New Type of Drug Discovery Isogenic Cell Models Created by CRISPR Genome Editing

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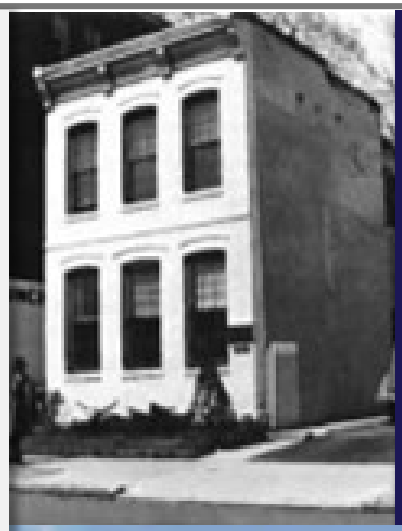
ATCC

Outline

- CRISPR technology in drug discovery
- The use of CRISPR/Cas9 to create new disease-model cell lines
  - EML4-ALK fusion in lung cancer
  - IDH1R132H mutant in glioma
  - IDH2R140Q mutant in leukemia
- The use of CRISPR/Cas9 to create new types of drug resistant cancer cell models
  - NRASQ61K mutant in drug-resistant melanoma
  - KRASG13D mutant in drug-resistant melanoma

ATCC Snapshot

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA (175,000 sf) and an R&D and Services center in Gaithersburg, MD (36,000 sf)
  - Worldwide brand name and quality recognition
- World's premiere biological materials resource and standards development organization
  - 4,000 cell lines
  - 70,000 microbes
- ATCC collaborates with and supports the scientific community with industry-standard and innovative biological solutions
  - Growing portfolio of products and services
  - Sales and distribution in 140 countries, 12 International distributors
- Talented team of 425+ employees; over one third with advanced degrees
- Multiple recognized accreditations including ISO 9001 and ISO 13485



Established partner to global researchers and scientists

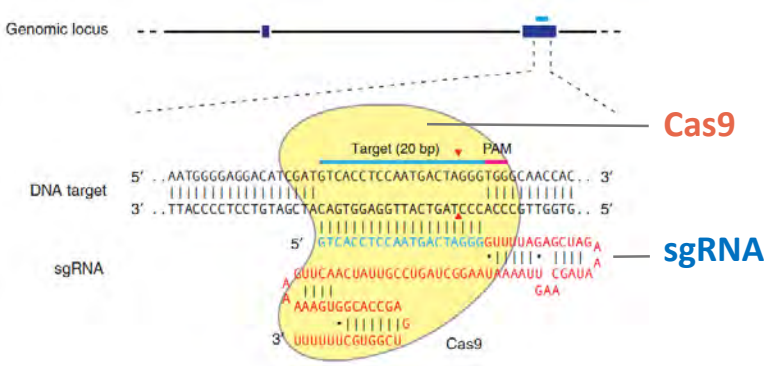
ATCC

CRISPR/Cas9 gene editing technology

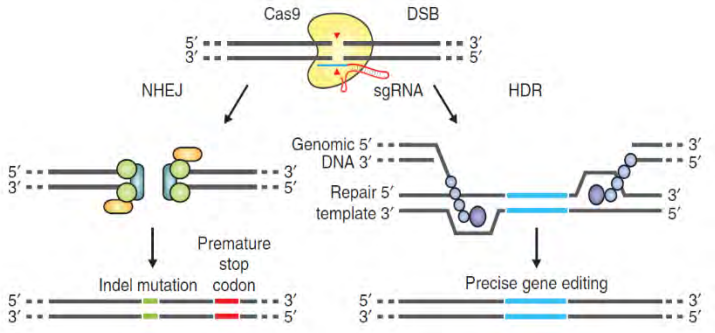
- Important novel gene editing technology
- A PubMed search will pull 5,477 articles about CRISPR
- The ‘single name’ journals have published issues with CRISPR on the cover
- There are many basic science and drug discovery applications for CRISPR/Cas9 gene editing

Use CRISPR/cas9 for gene editing

RNA-guided Cas9 nuclease

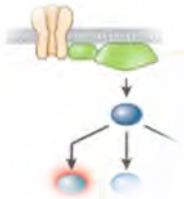


DNA repair promotes gene editing



Ran F, *et al*. Nat Protoc 8(11): 2281-308, 2013.

Application of CRISPR in drug discovery



Functional Screening

Genome-scale knockout, inhibition, and activation screens

Generate precision cell models

Isogenic cell lines for drug target validation, mechanistic analysis, and drug screening

Generate animal models of human disease

Generate therapeutics

Isogenic cells show great promise in drug testing or use in therapeutic regimens

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ATCC CRISPR/Cas9 gene editing platform

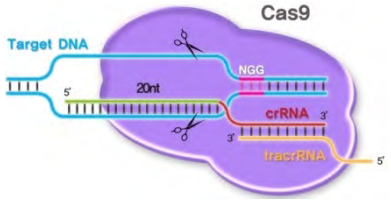
Molecular biology capability

- NGS
- Sanger sequencing
- ddPCR™ and qPCR
- CRISPR reagent design
- Primer design
- Expression vector toolbox
- Plasmids or oligos

Cell biology expertise

- Cell banking
- Cell line authentication
- Modification of extant cell lines
- Single cell cloning
- Cell line characterization

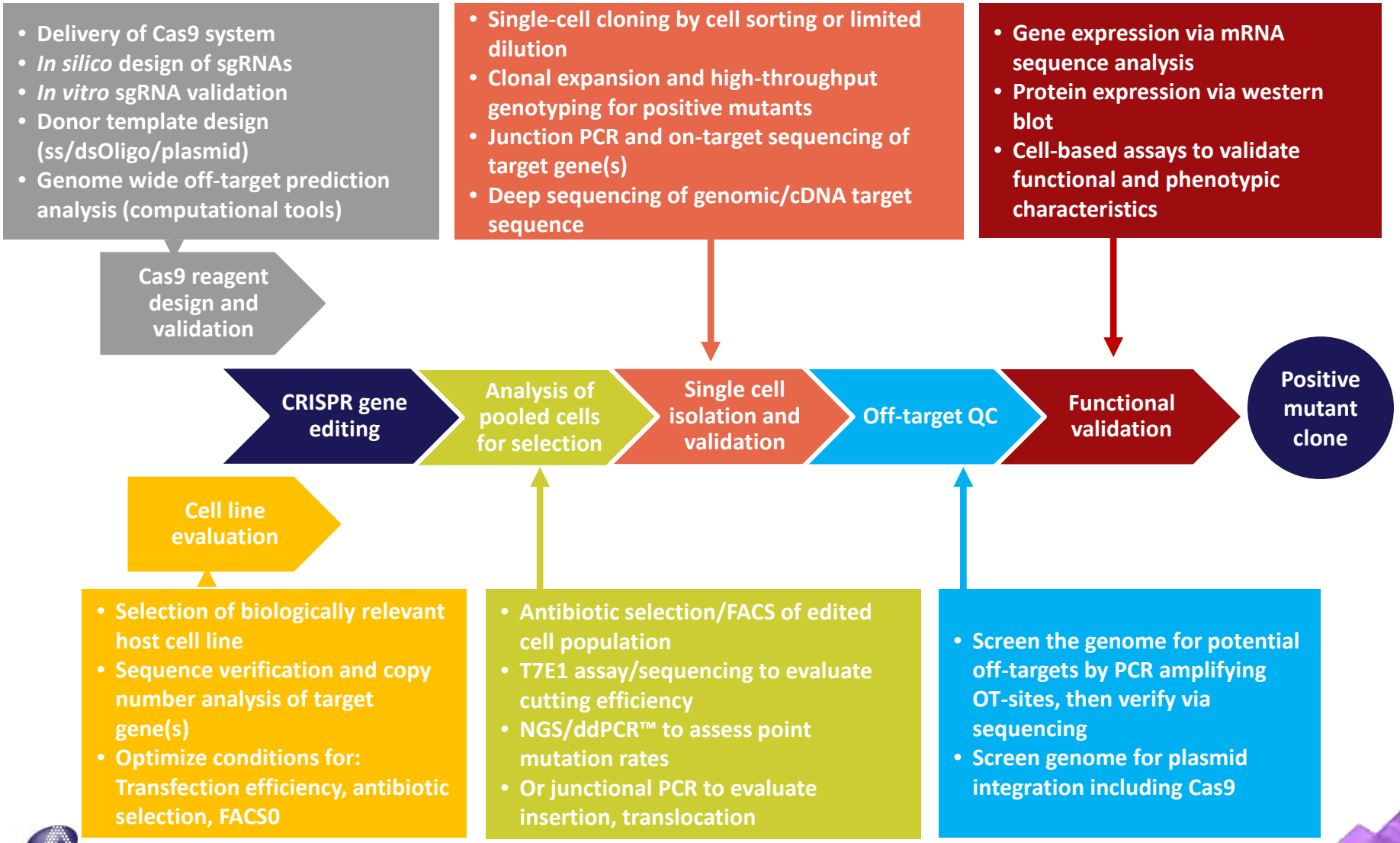
Use genome editing to create new cell models that contain rare mutations and biomarkers



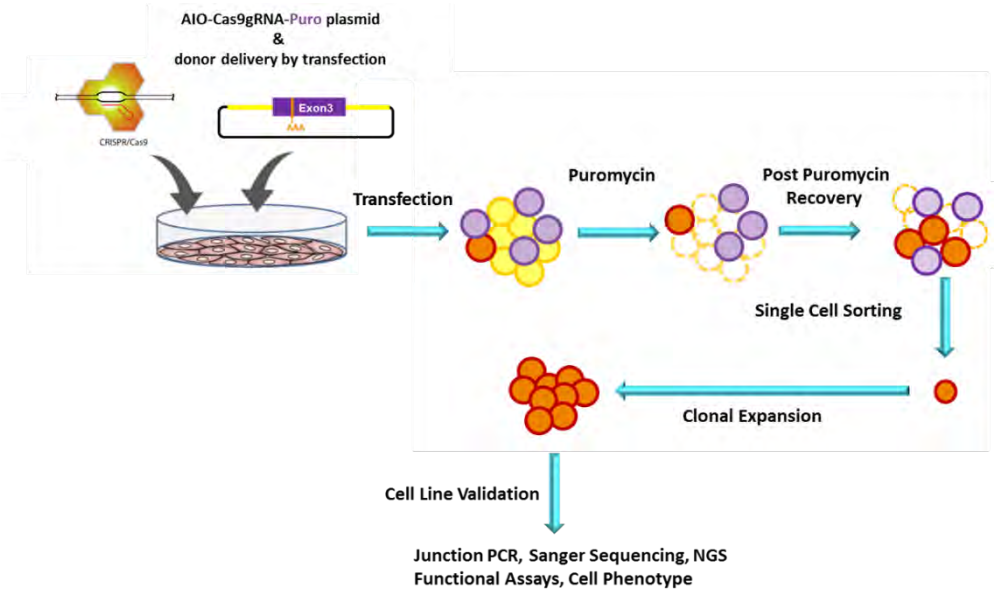
CRISPR platform

- Gene knock out
- Gene insertion
- Gene modification

ATCC gene editing workflow



Authenticated isogenic cell line in a vial



Positive clone → **Cell Banking** → **Distribution lot**

ATCC Quality and Standards

- Genetic verification
- Cell line purity and sterility confirmation
- Species and identify verification
- Post-freeze viability
- Functional/characterization test

Use of CRISPR system to create cancer disease models

ATCC precision cell models for the development of new anti-cancer therapeutics

Gene translocation

Project example
- EML4-ALK fusion in non-small cell lung cancer model (ATCC® CCL-185IG™)

Driver gene mutations

Project example
- $IDH1^{R132H}$ mutant glioma isogenic cell model (ATCC® HTB-14IG™)
- $IDH2^{R140Q}$ mutant leukemia isogenic cell model (ATCC® CRL-2003IG™)

Drug-resistant mutants

Project example
Drug-resistant melanoma models (ATCC® CRL-1619IG-1™, CRL-1619IG-2™)
- $NRAS^{Q61K}$ mutation KI with $BRAF^{V600E}$
- $KRAS^{G13D}$ mutation KI with $BRAF^{V600E}$

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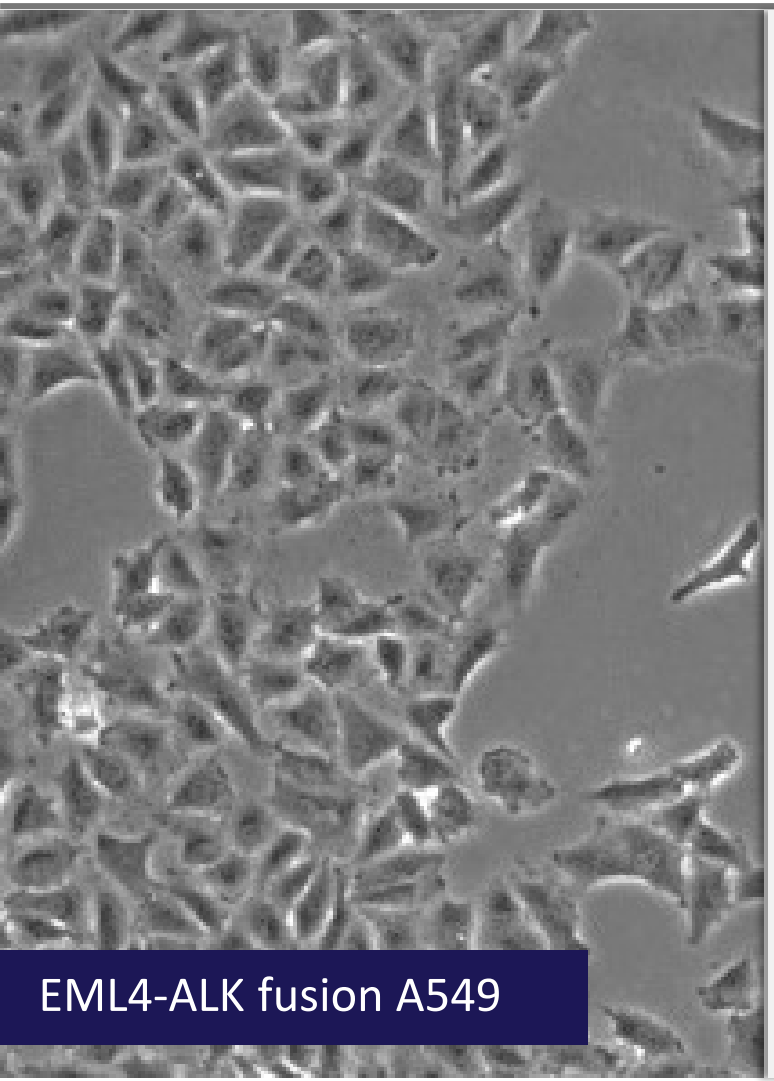
Case study #1: Creation of a gene translocation and fusion using CRISPR/Cas9



