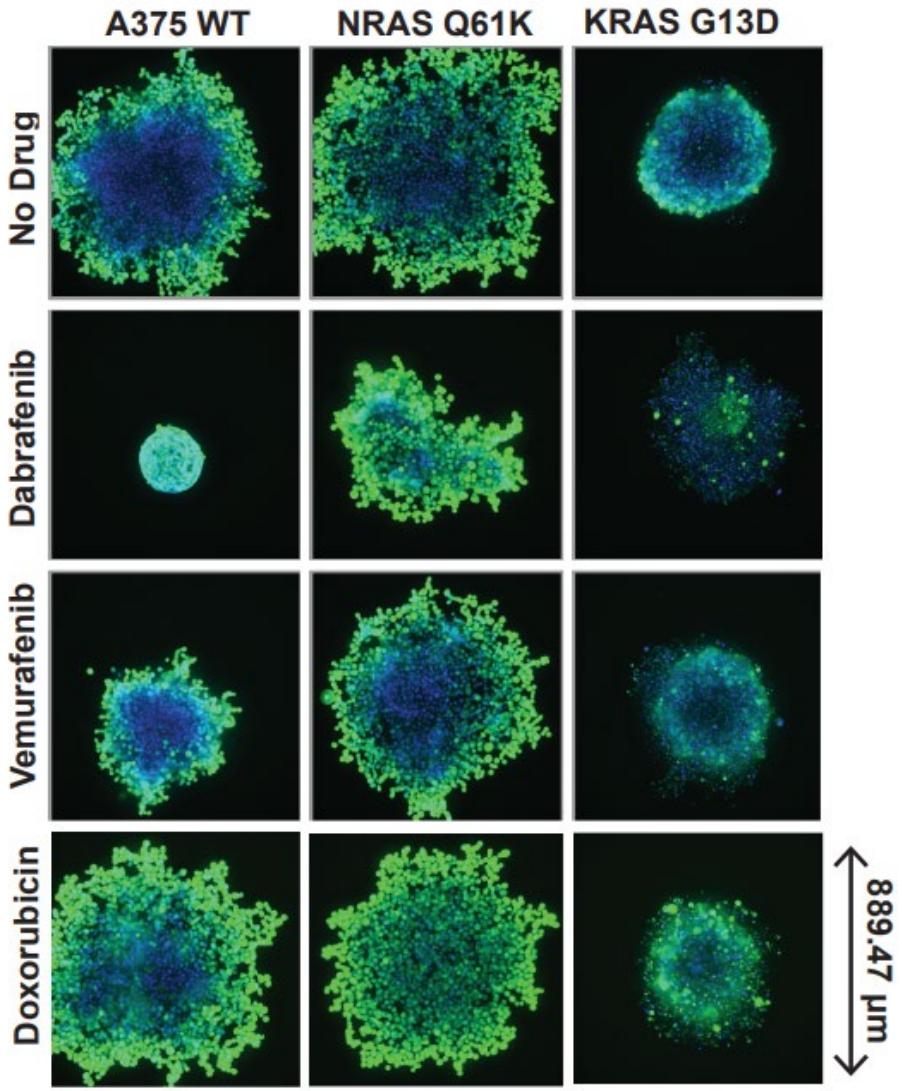
A375 isogenic lines for 2D drug screening

