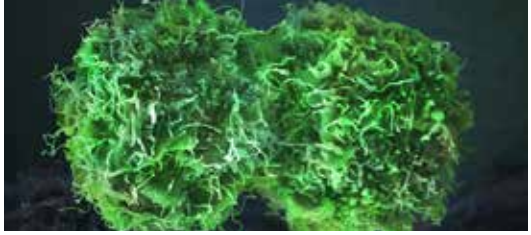
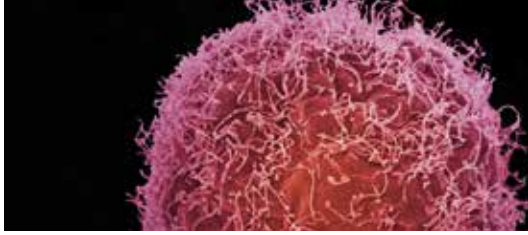
THE ESSENTIALS OF
LIFE SCIENCE RESEARCH
GLOBALLY DELIVERED™





ATCC® ISOGENIC CELL LINES

ATCC CRISPR/CAS9 GENE-EDITED ISOGENIC CELL LINES

Clinically relevant cell models are critical for studies of molecular and cellular mechanisms of tumors, as well as for drug screening for cancer. With genome editing tools such as CRISPR/Cas9, ATCC has created isogenic cell lines with mutants of key oncogenes, which are ideal for identifying novel, personalized treatment regimens.

- Precisely gene edited
- Engineered on commonly used tumor cell lines
- Highly relevant to diseases and drug targets
- Validated at genomic, transcript, and protein levels
- Biofunctional characterization with specific inhibitors
- Well suited for drug screening applications

ATCC® No.	Designation	Mutation	Parental Cell Line	Disease	Cancer Model
CCL-185IG™	EML4-ALK Fusion-A549	EML4-ALK fusion	A549	Non-small cell lung carcinoma	Gene translocation ALK inhibitor-sensitive mutant
CRL-1619IG-1™	KRAS Mutant-A375	KRAS G13D	A375	Melanoma	BRAF inhibitor-resistant mutant
CRL-1619IG-2™	NRAS Mutant-A375	NRAS Q61K	A375	Melanoma	BRAF inhibitor-resistant mutant
HTB-14IG™	IDH1 Mutant-U-87 MG	IDH1 R132H	U-87	Glioma	Driver gene mutation
CRL-2003IG™	IDH2 Mutant-TF-1	IDH2 R140Q	TF-1	Leukemia	Driver gene mutation

Start your drug screening studies with ATCC isogenic cell lines **www.atcc.org/isogenic**

DEVELOPMENT OF CRISPR GENE-EDITING

ATCC has mastered the science and art of CRISPR gene-editing. We take highly authenticated cell lines from our collection and introduce the disease-relevant mutations using single guide RNAs (sgRNAs) that are designed to guide Cas9 to the targeted regions. The parental cell lines are cotransfected with sgRNA and Cas9. The transfected cells are sorted into single cells and expanded for testing. The gene-edited isogenic cell clones are then rigorously screened.

Genetic and transcript validation:

- Sanger sequencing
- Next-generation sequencing (NGS)
- PCR and RT-PCR

Protein expression and function validation:

- Western blot (when appropriate)
- Drug responses
- Other biofunctional assays

TYPICAL VALIDATION OF ATCC ISOGENIC CELL LINES

Below is an example of the genomic and transcriptional validation of ATCC isogenic cell lines, using the NRAS Mutant-A375 cell line. The chromatograms show via Sanger sequencing the point mutation of glutamine to lysine at position 61 (Figure 1). These results were confirmed by NGS (data not shown) and inhibitor studies. Detailed functional validation of all of the isogenic cell lines in ATCC's collection is shown on the following pages.