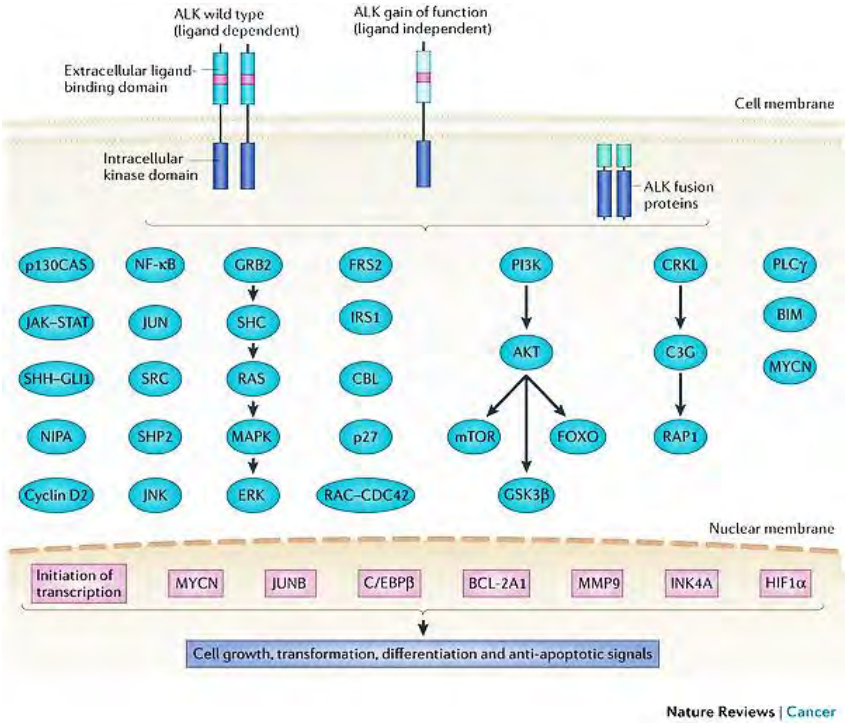
EML4-ALK fusion A549

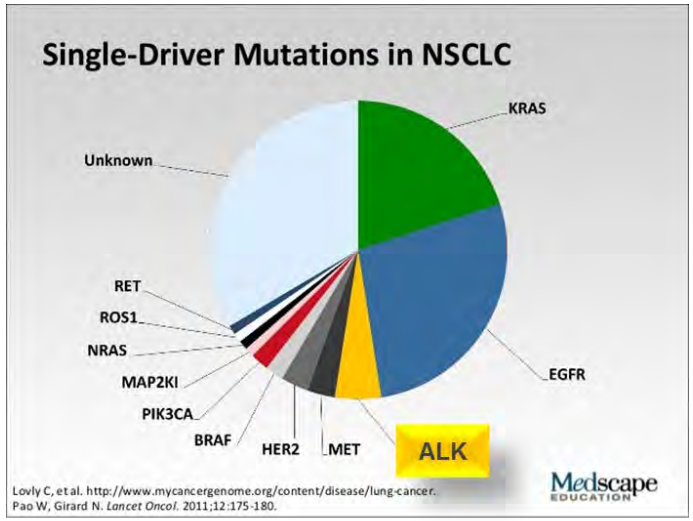
EML4-ALK fusion, isogenic non-small cell lung cancer (NSCLC) model in A549 cells

- ATCC® CCL-185IG™

ALK is a drug target and diagnostic marker

Anaplastic lymphoma kinase (ALK) regulates the cell signaling pathway linked to cell growth, transformation, and metastasis. ALK fusion events are gain-of-function tumorigenic mutations found in NSCLC.





- EML4- ALK fusions have been identified as important drug targets and diagnostic biomarkers
- Lung cancer model cell lines containing EML4-ALK fusion are required for both basic oncology research and clinical drug discovery

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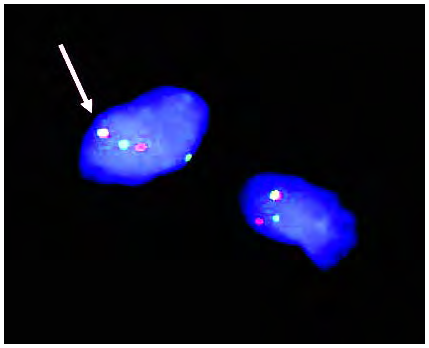
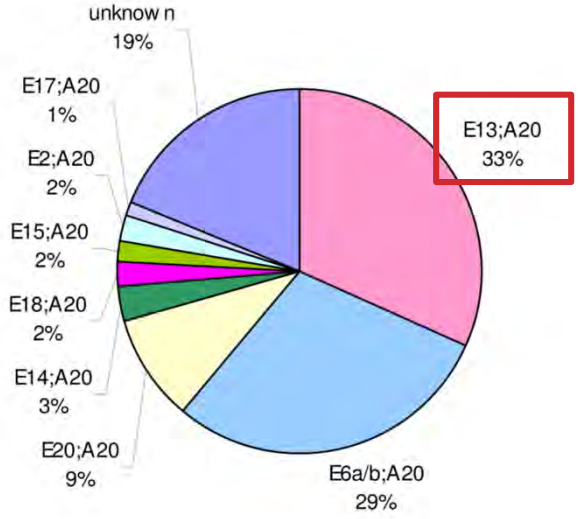
Generation of EML4-ALK fusion in NSCLC A549

EML4-ALK fusion in clinical samples

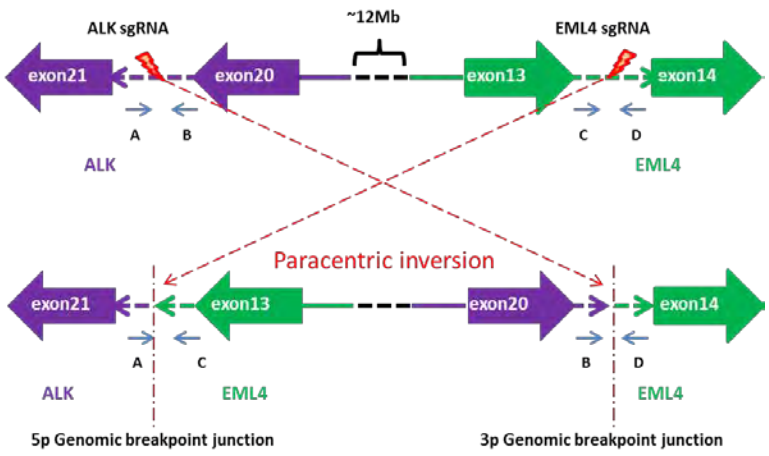


Clin Cancer Res 14(13): 4275-4283, 2008.



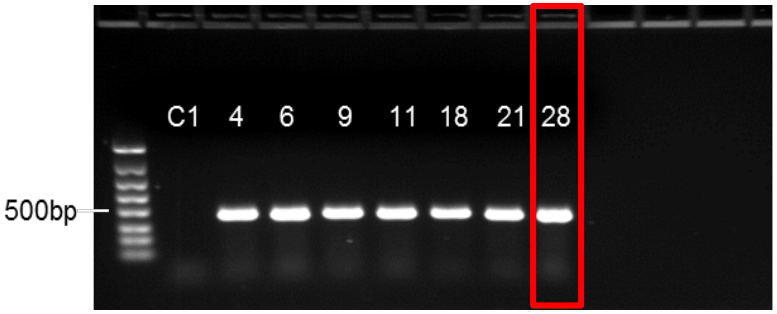
Eur J Cancer 46(10): 1773–1780, 2010.

EML4-ALK variant 1 genomic inversion

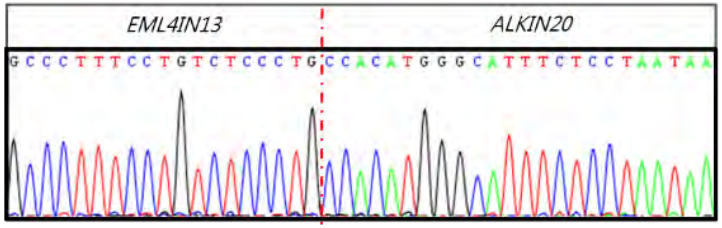


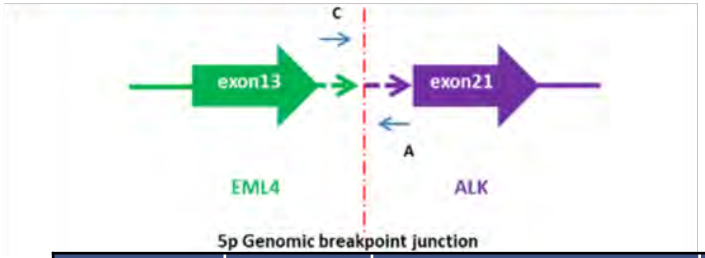
Confirm the designed gene fusion event

5’ Genomic breakpoint junction



A. Sequence analysis verifying junction of single clone

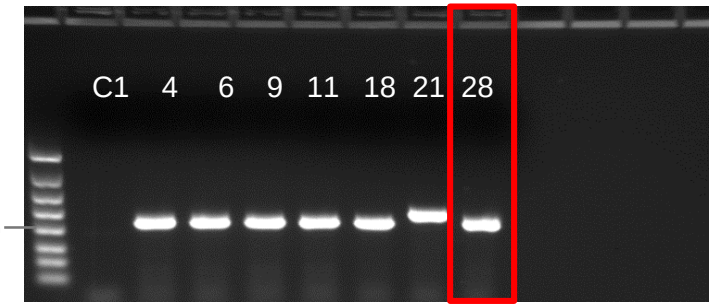




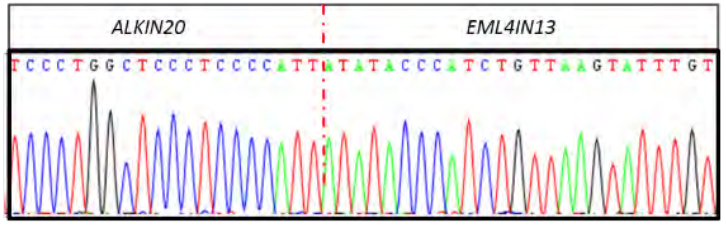
5p Genomic breakpoint junction

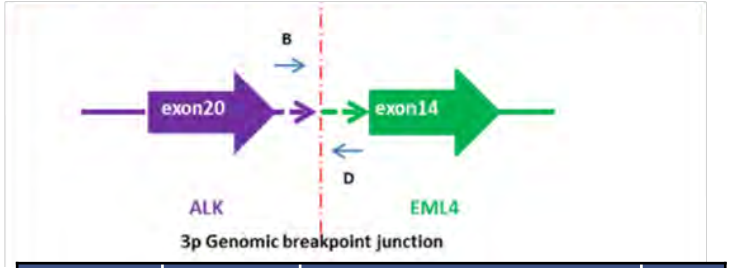
Primer name	Genomic target	Primer sequence (5’ to 3’)	Size
5pEML4IN13	5’EML4-ALK	TGAAACTCCCACACCTTTGCTTTTTG TGTTTTCTTAC	545bp
3pALKIN20	5’EML4-ALK	TGCAGCCATTTGGAATGTCCCCTTT AAATTTAGAAACAG	

3’ Genomic breakpoint junction



B. Sequence analysis verifying junction of single clone





3p Genomic breakpoint junction

Primer name	Genomic target	Primer sequence (5’ to 3’)	Size
5pALKIN20	3’EML4-ALK	GACCTGGTCCTCATGGCTCAGCTTG	577bp
3pEML4IN13	3’EML4-ALK	GGCATACTTCAACAGGGTAGATTTCA TGGTATGTGAATTATATATCG	

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Off-target site evaluation by sequencing

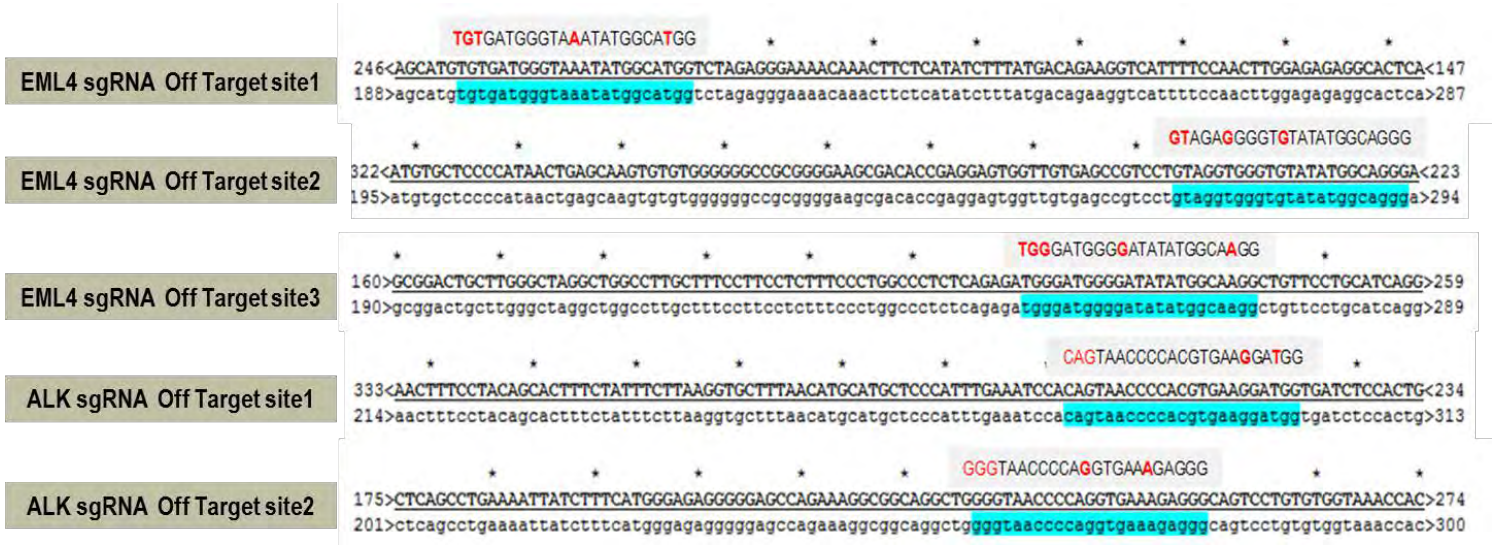
Top potential off target sites:

EML4 sg Target sites/Off Target sites	Target sites/Off Target sites Sequence	On/Off-target score	Gene	Gene Segment	Chromosome
EML4 sgRNA Target site	ACAGATGGGTATATATGGCAGGG	1	EML4	I	2
EML4 sgRNA Off Target site1	TGTGATGGGTAAATATGGCATGG	0.568575	AGA	IG	4
EML4 sgRNA Off Target site2	GTAGAGGGGTGTATATGGCAGGG	0.5054	ASCL1	I	11
EML4 sgRNA Off Target site3	TGGGATGGGGATATATGGCAAGG	0.568575	ETS1	I	11

ALK sg Target sites/Off Target sites	Target sites/Off Target sites Sequence	On/Off-target score	Gene	Gene Segment	Chromosome
ALK sgRNA Target site	GTATAACCCCACGTGAACGAGGG	1	ALK	I	2
ALK sgRNA Off Target site1	GTATAACCCCACGTGAAGGATGG	0.16245	RP11-749H17.1	I	18
ALK sgRNA Off Target site2	GTATAACCCCAGGTGAAAGAGGG	0.113715	CPE	I	4

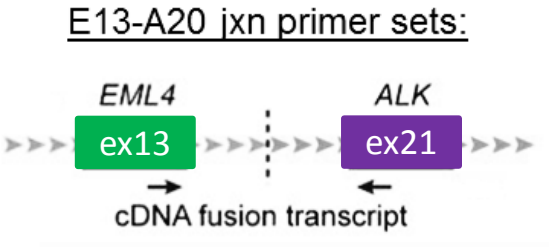
Red: potential mismatched bases

Genome screening for off-target cut sites within the selected isogenic clone:

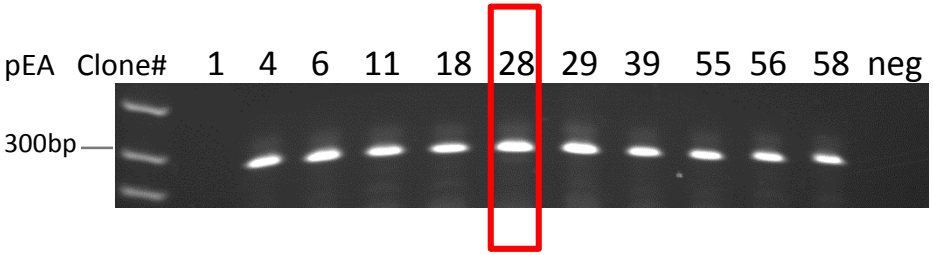


No off-target indels were detected in the EML4-ALK isogenic clone.