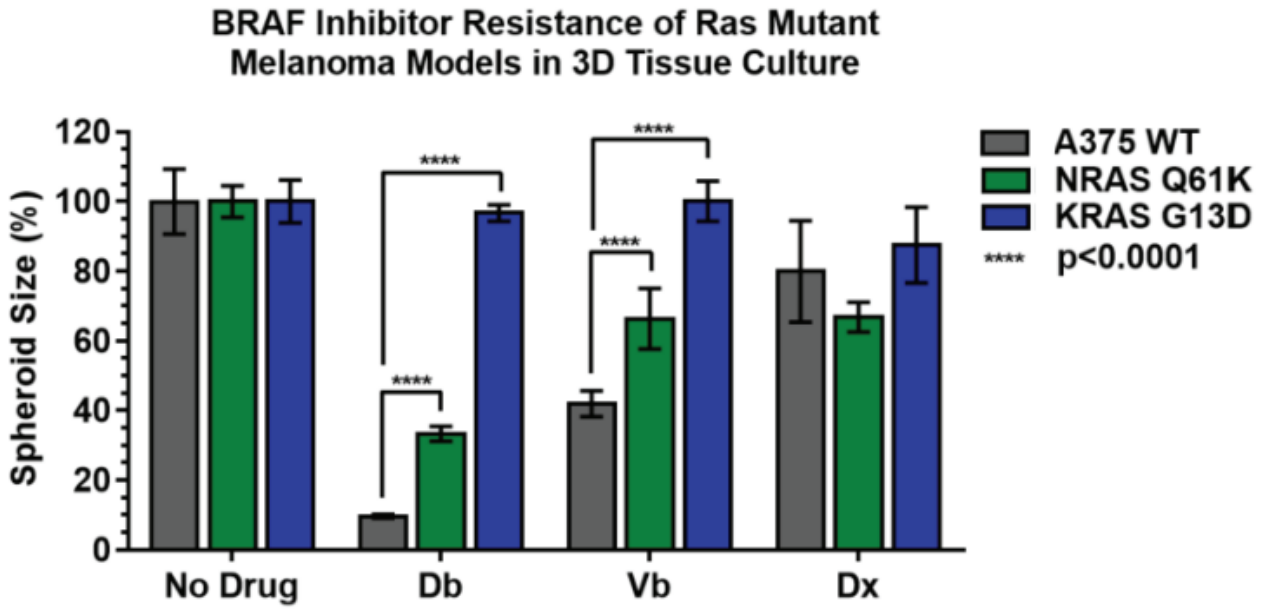
Cell Line Name	ATCC® No.	BRAF V600E	Engineered Mutation	Engineered Genotype	BRAF Inhibitor Resistance	MEK Inhibitor Resistance	3D Functional Validation
Unedited A375	CRL-1619™	+	N/A	N/A	−	−	+
KRAS Mutant-A375 Isogenic	CRL-1619IG-1™	+	KRAS G13D	heterozygous	+	−	+
NRAS Mutant-A375 Isogenic	CRL-1619IG-2™	+	NRAS Q61K	heterozygous	+	−	+
MEK1 Mutant-A375 Isogenic	CRL-1619IG-3™	+	MEK1 Q56P	homozygous	+	+	+