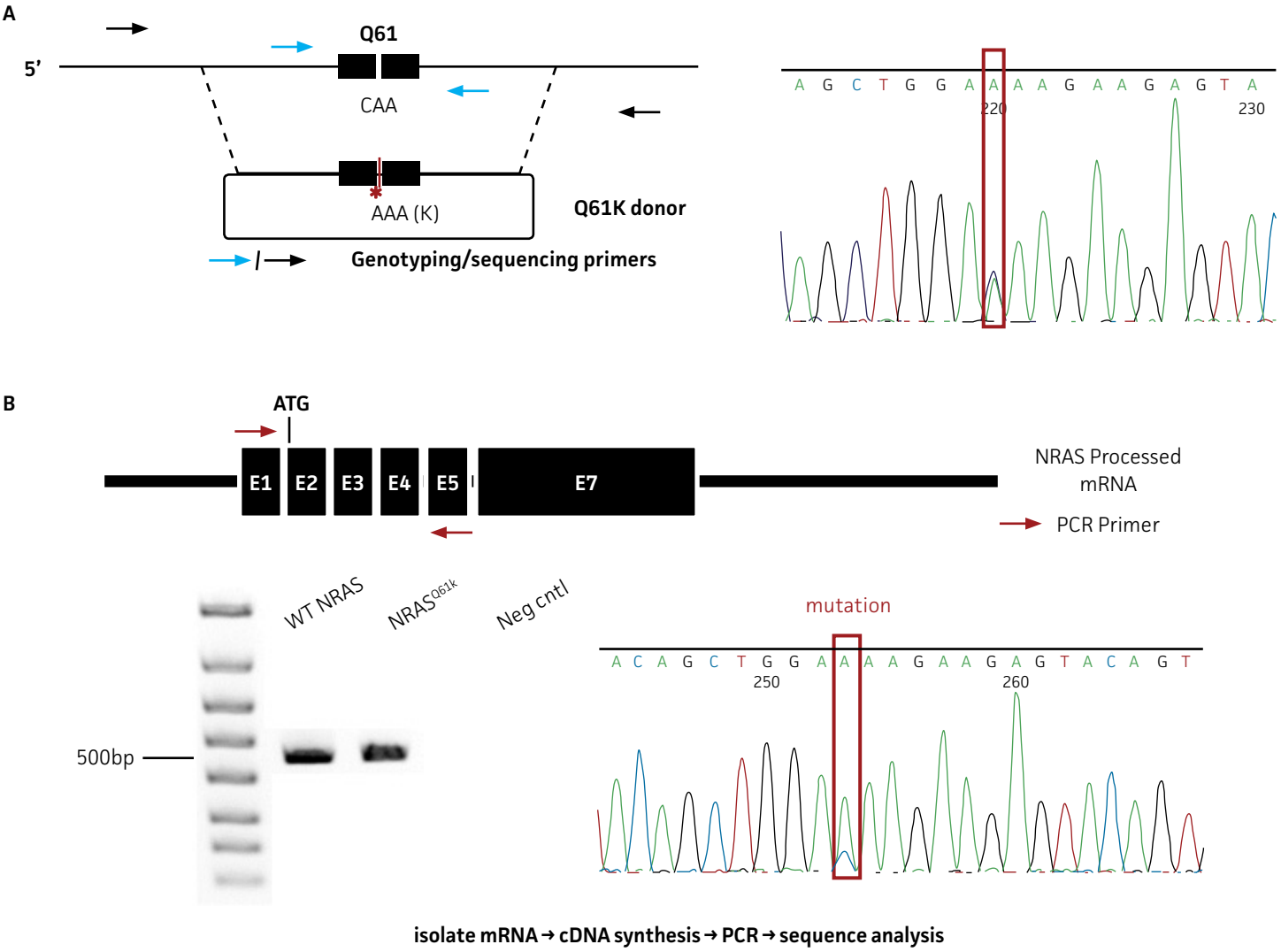
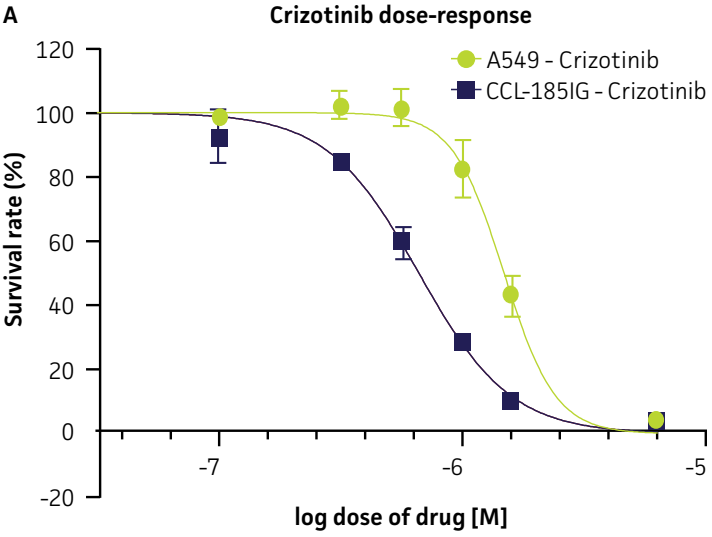