Molecular and functional validation of EML4-ALK

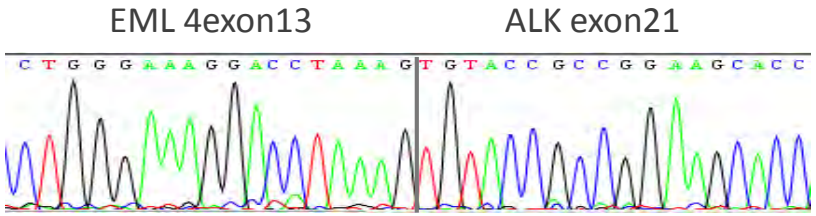


isolate mRNA → cDNA synthesis → junctional PCR

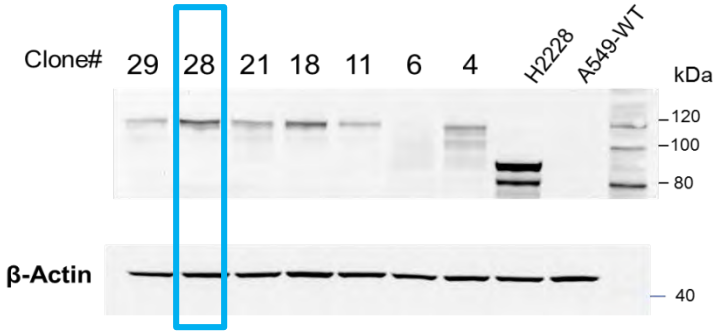


Breakpt jxn (293bp)

Sequence of EML4-ALK v1 fusion transcript across cDNA breakpoint



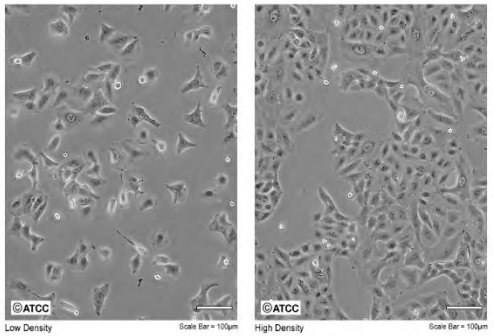
Verify that the EML4-ALK v1 fusion gene express EML4-ALK v1 fusion protein



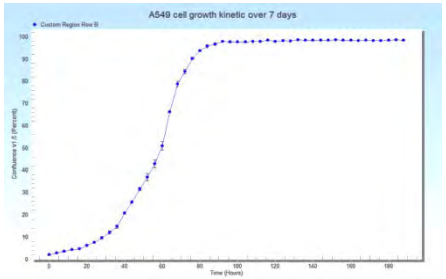
Additional functional validation

- Cell morphology
- Cell growth kinetics
- Cell STR profile
- Cell response to therapeutics

Parental cell line A549

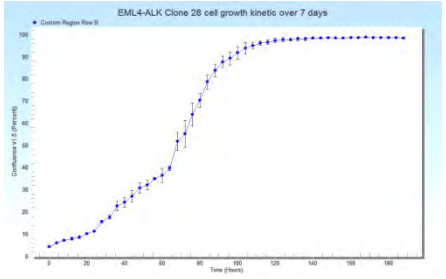


Parental cell line A549



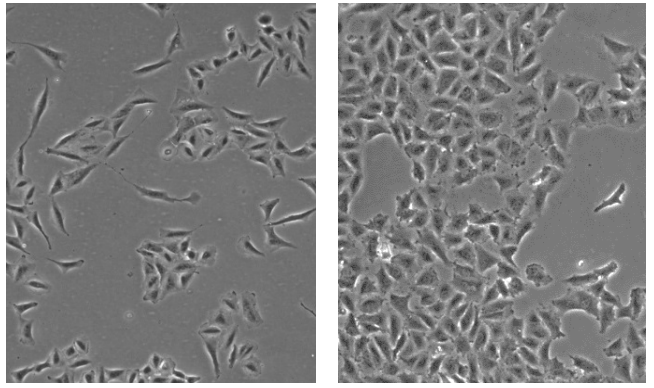
Doubling time =19.30 h

EML4-ALK isogenic cell line



Doubling time =21.07 h

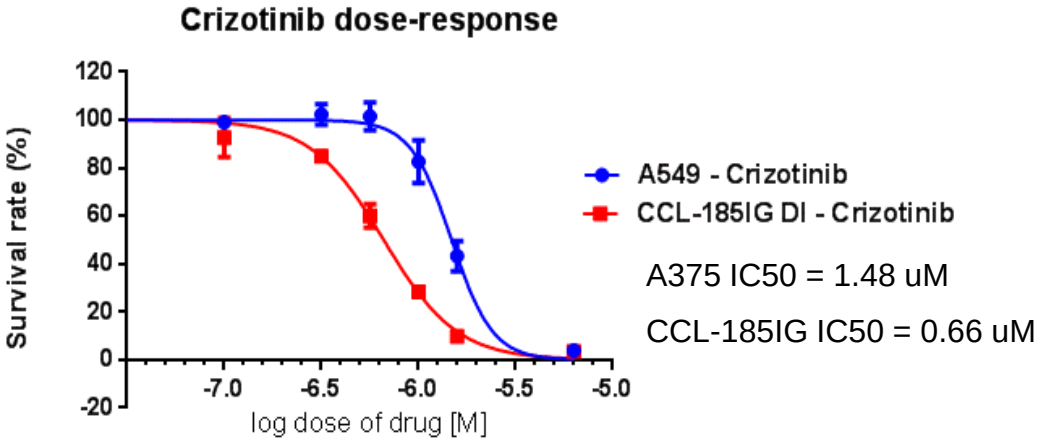
Isogenic cell line CCL-185IG



EML4-ALK isogenic cell line drug response

EML4-ALK isogenic line (ATCC® CCL-185IG™) is more sensitive to ALK inhibitor than its parental line

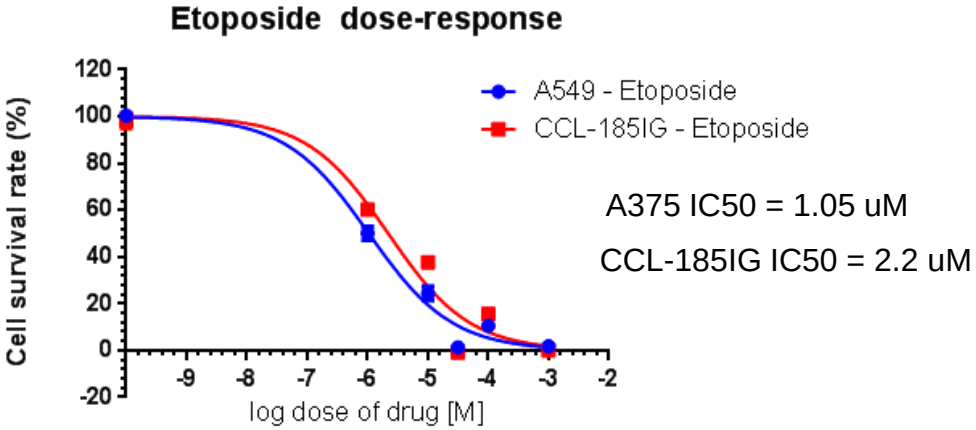
ALK specific inhibitor



A375 IC50 = 1.48 uM

CCL-185IG IC50 = 0.66 uM

Non-specific chemotherapy drug

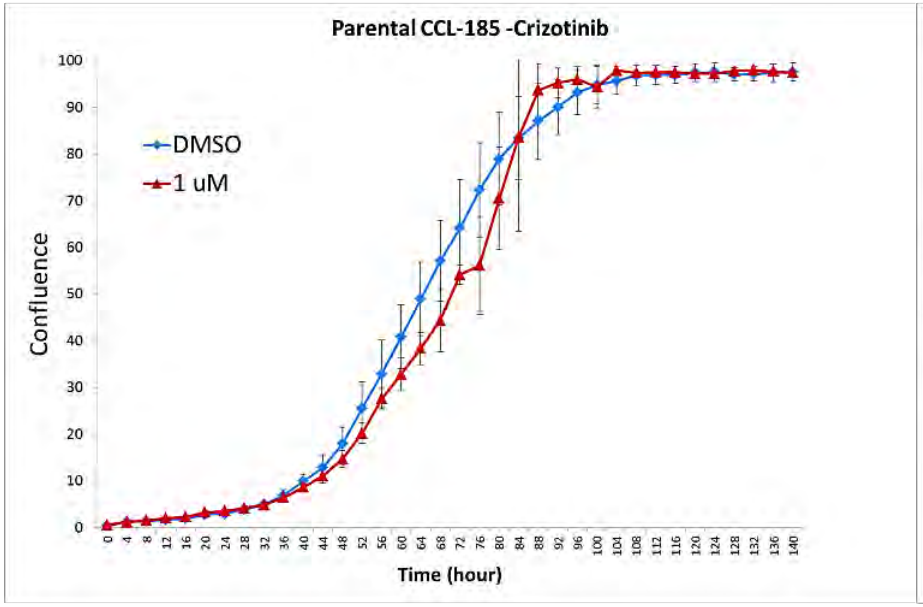


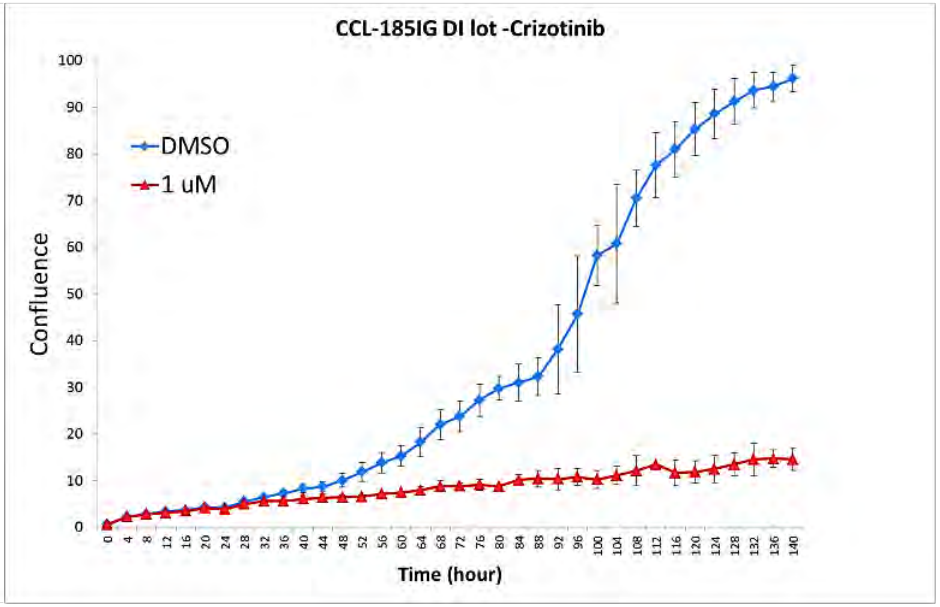
A375 IC50 = 1.05 uM

CCL-185IG IC50 = 2.2 uM

CCL-185IG EML4-ALK is sensitive to ALK inhibitors

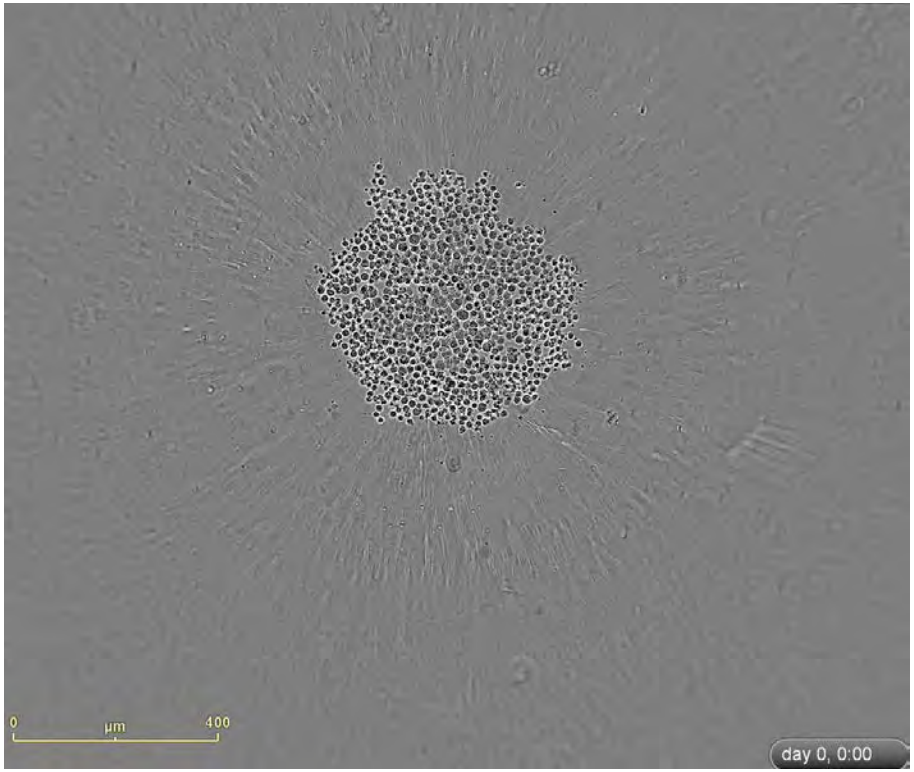
EML4-ALK isogenic line (ATCC® CCL-185IG™) is more sensitive to ALK inhibitor than its parental line



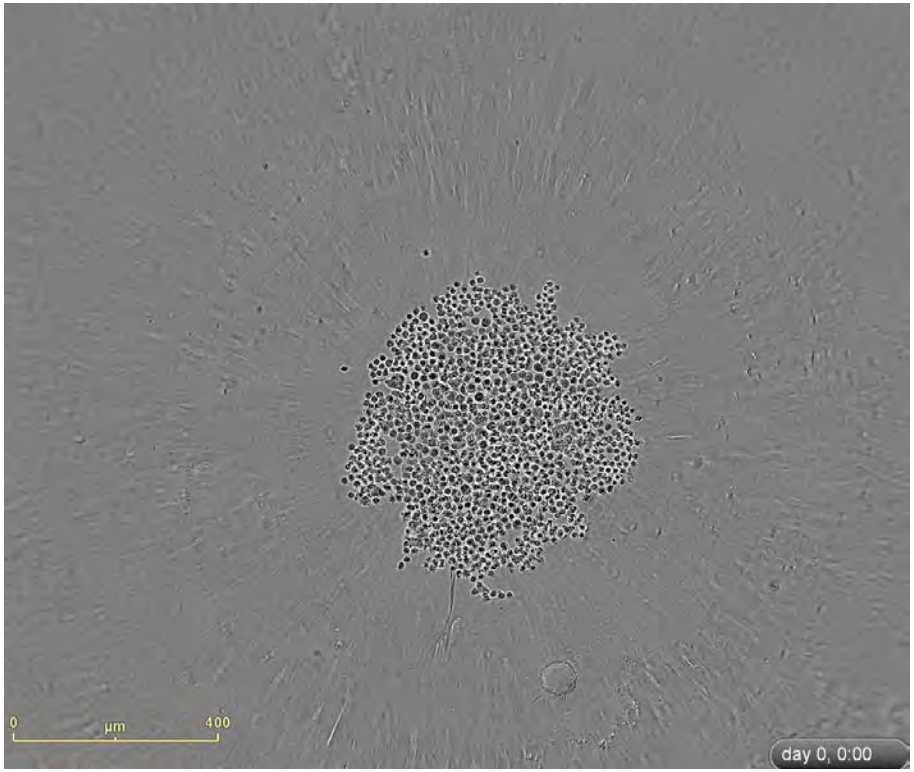


Isogenic pair in 3D culture

A549 parental cell line (ATCC® CCL-185 ™)



EML4-ALK isogenic line (ATCC® CCL-185IG™)



Video clips of the cell growth in 3D culture environment (1-9 days). The EML4-ALK isogenic line (right) grows more aggressively in 3D culture than the parental line (left).