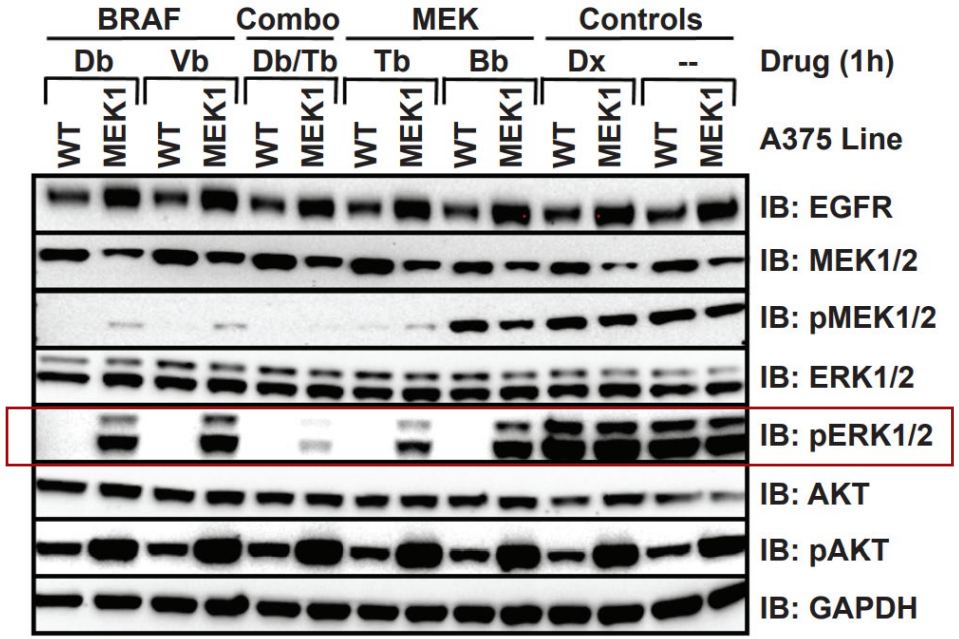


A375 isogenic lines for 3D culture drug screening

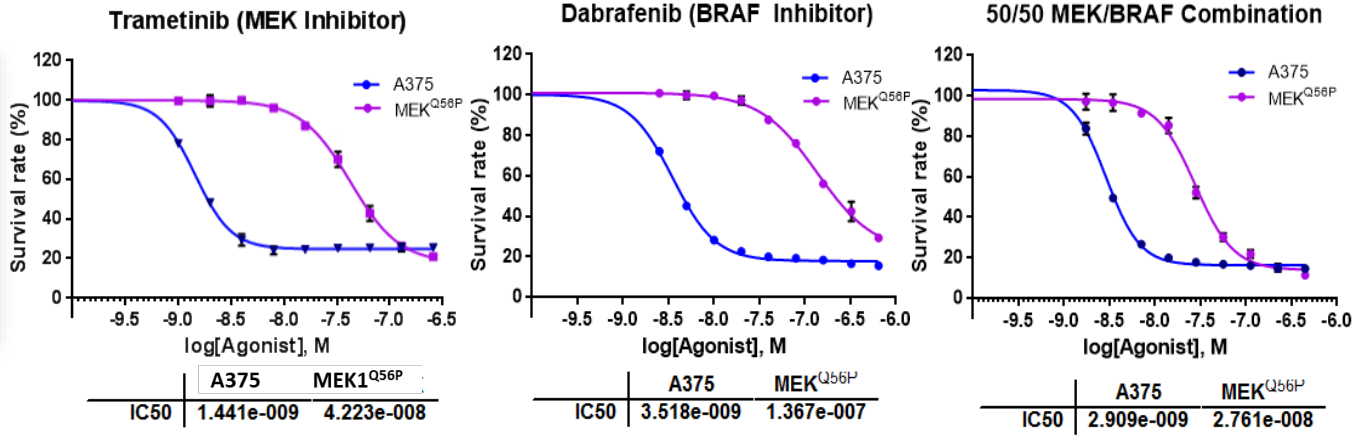




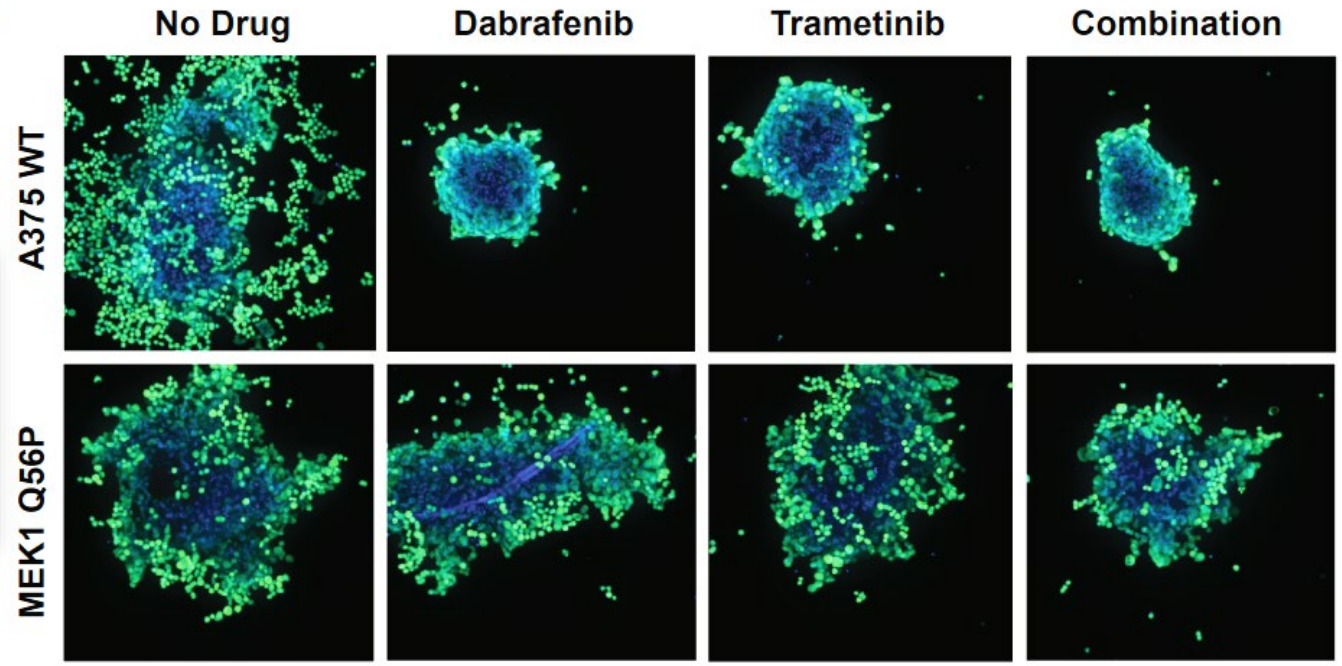
MEK1 isogenic A375 lines for combination therapy study