Figure 1. Validation of point mutation at genomic and transcript level. A) Screening for the NRASQ61K point mutation in the genome of the edited clones was carried out using PCR primers as shown left. Introduction of the NRASQ61K point mutation was then confirmed via sequencing shown on the right. Boxed in red is the expected C>A mutation. B) Validating the transcripts of NRASQ61K point mutation in the edited clones was carried out via cDNA generation from cells and PCR (red arrows; top image). The bottom left is the gel image of the PCR products. The bottom right is the Sanger sequencing chromatogram. Boxed in red is the expected point mutation.

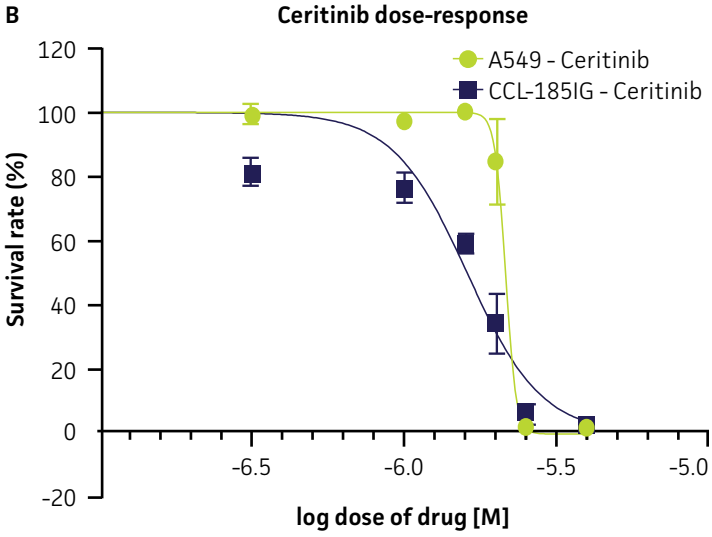
EML4-ALK FUSION ISOGENIC CELL LINE

Introduction: The anaplastic lymphoma kinase (ALK) gene product regulates cell growth and plays an essential role in the development of the brain by helping with the proliferation of nerve cells. When the ALK gene forms a fusion with other genes such as echinoderm microtubule-associated protein-like 4 (EML4), the resulting EML4-ALK genetic abnormality becomes a key oncogenic driver in non-small cell lung carcinoma (NSCLC).

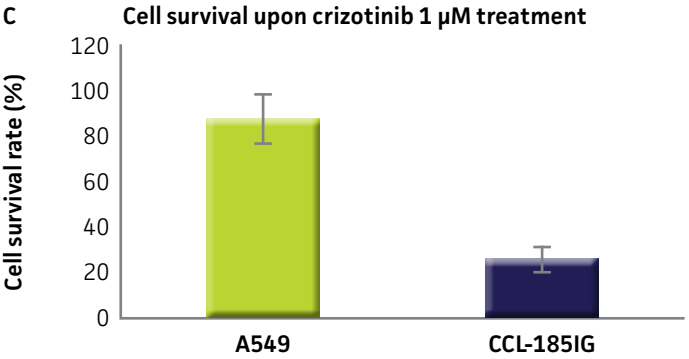
The CRISPR/Cas9 genome editing platform was used to generate the desired targeted genomic rearrangement in the A549 (ATCC® CCL-185™) lung cancer cell line. Precise cuts were made by Cas9 to the appropriate translocation breakpoints in the EML4 and ALK genes. The cancer-relevant EML4-ALK genomic rearrangements occurred via the cell's own homologous DNA repair system. To assess the biofunctional response of the EML4-ALK-fusion A549 Isogenic cell line (ATCC® CCL-185IG™) we challenged the cell line with known Food and Drug Administration-approved inhibitors of ALK.