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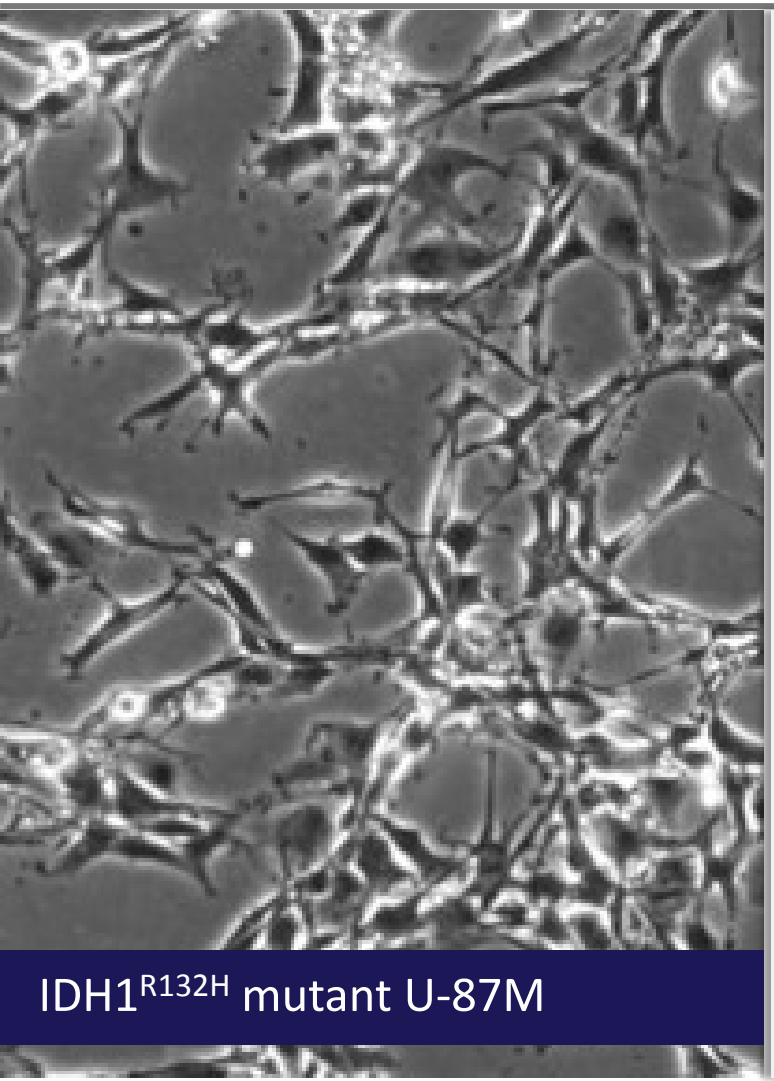
Case study #2: Creation of driver gene mutations using CRISPR/Cas9



IDH1^{R132H} mutant U-87M

IDH1^{R132H} mutant glioma isogenic cell model in U-87MG

- ATCC® HTB-14IG™

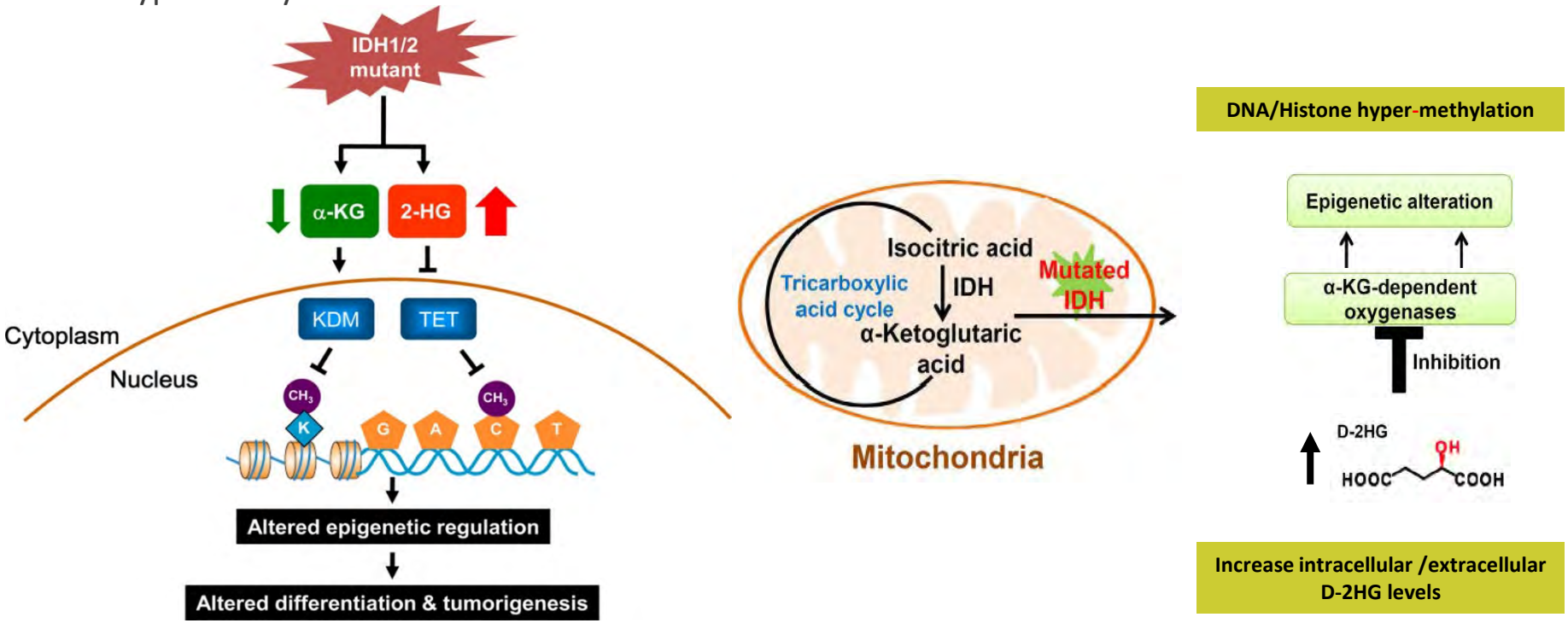
IDH2^{R140Q} mutant leukemia isogenic cell model in AML TF-1

- ATCC® CRL-2003IG™

IDH1/2 mutations in cancer

The Role of IDH1^{R132H} & IDH2^{R140Q} Gene Mutations in Cancer

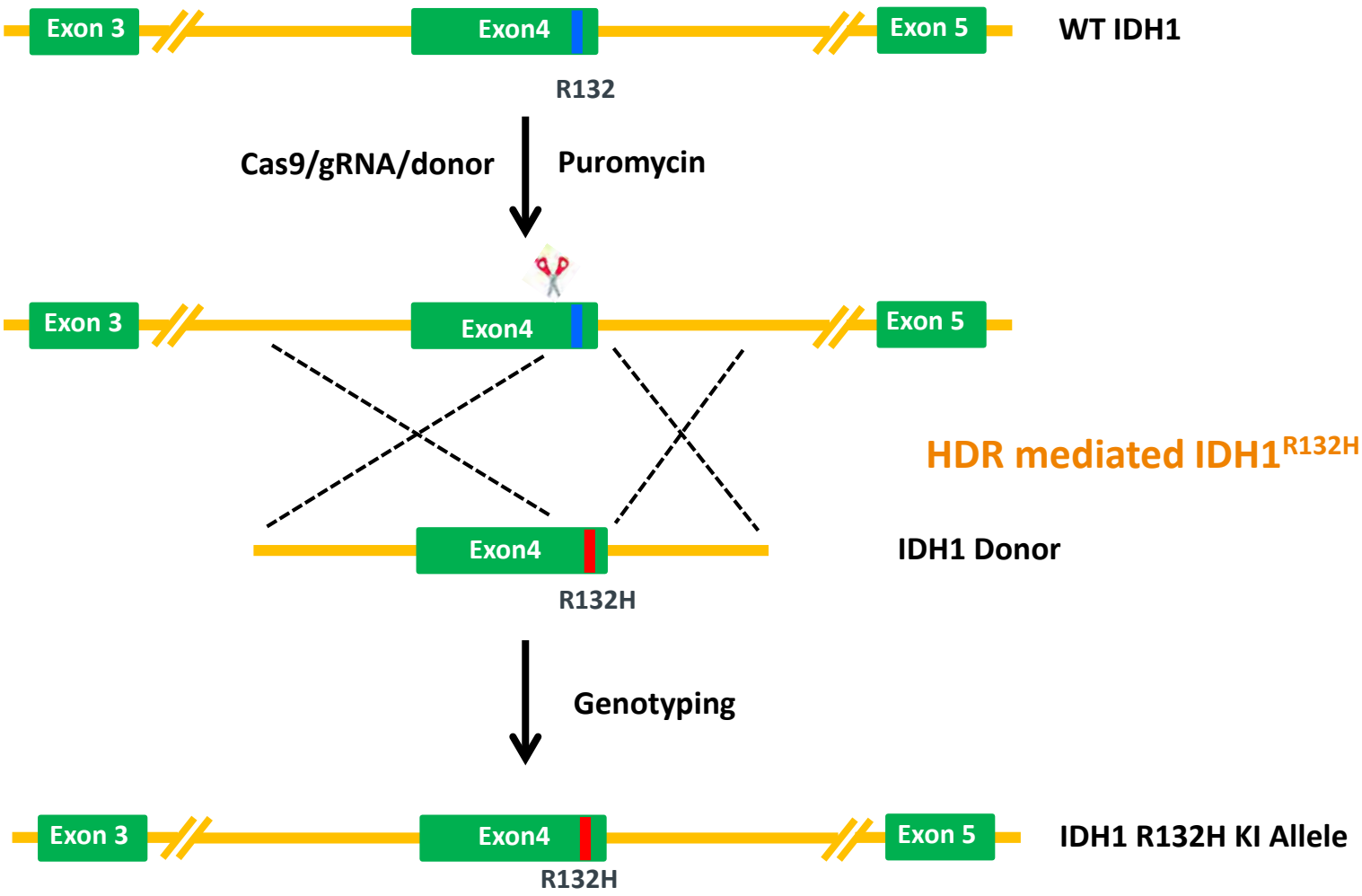
- IDH1 and IDH2 genes are mutated in glioma, acute myeloid leukemia, and other types of human cancer.
- IDH1/2 mutations cause a gain-of-function in cancer cells resulting in accumulation and secretion of the oncometabolite (2HG). This causes dysregulation of demethylases leading to histone and DNA hypermethylation.



Yang H, *et al*. Clin Cancer Res 18(20): 5562–5571, 2012.

Wang F, *et al.* Science 340 (6132), 622-666, 2013.

Generation of the IDH1^{R132H} isogenic glioma U-87MG line



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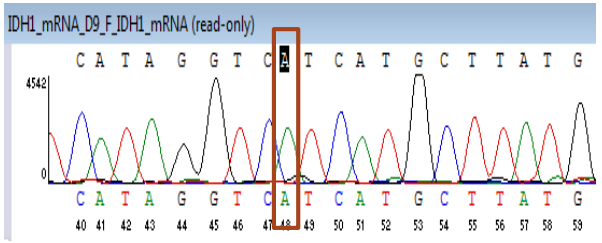
Generation of the IDH1^{R132H} isogenic glioma U-87MG line

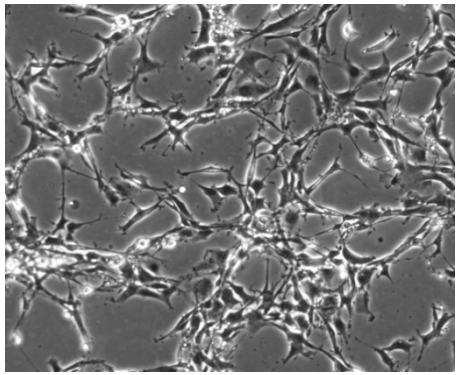
IDH1^{R132Q} mutant TF-1 Isogenic Cell Line (ATCC® HTB-14IG™)

- Sequence gDNA to confirm IDH1^{R132H} knock-in
- mRNA level validation
- Off-target screening
- Cell morphology
- Cell growth kinetics
- Cell STR profile
- Bio-functional assays

Isogenic line IDH1^{R132H} transcript



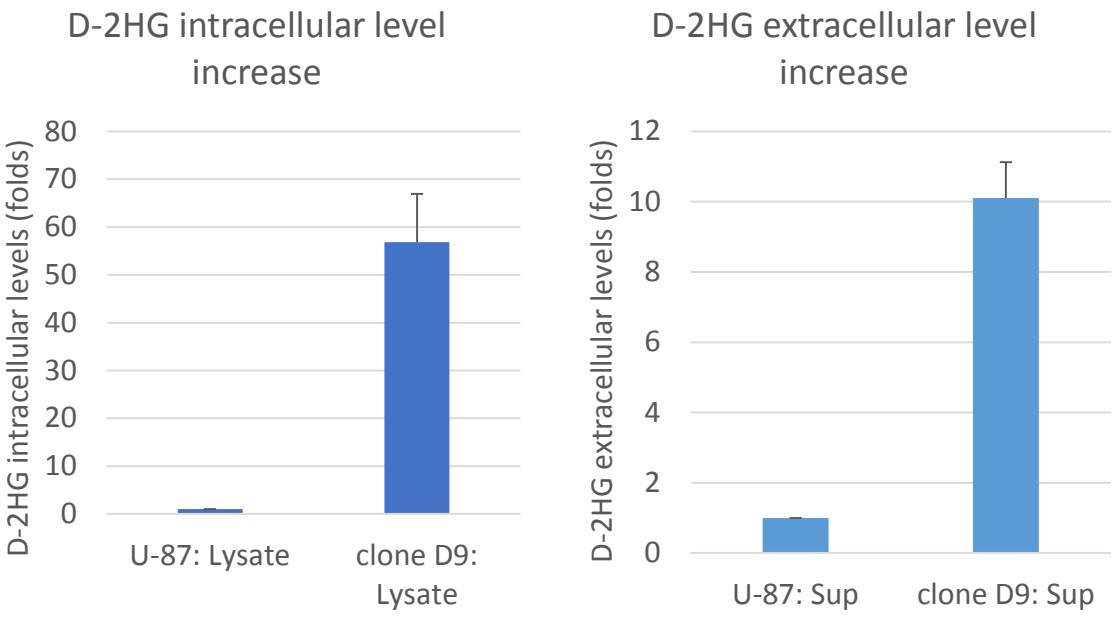
HTB-14IG



Doubling time = 25.6 h

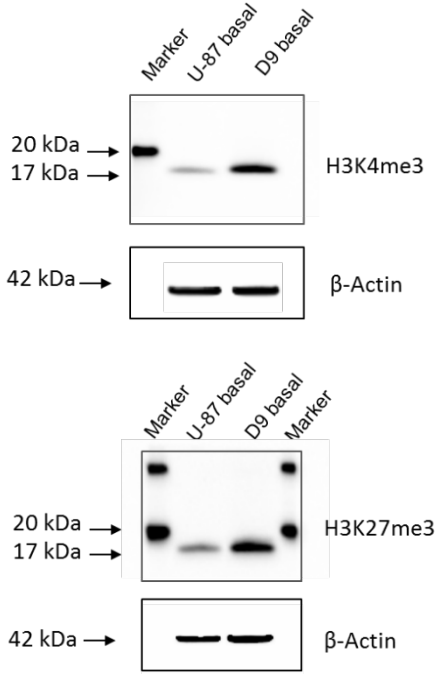
Bio-functional validation of the IDH1^{R132H} isogenic line

Significantly increased intracellular and extra-cellular 2-HG levels in HTB-14IG



Pico-Probe assay was used to detect D-2HG level.