2D culture

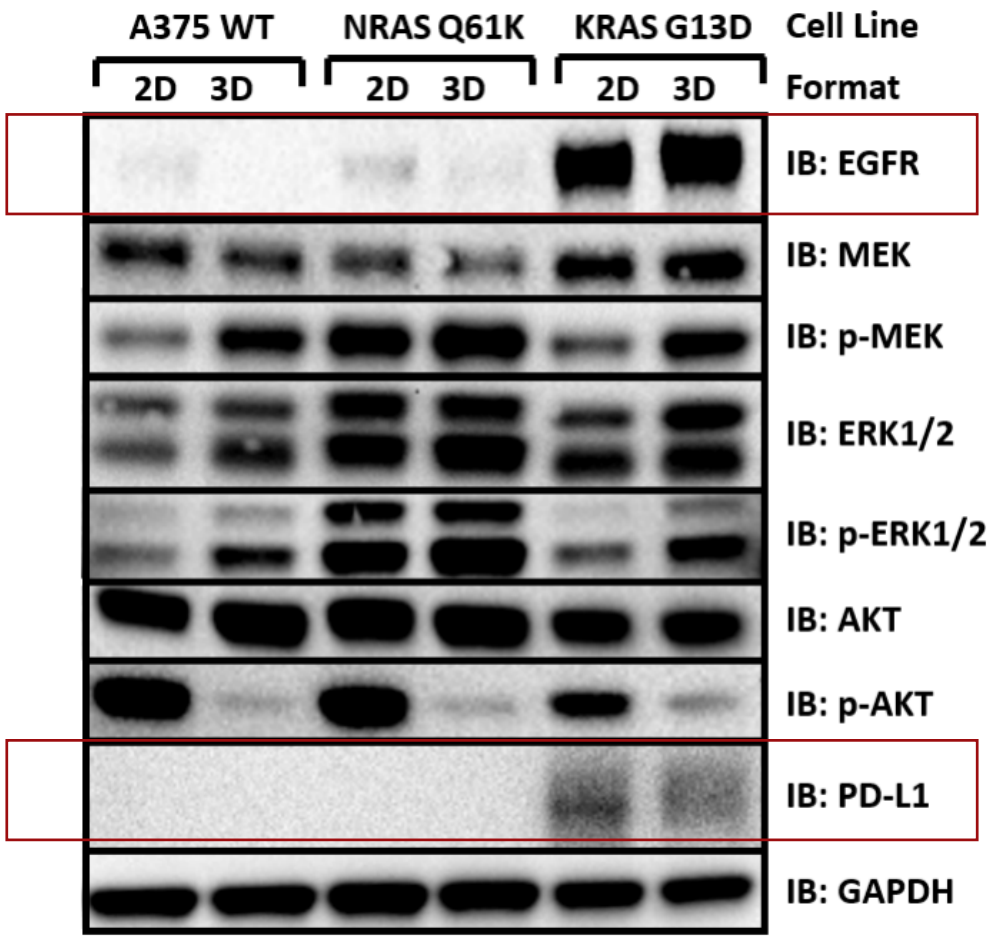


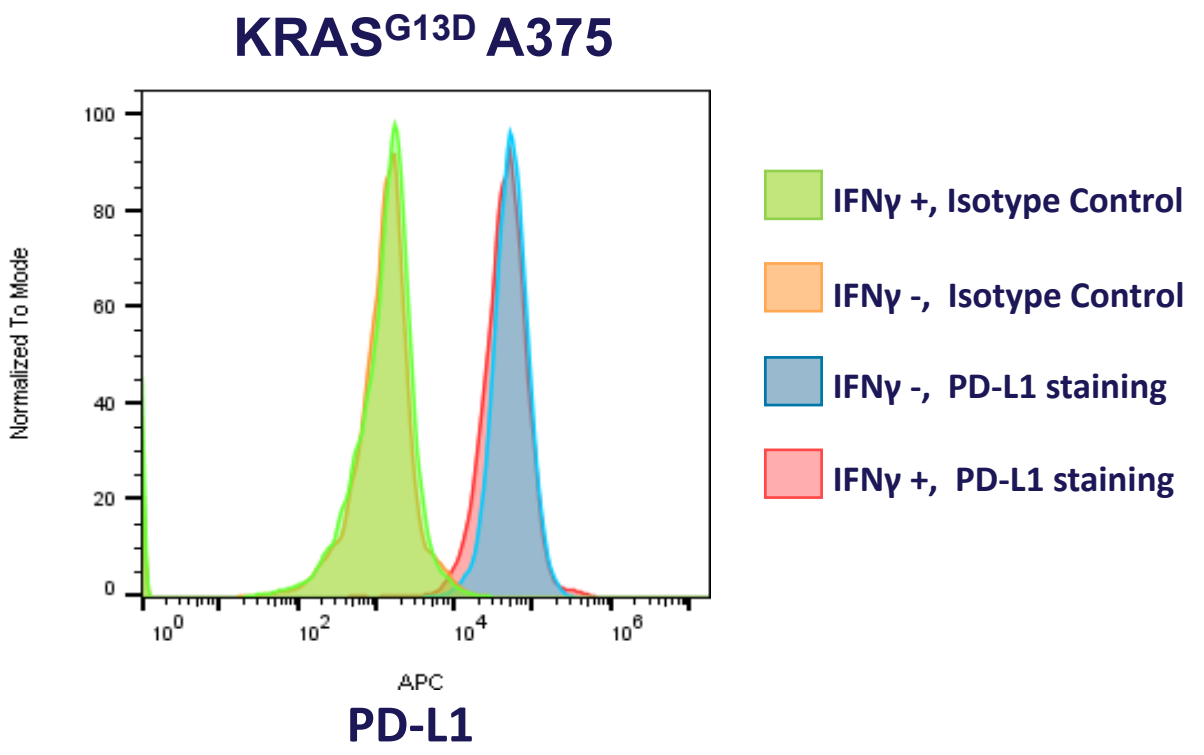