	A549 - Crizotinib	CCL-185IG - Crizotinib
IC50	1.482e-006	6.627e-007



	A549 - Ceritinib	CCL-185IG - Ceritinib
IC50	2.139e-006	1.603e-006



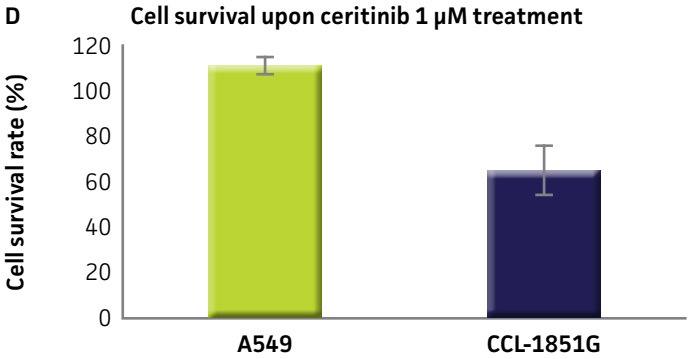
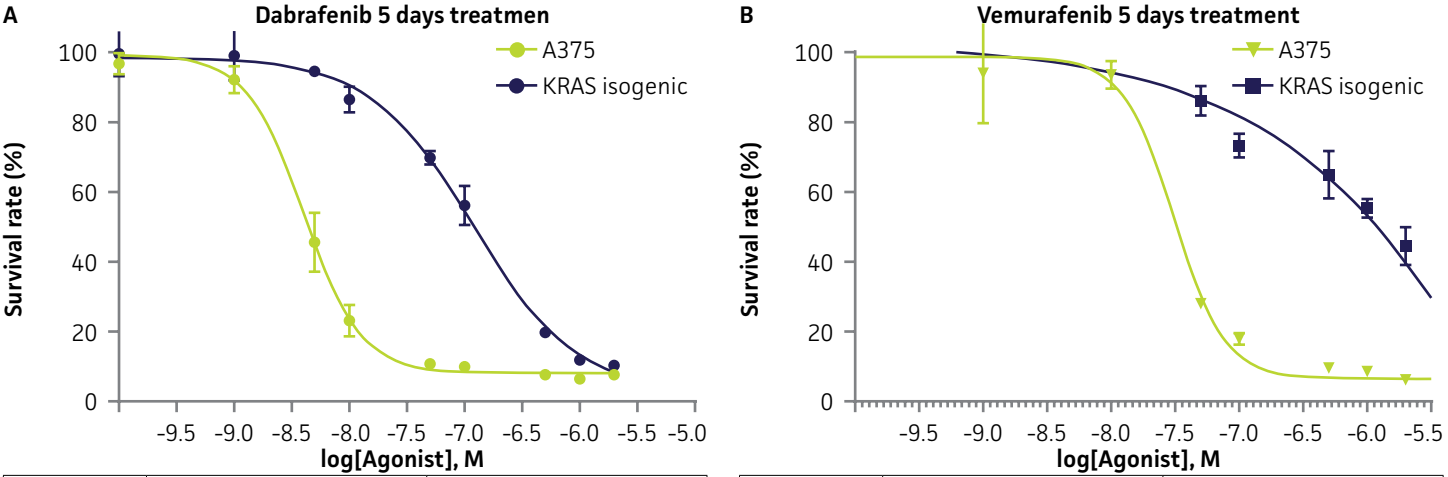


Figure 2. CCL-185IG is sensitive to ALK inhibitor drugs. (A, B) A549 and CCL-185IG cells were treated with the indicated concentrations of ALK inhibitors crizotinib and ceritinib and cell survival was determined via live cell analysis. (C, D) A549 and CCL-185IG cells were treated with 1 µM of same compounds and cell survival was confirmed by cell viability assay.