Histone hyper-methylation in HTB-14IG

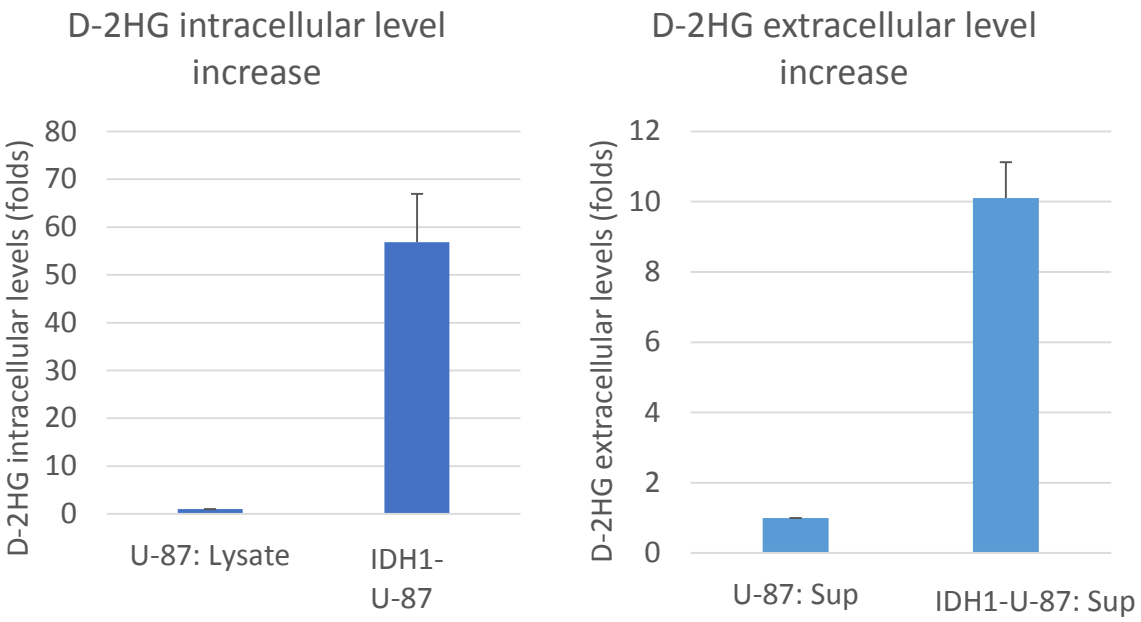


IDH1 mutations cause a gain-of-function in cancer cells, resulting in accumulation and secretion of the oncometabolite D-2-Hydroxyglutarate (D-2HG). This causes inhibition of proteins involved in epigenetic regulation leading to DNA and histone hypermethylation.

ATCC

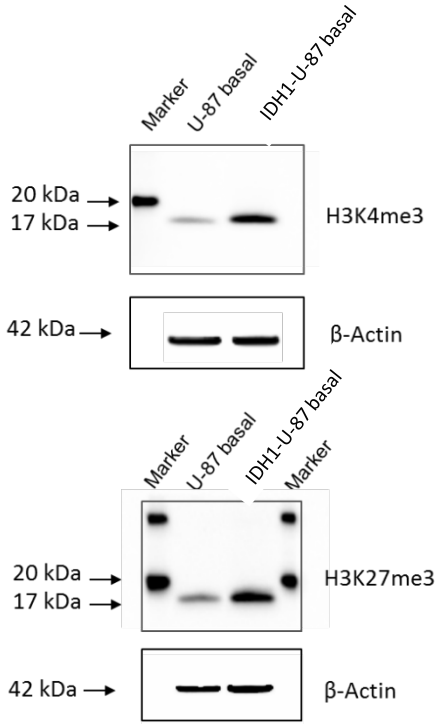
Bio-functional validation of the IDH1^{R132H} isogenic line

Significantly increased intracellular and extra-cellular 2-HG levels in HTB-14IG



Pico-Probe assay was used to detect D-2HG level.

Histone hyper-methylation in HTB-14IG



IDH1 mutations cause a gain-of-function in cancer cells, resulting in accumulation and secretion of the oncometabolite D-2-Hydroxyglutarate (D-2HG). This causes inhibition of proteins involved in epigenetic regulation leading to DNA and histone hypermethylation.

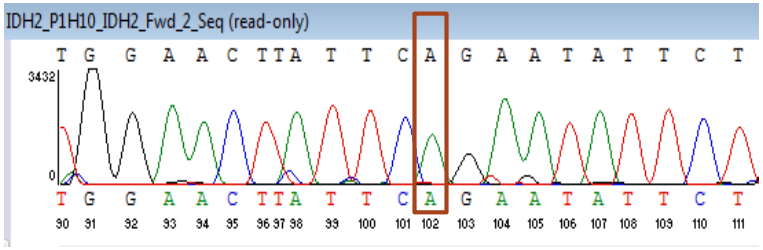
ATCC

Generation and validation of the IDH2^{R140Q} isogenic TF-1 line

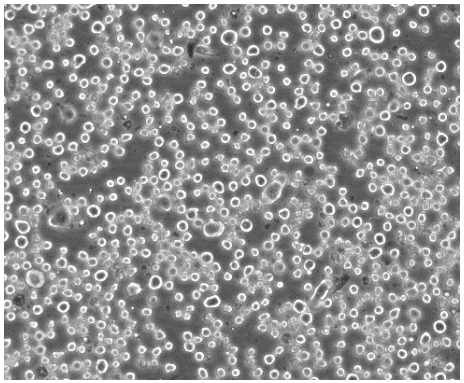
IDH2^{R140Q} mutant TF-1 Isogenic Cell Line (ATCC® CRL-2003IG™)

- Sequence gDNA to confirm IDH2^{R140Q} knock-in
- mRNA level validation
- Off-target screening
- Cell morphology
- Cell growth kinetics
- Cell STR profile
- Cell response to therapeutics

Isogenic line IDH2^{R140Q} transcript



CRL-2003IG



Doubling time = 21.4 h

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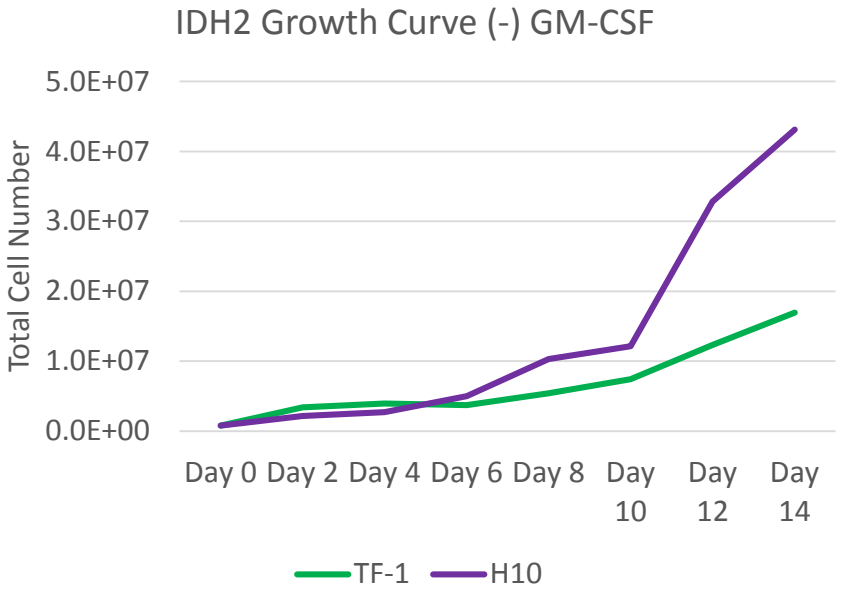
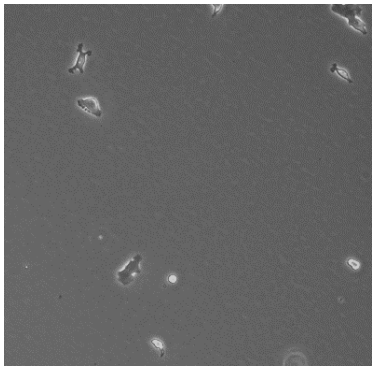
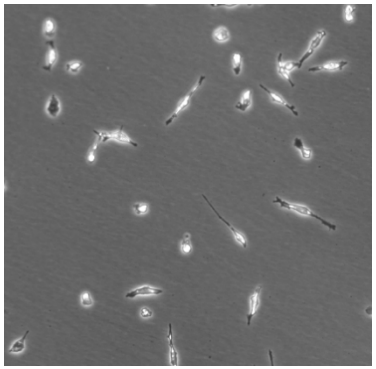
Impact of the IDH2^{R140Q} mutation on cell growth and differentiation



TF-1 Parental (ATCC® CRL-2003™)



IDH2^{R140Q} (ATCC® CRL-2003IG™)



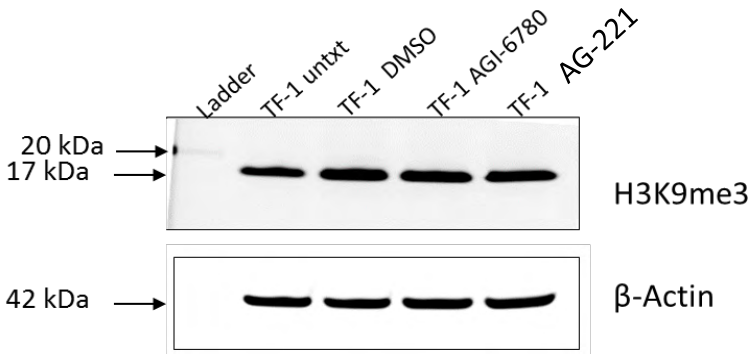
TF1 cells are GM-CSF-dependent, while IDH2^{R140Q} mutants exhibit GM-CSF-independent proliferation

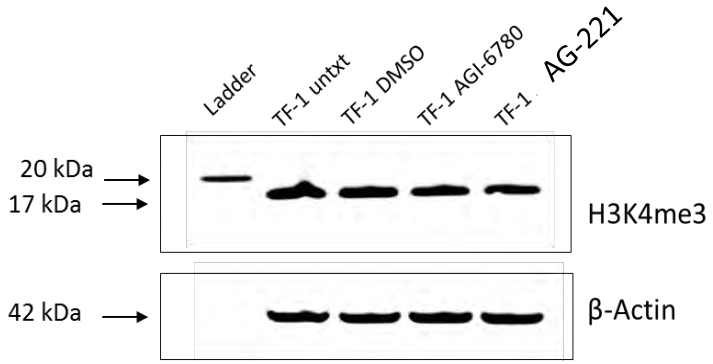
IDH2^{R140Q} mutant cells attach to plate, exhibit spindle-like (undifferentiated mesenchymal) morphology

ATCC

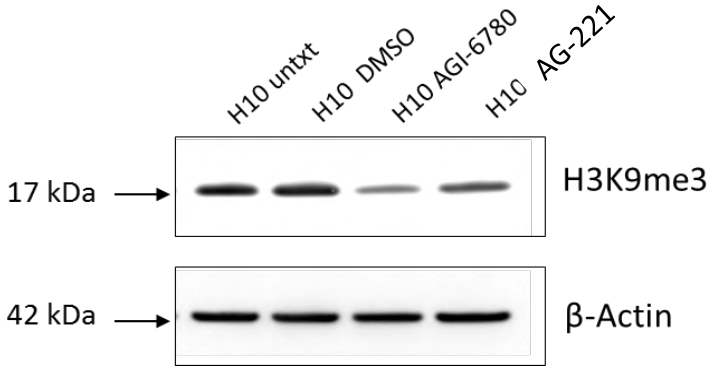
IDH2 inhibitors decrease histone methylation in the IDH2^{R140Q} isogenic line

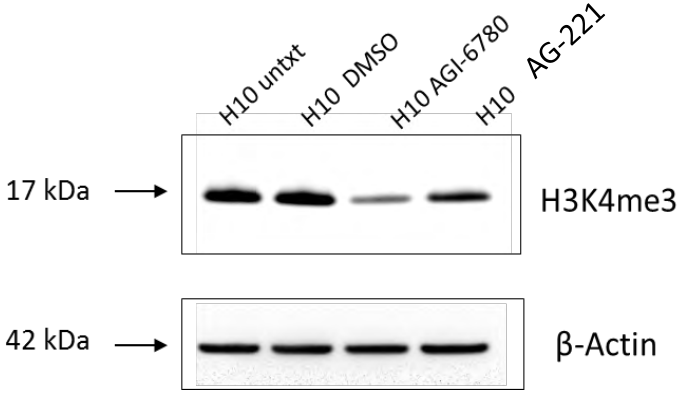
TF-1 Parental line (ATCC® CRL-2003™)