3D culture



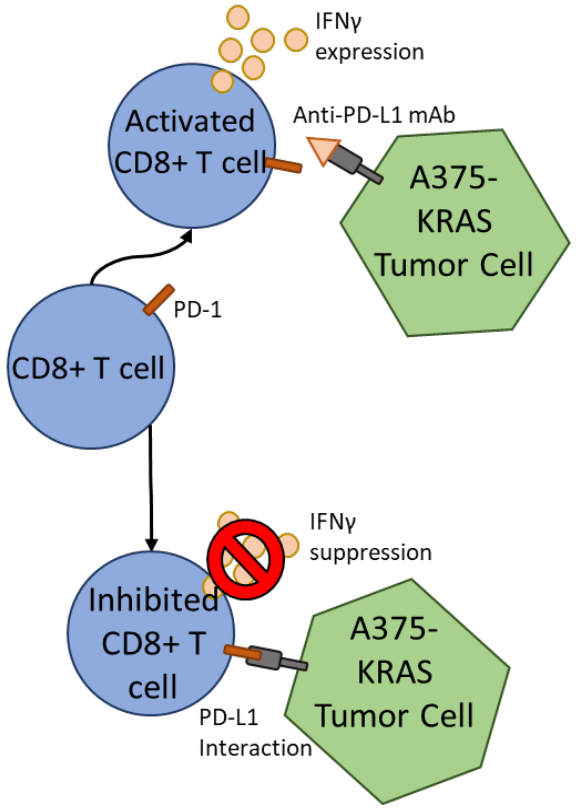
Unique features of KRAS isogenic A375 line