RAS MUTANT ISOGENIC CELL LINES

Introduction: The Rat Sarcoma (RAS) proto-oncogene encodes for proteins such as KRAS and NRAS that belong to the small GTPase superfamily. RAS proteins recruit and activate downstream effectors, such as those of the AKT and ERK pathways that in turn affect cell growth, differentiation, and survival. Mutations in the RAS gene have been identified in melanoma, and may be predictive of a very poor response to BRAF inhibitors.

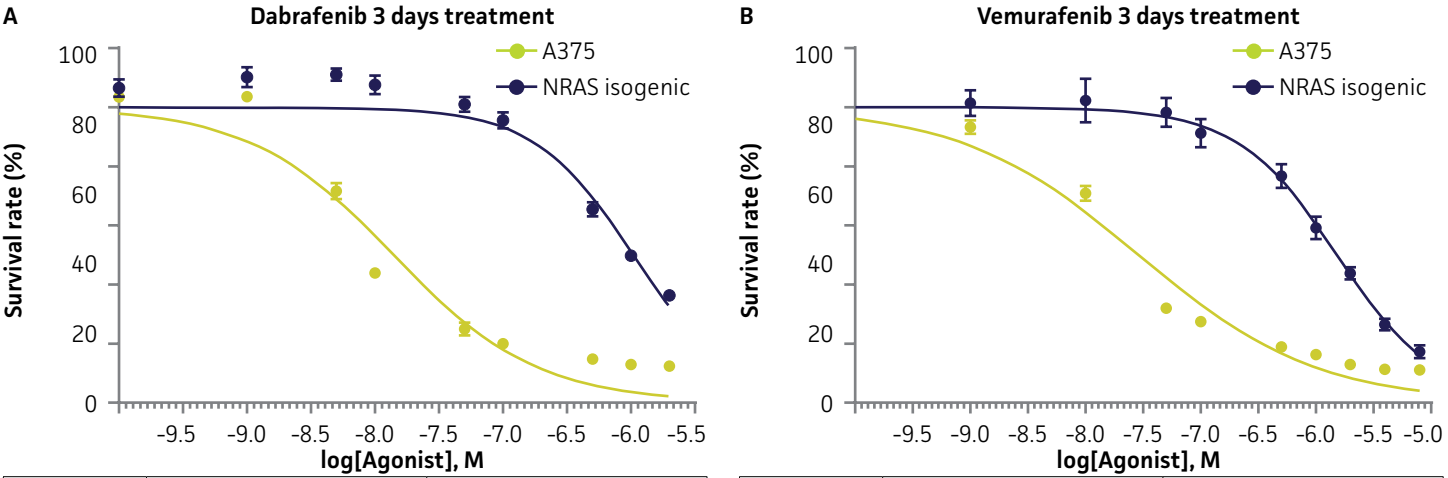
CRISPR/Cas9 gene editing was used to create mutants in the A375 (ATCC® CRL-1619™) cell line, one of the most commonly used *in vitro* models of melanoma. We mutated glycine position 13 to aspartic acid in KRAS (KRASG13D) in A375 cells to create the KRAS mutant-A375 Isogenic Cell Line (ATCC® CRL-1619IG-2™). The NRAS mutant-A375 Isogenic Cell Line (ATCC® CRL-1619IG-2™) was created by replacing the glutamine at position 61 with a lysine (NRASQ61K). To assess the biofunctional responses of the KRAS- and NRAS-mutant Isogenic Cell Lines, we challenged them with the BRAF inhibitors.



	A375	KRAS isogenic
IC50	4.056e-009	1.184e-007

	A375	KRAS isogenic
IC50	3.094e-008	6.55e-005

Figure 3. The KRAS isogenic cell line is more resistant to BRAF inhibitors than the parental A375 cell line. A375 and KRAS Mutant-A375 Isogenic Cells were treated with the indicated concentrations of either A) dabrafenib or B) vemurafinib for three days. Cell survival was monitored via cell viability assay.



	A375	NRAS isogenic
IC50	1.407e-008	1.042e-006

	A375	NRAS isogenic
IC50	2.907e-008	1.519e-006

Figure 4. The NRAS isogenic cell line is more resistant to BRAF inhibitors than the parental A375 cell line. A375 and NRAS Mutant-A375 Isogenic Cells were treated with the indicated concentrations of either A) dabrafenib or B) vemurafinib for three days. Cell survival was monitored via cell viability assay.