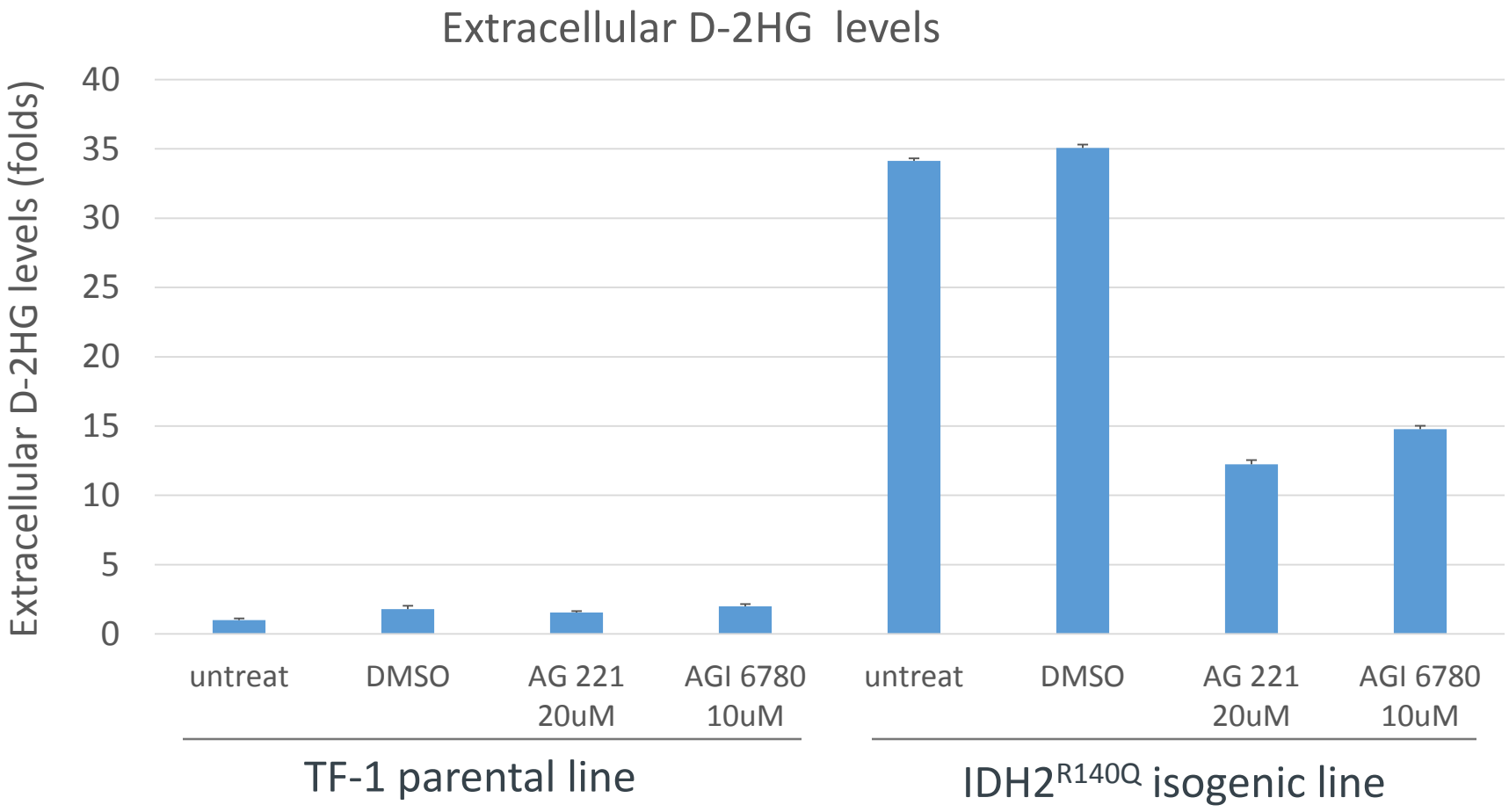


IDH2^{R140Q} Isogenic line (ATCC® CRL-2003IG™)





IDH2 inhibitors decrease 2-HG levels in the IDH2^{R140Q} isogenic line



Parental and IDH2 isogenic cell lines were cultured with or without IDH2-specific inhibitors (AG221 and AGI6780) in triplicate for 3 days. Pico-ProbeTM D-2HG assay kit (BioVision) was used to detect D-2HG levels days post drug treatment showing several fold reduction in extracellular D-2HG levels.

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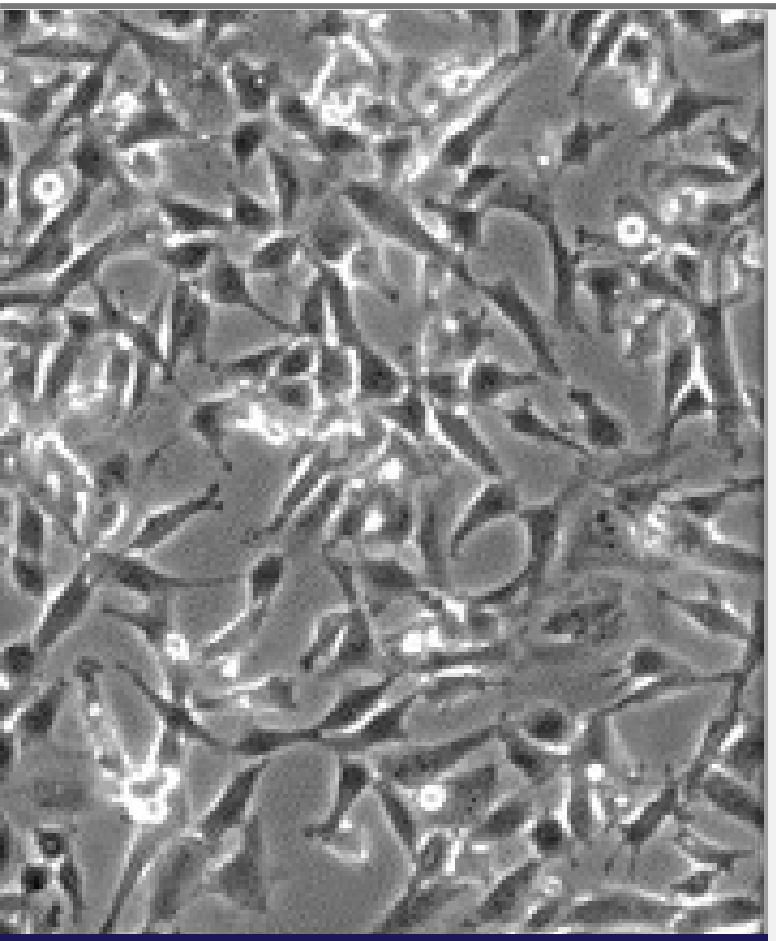
Case study #3: Creation of drug-resistant mutations using CRISPR/Cas9



KRAS^{G13D} mutant A375

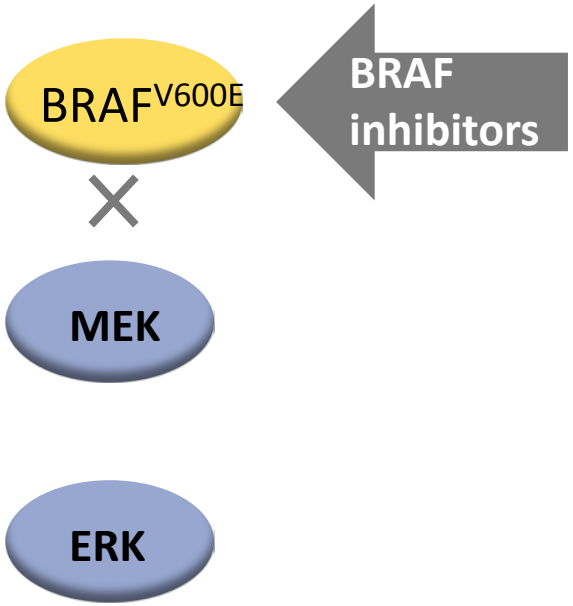
BRAF inhibitor-resistant melanoma models (ATCC® CRL-1619IG-1™, CRL-1619IG-2™)

- NRAS^{Q61K} mutation in A375 BRAF^{V600E} melanoma line
  - Available now
- KRAS^{G13D} mutation in A375 BRAF^{V600E} melanoma line
  - Coming soon

ATCC

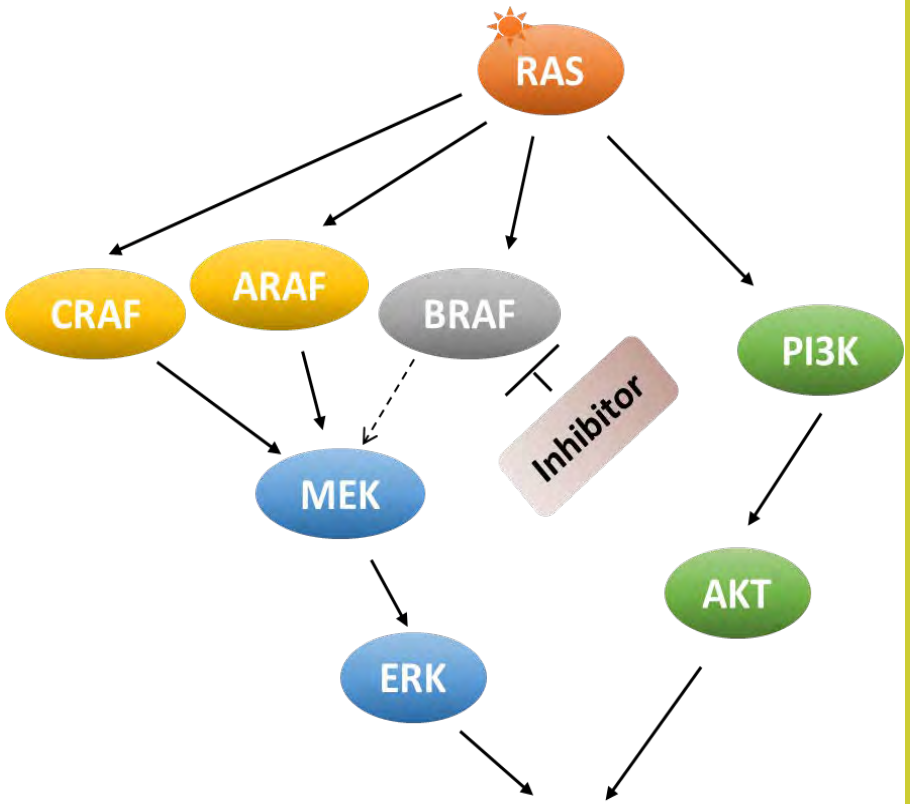
Therapeutic targeting of $BRAF^{V600E}$ in melanoma

Role of mutant $BRAF^{V600E}$ in driving cell survival: Oncogenic BRAF signaling



Decrease cell growth and survival

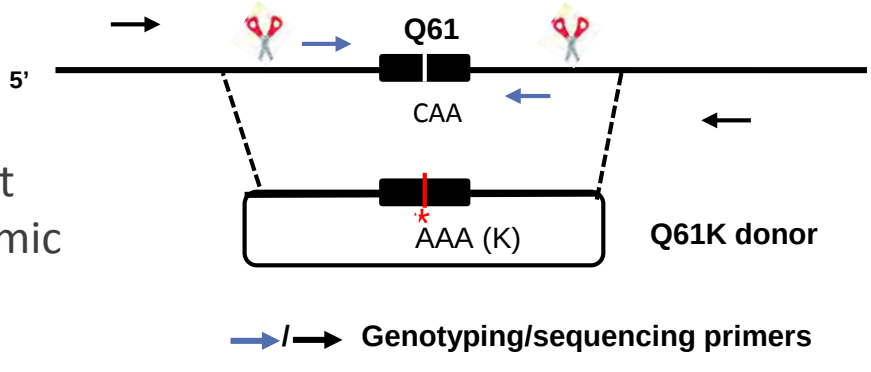
RAS mediated resistance to BRAF inhibitor in melanoma

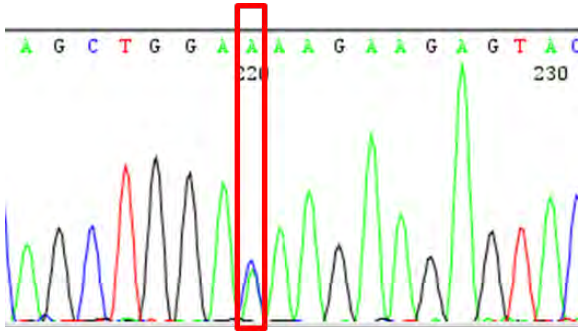


Cell proliferation, cell survival

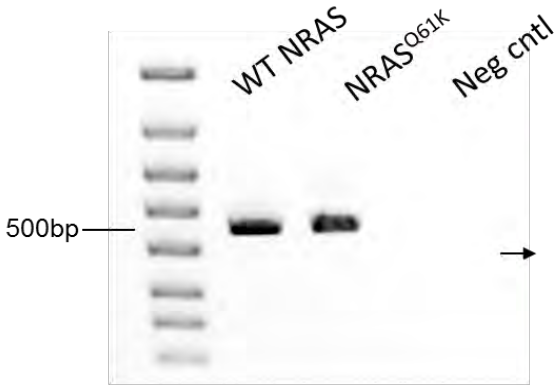
Generation of NRAS^{Q61K} isogenic melanoma A375 line

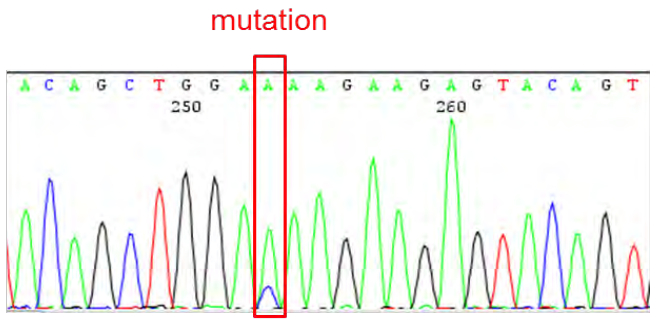
Validation of point mutation at genomic level





Validation of point mutation at transcript level

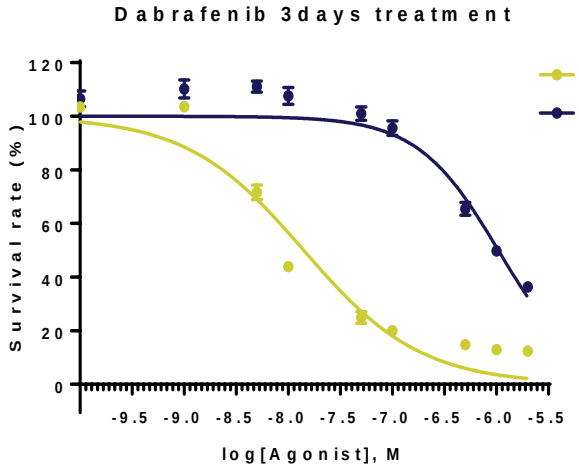




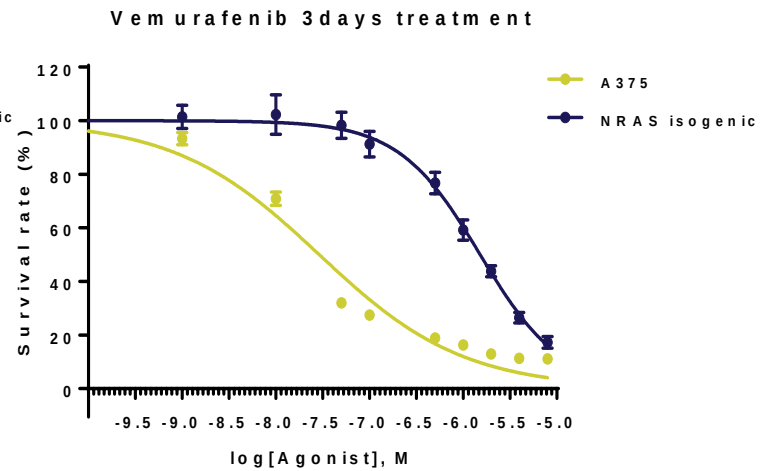
isolate mRNA → cDNA synthesis →PCR → sequence analysis