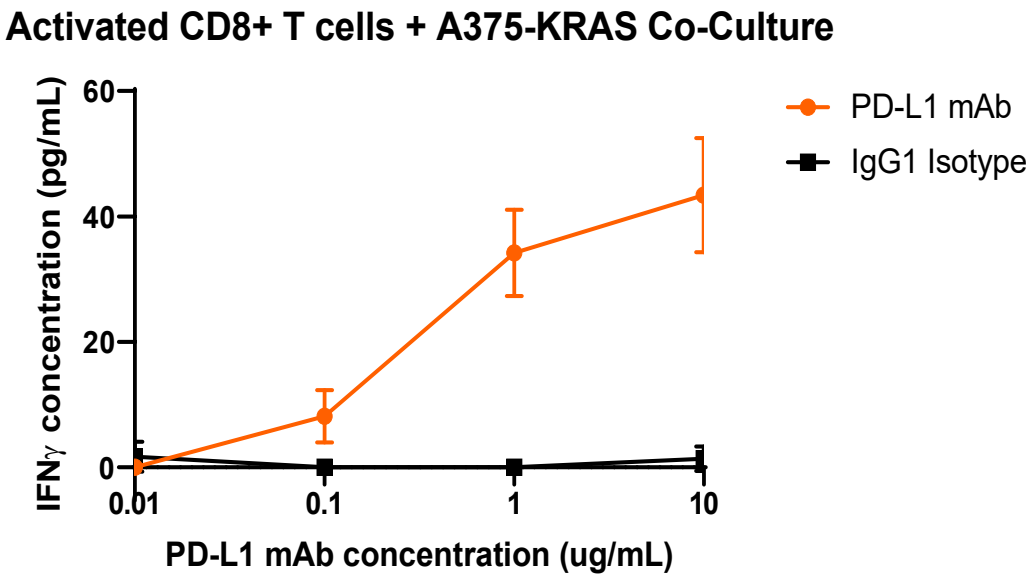
$KRAS^{G13D}$ A375 isogenic line highly expressing EGFR and PD-L1





Isogenic A375 lines for IO and combination therapy study

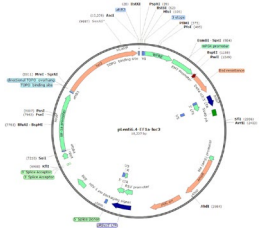




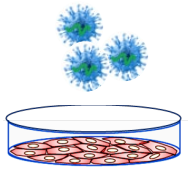
- KRAS^{G13D} A375 Isogenic line harbors BRAF^{600E}, KRAS^{G13D} mutations, highly expresses EGFR and IO checkpoint PD-L1
- Together with it's parental cell line, KRAS^{G13D} A375 Isogenic line can be an ideal drug resistant melanoma model for the studies of commination therapy using BRAF inhibitor and IO checkpoint blockade

Drug-resistant isogenic A375 lines for in vivo study

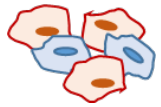
Generation of Luciferase Expressing isogenic A375 Cell Line



Lenti-Luc2 plasmid



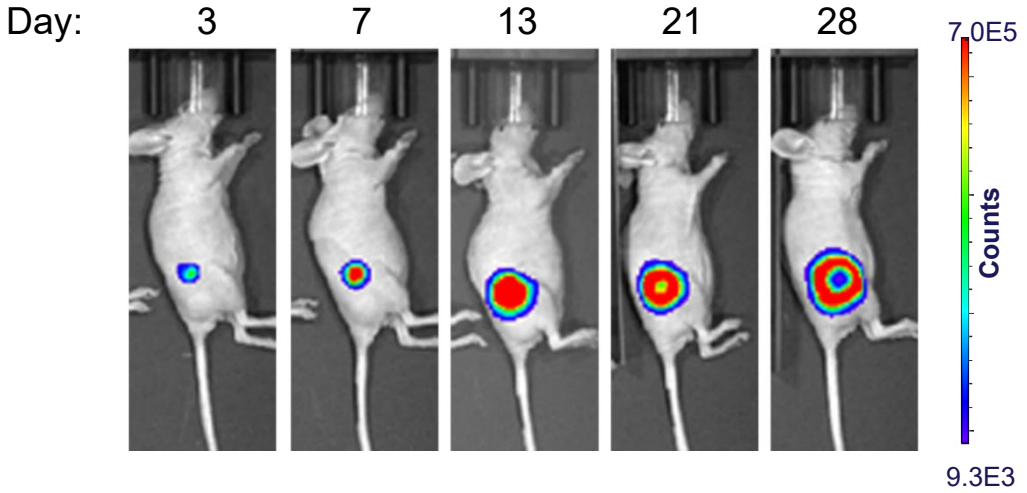
Viral transduction



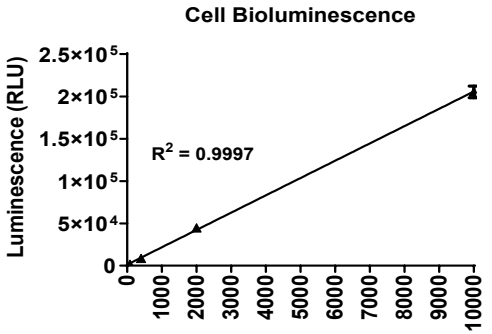
Pool evaluation and selection

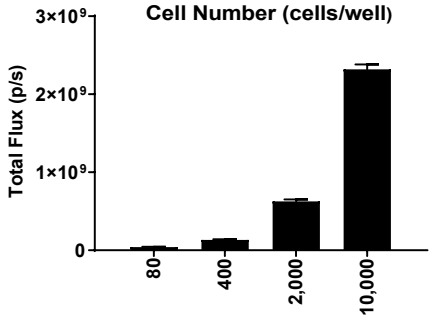
Expansion and validation of single clones

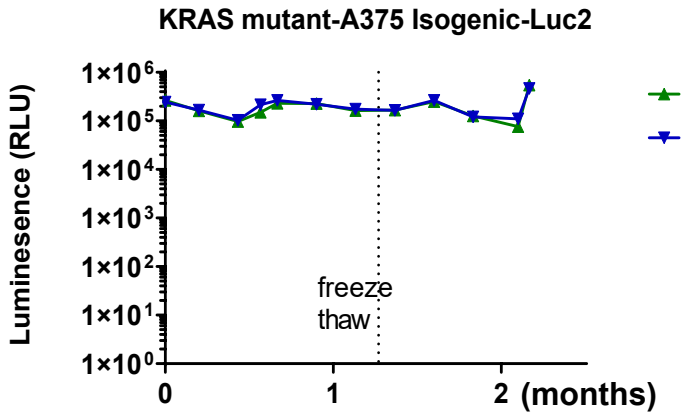
KRAS^{G13D} A375 Isogenic-Luc2 Tumor Model and *in vivo* Bioluminescence Imaging

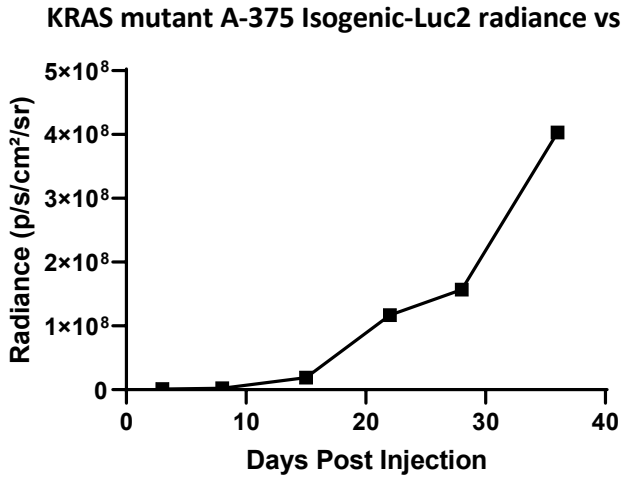


In vitro Confirmation of Luciferase Expression and Stability