Drug resistance of NRAS^{Q61K} isogenic melanoma A375 line CRL-1619IG-2

BRAF specific inhibitor

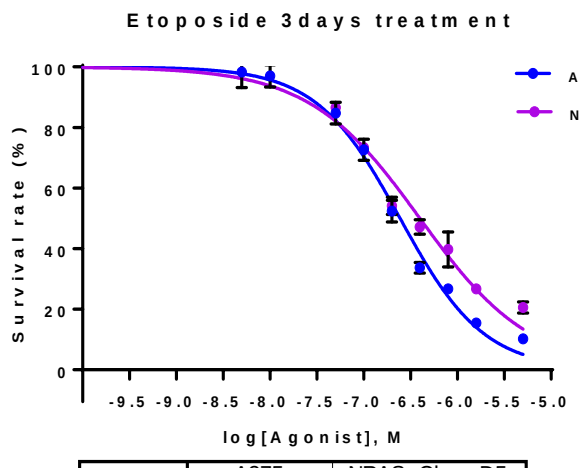


	A375	NRAS Clone D5
IC50	1.407e-008	1.042e-006

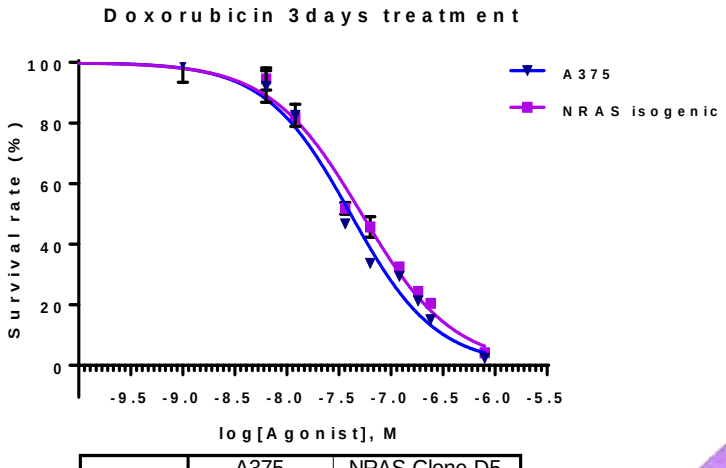


	A375	NRAS Clone D5
IC50	2.907e-008	1.519e-006

Non-specific chemotherapy drug

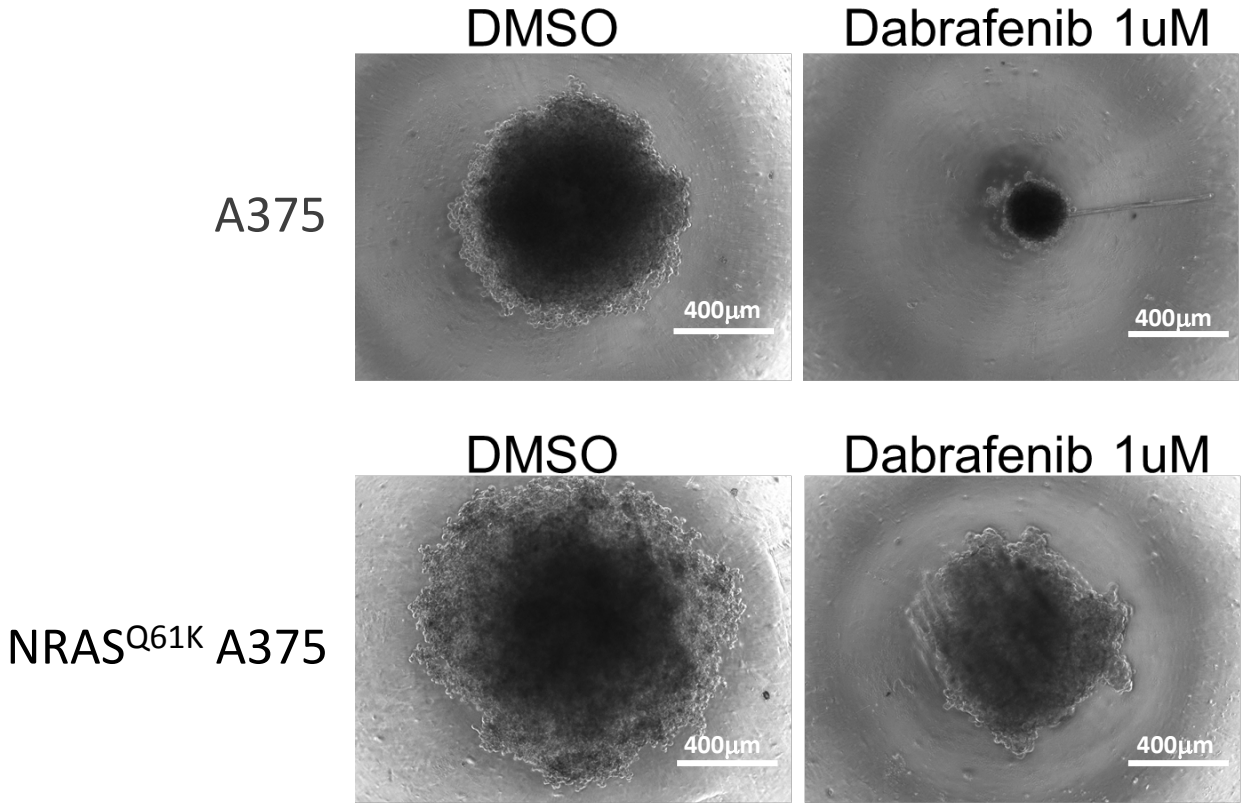


	A375	NRAS Clone D5
IC50	2.447e-007	3.98e-007



	A375	NRAS Clone D5
IC50	4.093e-008	5.238e-008

3D culture evaluation of isogenic pair



3D culture spheroids were generated by seeding cells on an ultra-low attachment plate and culturing for 4 days. Spheroids were then incubated for 10 days in the presence or absence of 1 µM BRAF inhibitor debrafenib and then imaged. The NRAS^{Q61K} isogenic line displays resistance to BRAF inhibitor, mimicking clinical emergence of BRAF-inhibitor resistant cancers.

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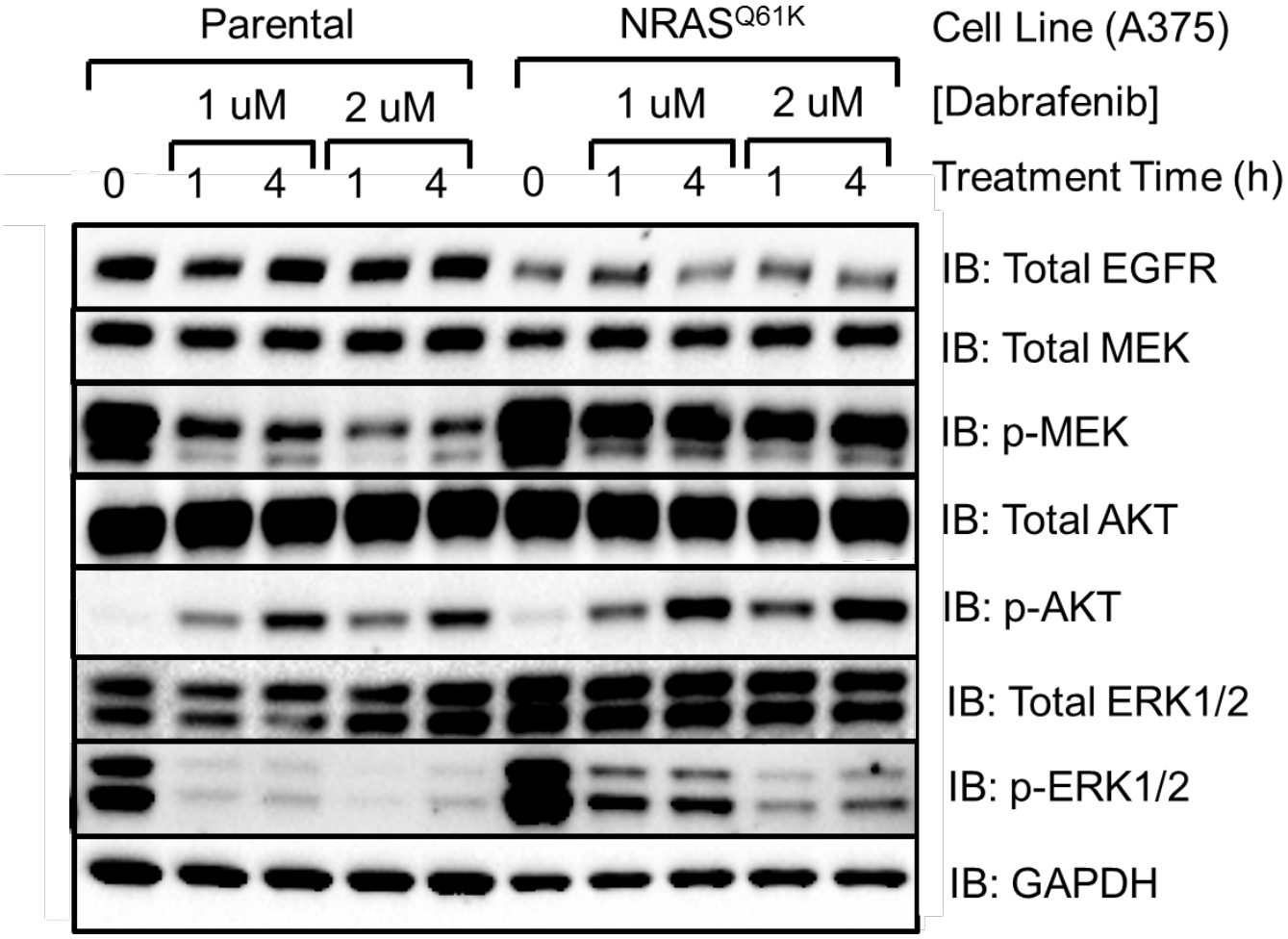
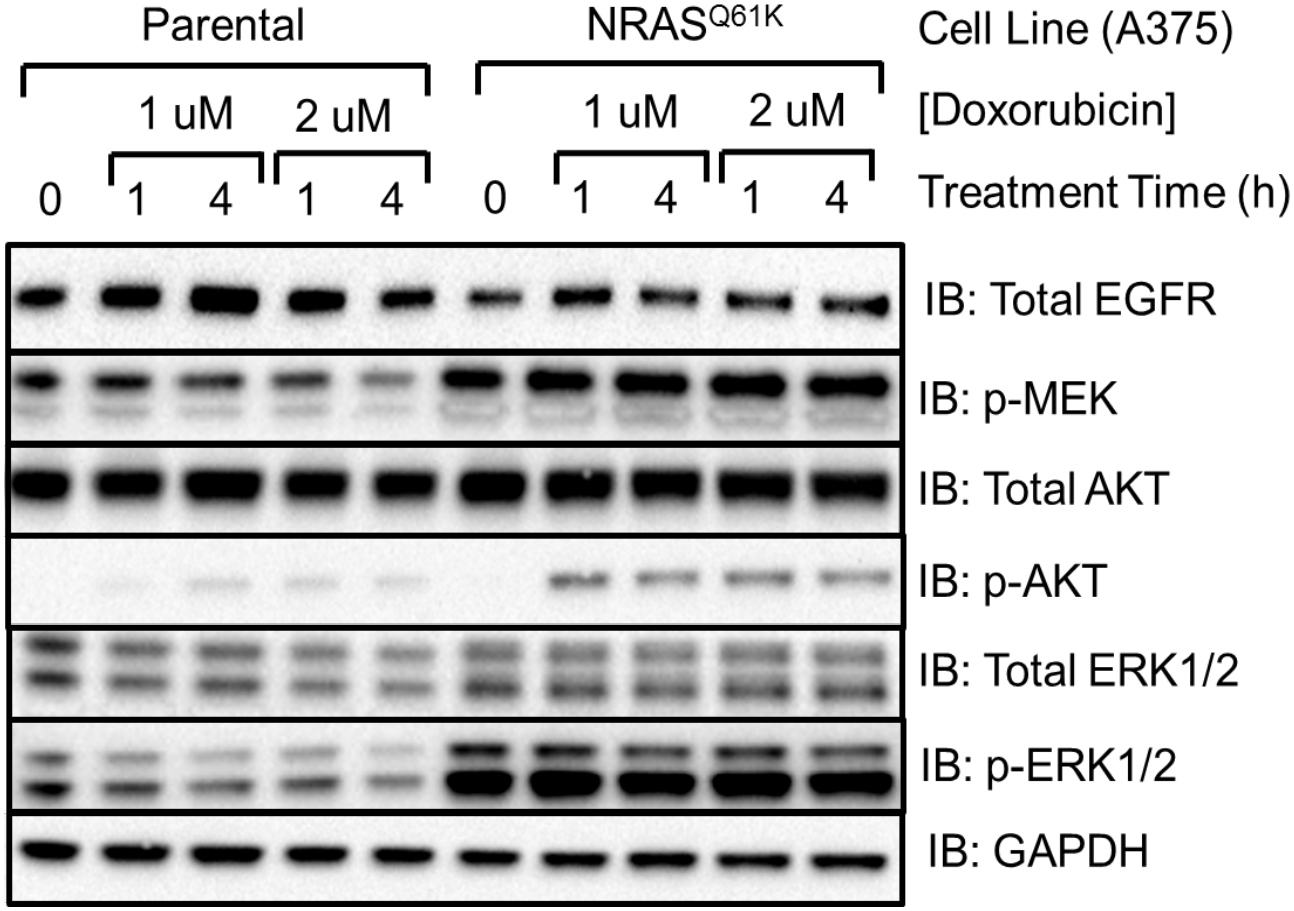
ERK and AKT activation within NRAS^{Q61K} Mutant A375 Isogenic Cell Line

BRAF specific inhibitor treatment



ERK and AKT activation within NRAS^{Q61K} Mutant A375 Isogenic Cell Line

Non-specific chemotherapy drug treatment

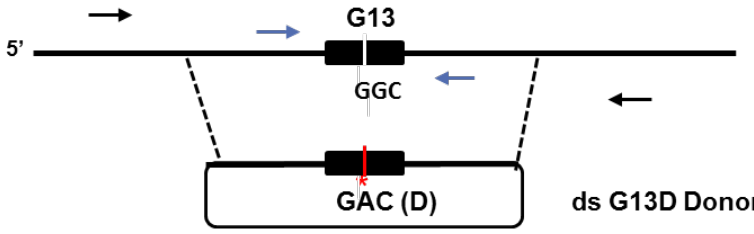


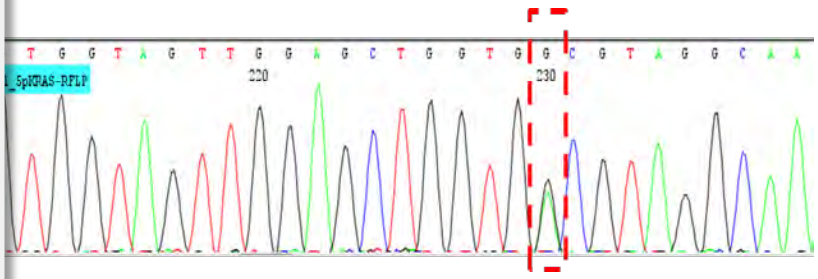
ATCC

Generation and validation of the KRAS^{G13D} mutant A375 isogenic cell line

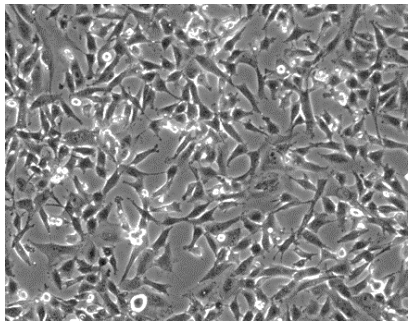
KRASG13D isogenic A375 melanoma line (ATCC® CRL-1619IG-1™)

- Sequence gDNA to confirm KRASG13D knock-in
- mRNA level validation
- Off-target screening
- Cell morphology
- Cell growth kinetics
- Cell STR profile
- Cell response to therapeutics