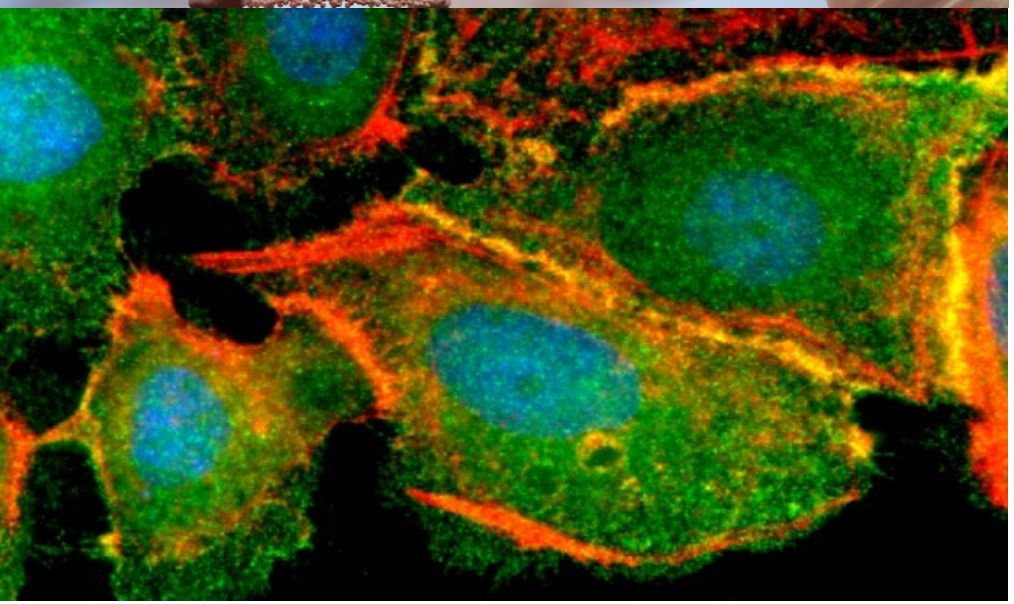






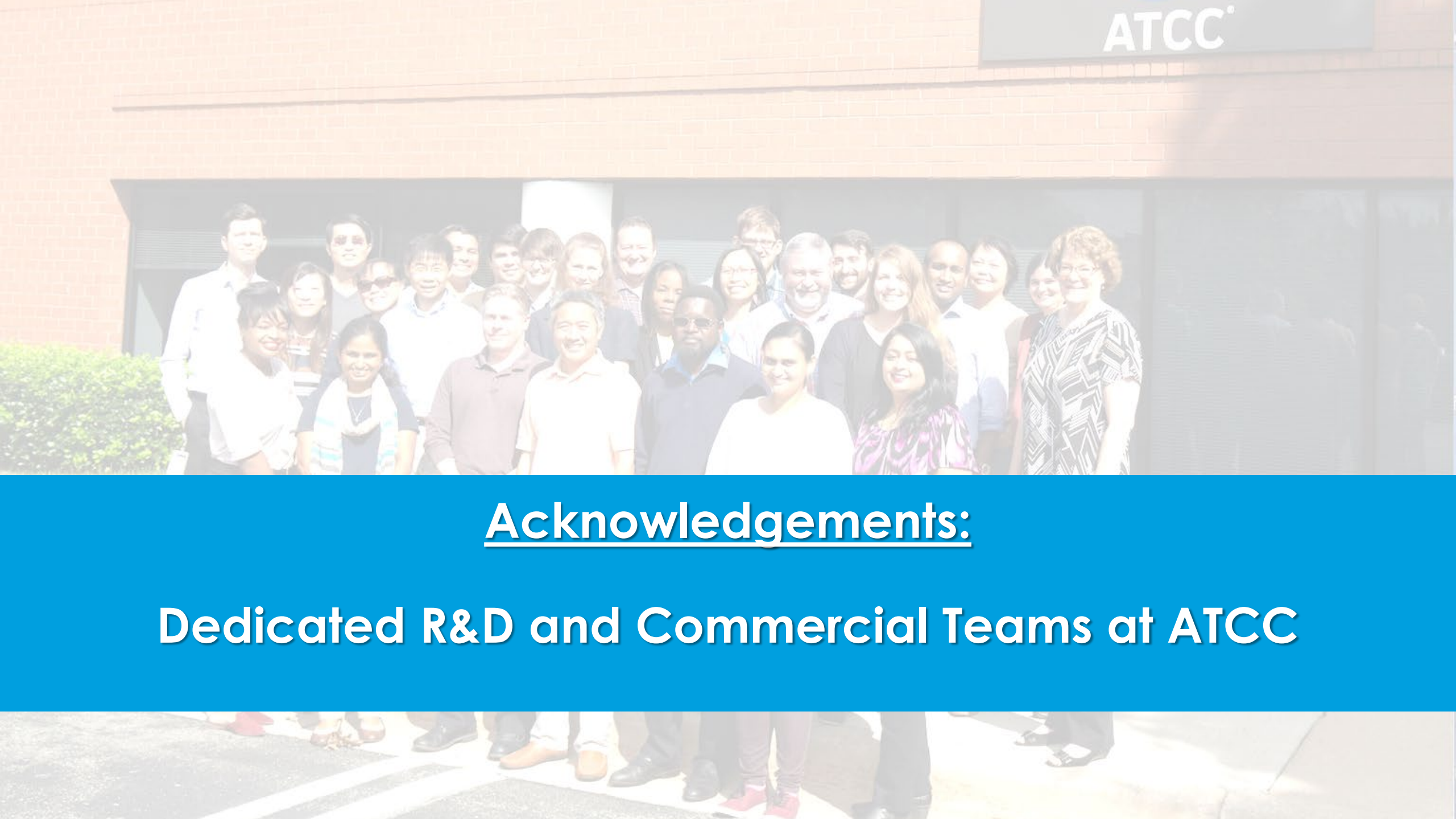


Summary



- 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.

ATCC



Acknowledgements:

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