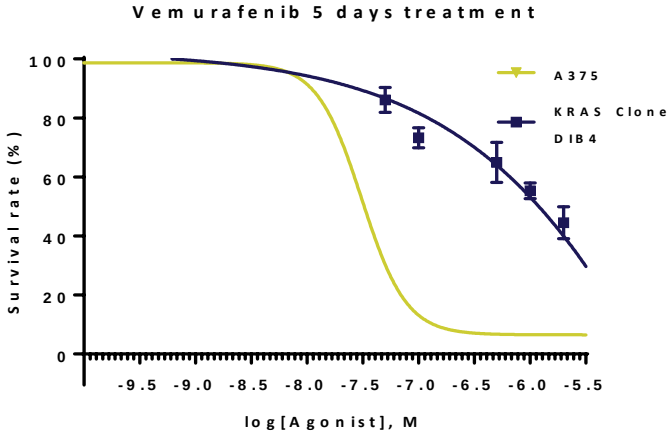


(ATCC® CRL-1619IG-1™)

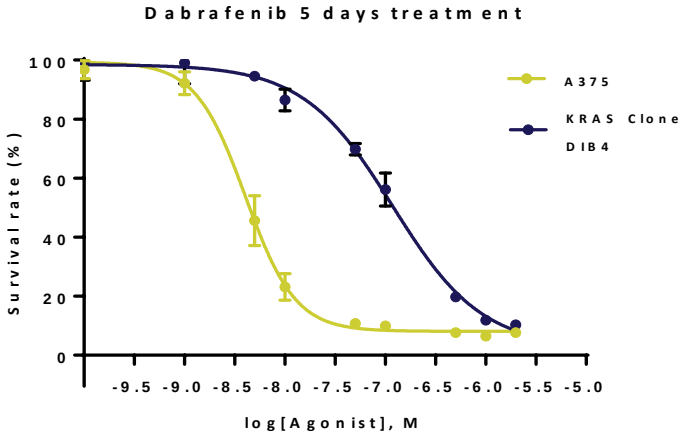


Doubling time = 20.58 h

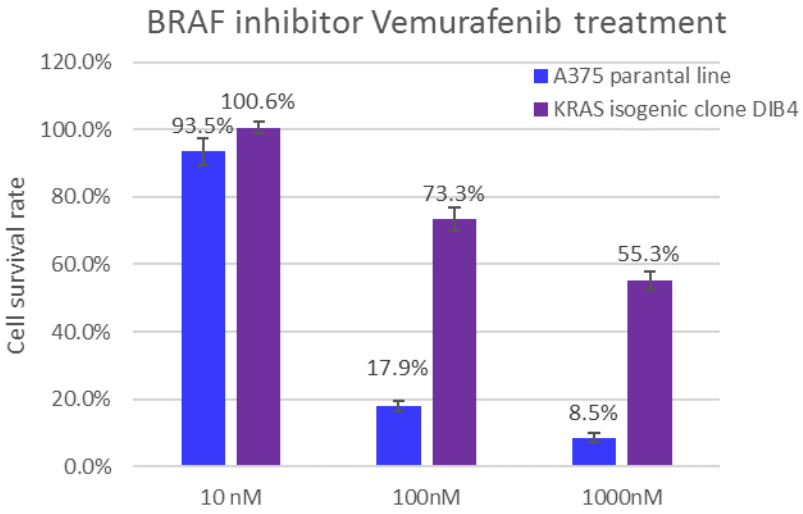
$KRAS^{G13D}$ mutant A375 Cell Line exhibits significant resistance to BRAF inhibitors

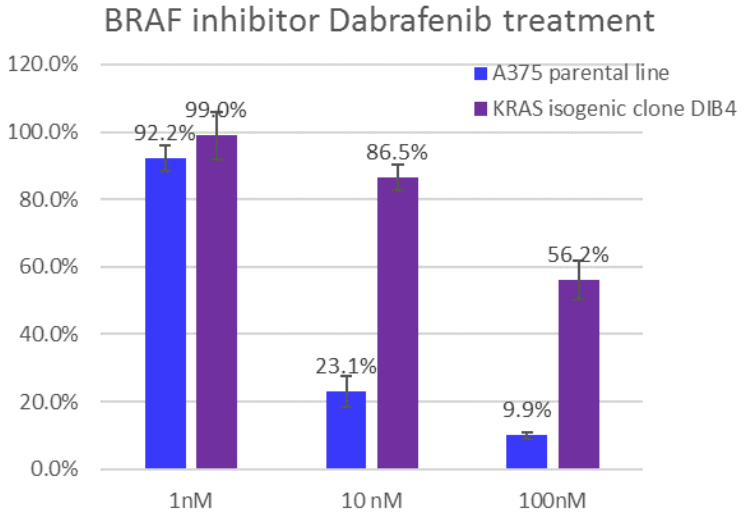


	A375	KRAS Clone DIB4
IC50	3.094e-008	6.55e-005

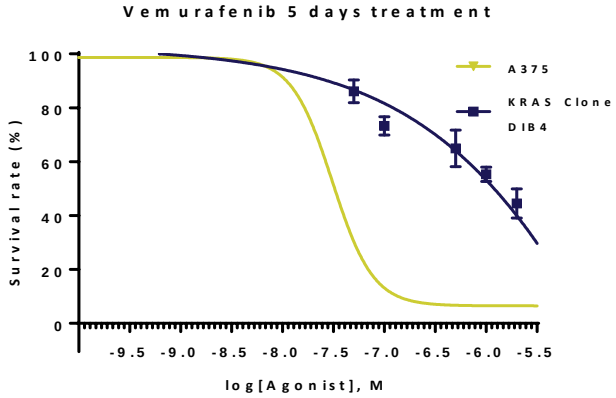


	A375	KRAS Clone DIB4
IC50	4.056e-009	1.184e-007

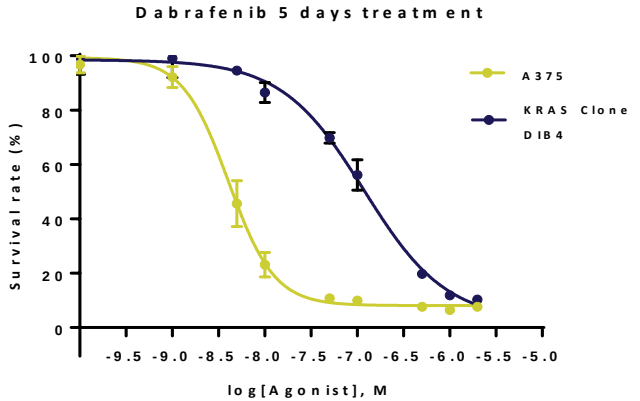




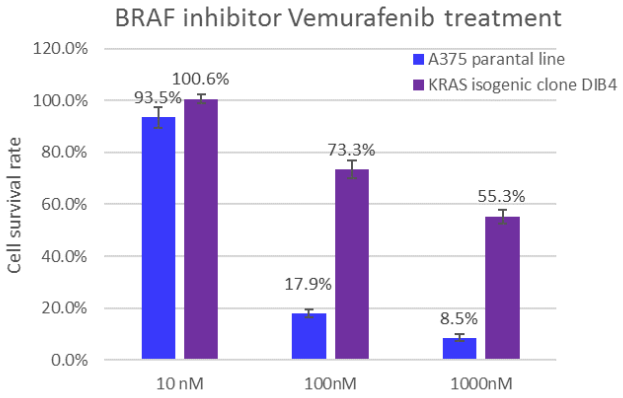
KRAS^{G13D} mutant A375 Cell Line exhibits significant resistance to BRAF inhibitors

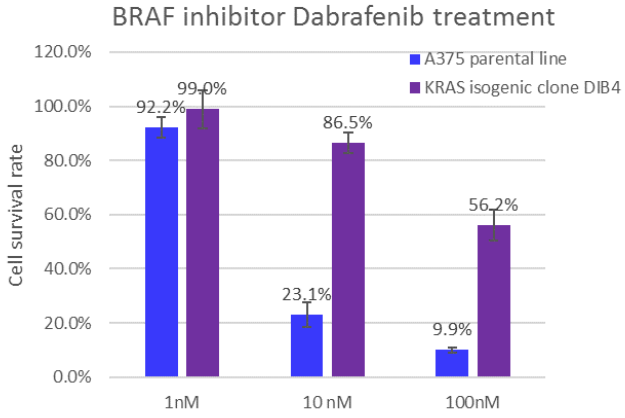


	A375	KRAS Clone DIB4
IC50	3.094e-008	6.55e-005



	A375	KRAS Clone DIB4
IC50	4.056e-009	1.184e-007





The KRAS isogenic cell line is more resistant to BRAF inhibitors than the parental A375 cell line. A375 cells (ATCC® CRL-1619™ and KRAS Mutant-A375 Isogenic Cells (ATCC® CRL-1619IG-1™) were treated with the indicated concentrations of either dabrafenib of vemurafenib for three days. Cell survival was monitored via cell viability assay.

Use of CRISPR system to create cancer disease models

ATCC precision cell models for the development of new anti-cancer therapeutics

Gene translocation

- EML4-ALK fusion A549 (ATCC® CCL-185IG™) **Now Available!**

Driver gene mutations

- $IDH1^{R132H}$ mutant U-87 (ATCC® HTB-14IG™)
- $IDH2^{R140Q}$ mutant TF-1 (ATCC® CRL-2003IG™)

Drug-resistant mutants

- $NRAS^{Q61K}$ mutant A375 (ATCC® CRL-1619IG-1™)
- $KRAS^{G13D}$ mutant A375 (ATCC® CRL-1619IG-2™)

ATCC

Conclusion

Clinically relevant cancer cell models are critical both for studies of molecular and cellular mechanisms of tumorigenesis and for the design and screening of novel cancer therapeutics. With new genome editing tools such as CRISPR/Cas9, ATCC can now use its extensive cell-banking resources to generate novel isogenic disease model cell lines. We have engineered isogenic lines with mutations in key oncogenes that are ideally suited for the identification of novel, personalized treatment regimens.

Key features of ATCC isogenic cell lines:

- Parental lines are carefully selected to be highly relevant to diseases and drug targets
- Precisely edited isogenic cell lines have been thoroughly validated at genomic, transcript, and protein levels
- Additional bio-functional characterization with specific inhibitors to demonstrate drug-screening applicability
- Together with authenticated parental lines, CRISPR/Cas9-edited isogenic lines provide useful *in vitro* models for both basic and translational research

ATCC

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Diana Douglas

John Foulke

Michael Jackson

Stephen King

Catherine Nguyenngo

Weiguo Shu

Elizabeth Turner

Lysa-Anne Volpe

Monica Wood

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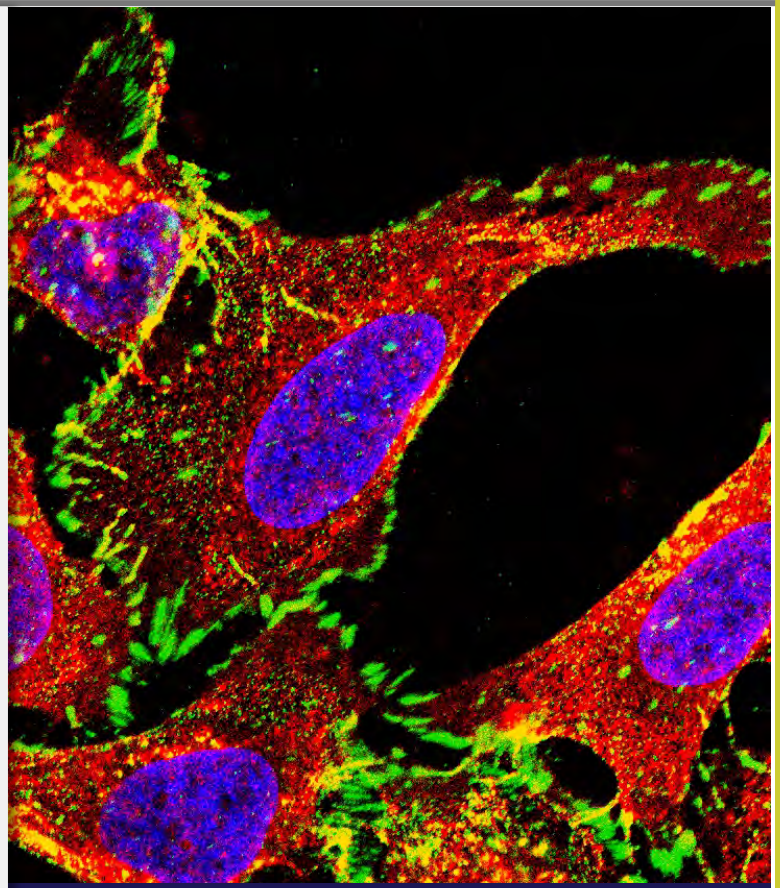
Disclaimers



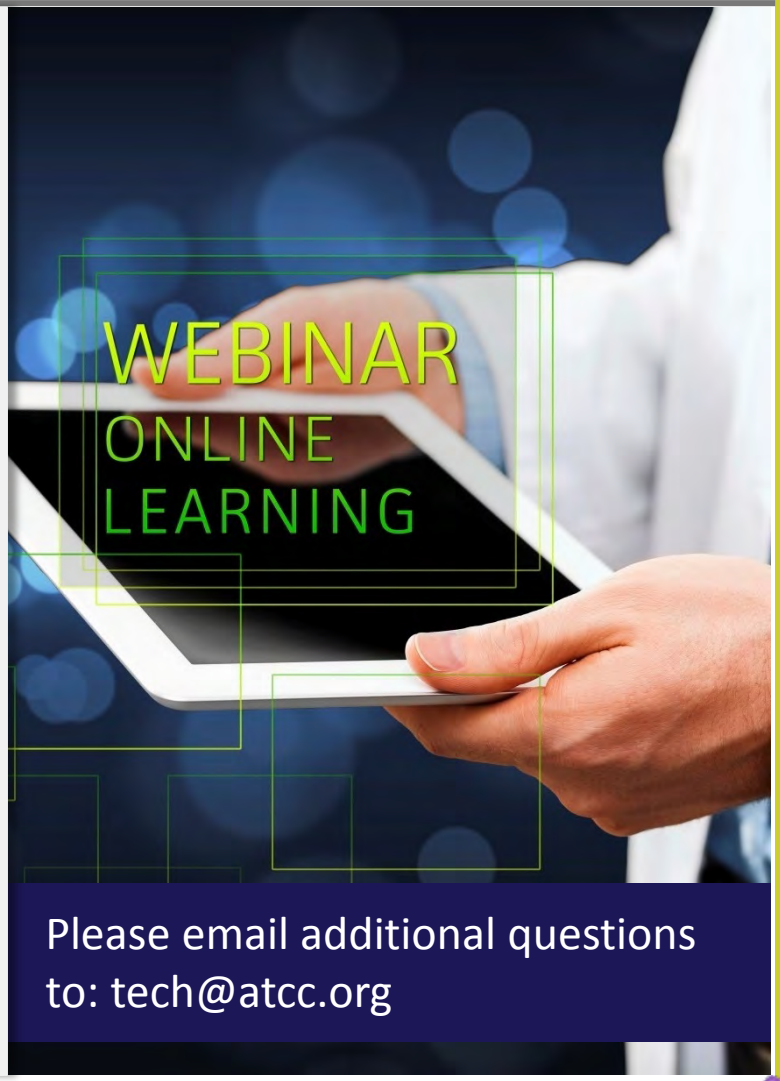
A549 cells, image courtesy of Christopher Chin

Thank you for joining today!

Register for more ATCC webinars at
www.atcc.org/webinars

Learn more about isogenic cell lines at
www.atcc.org/isogenics

For more cancer research resources visit
www.atcc.org/cancer



Please email additional questions to: tech@atcc.org

ATCC