IDH1 AND IDH2 MUTANT ISOGENIC CELL LINES

Introduction: The isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) genes code for proteins that are normally involved in metabolism, taking part in fatty acid synthesis or the TCA cycle. Once mutated these proteins can promote tumorigenesis, in part via histone methylation.

CRIPSR/Cas9 gene editing was employed to create mutants in the U-87 MG (ATCC® HTB-14™) and TF-1 (ATCC® CRL-2003™) cell lines, commonly used *in vitro* models of brain and leukemic cancers, respectively. Arginine position 132 was mutated to threonine in IDH1 (IDH1R132H) in U-87 MG cells to create the IDH1 mutant-U-87 MG Isogenic Cell Line (ATCC® HTB-14IG™). The IDH2 mutant-TF-1 Isogenic Cell Line (ATCC® CRL-2003IG™) was created by replacing the arginine at position 14 with a glutamine (IDH2R14Q). Two different tests were used to confirm the biofunctional responses of the IDH mutants.

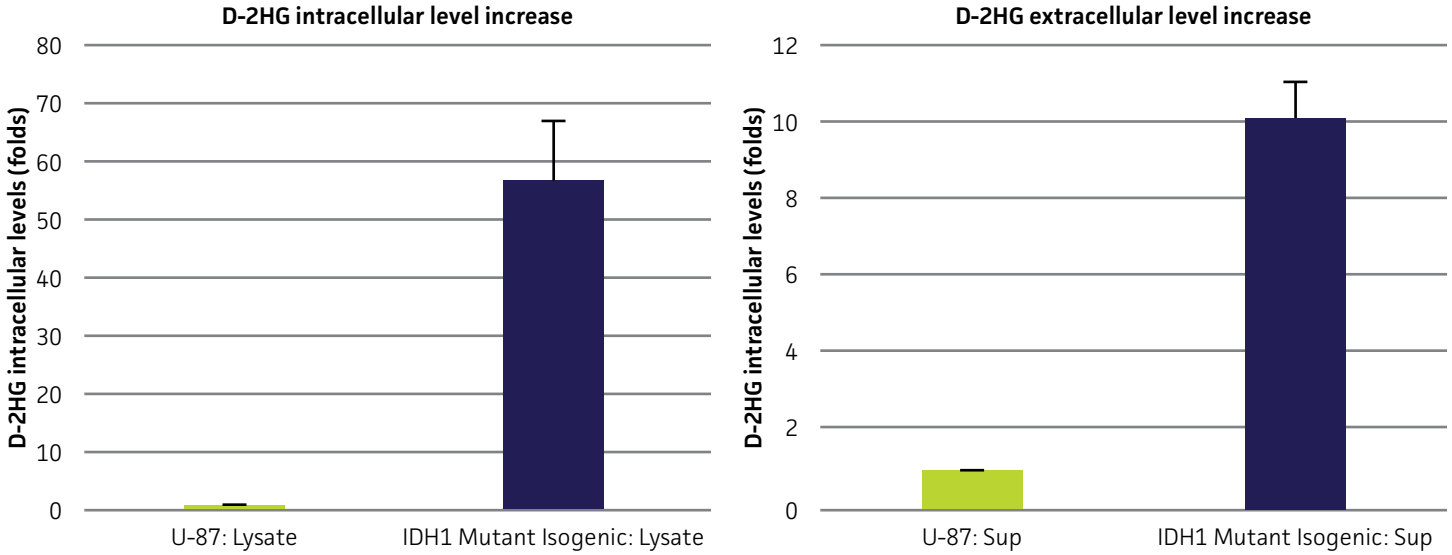


Figure 5. IDH1-mutant-U-87 MG Isogenic cells display increased oncometabolite expression. To assess the biofunctional response of the IDH1 mutant we monitored the accumulation and secretion of the oncometabolite D-2-hydroxyglutarate (D-2HG). The parental and IDH1 mutant lines were tested for intracellular and extracellular D-2HG levels after seven days post seeding.

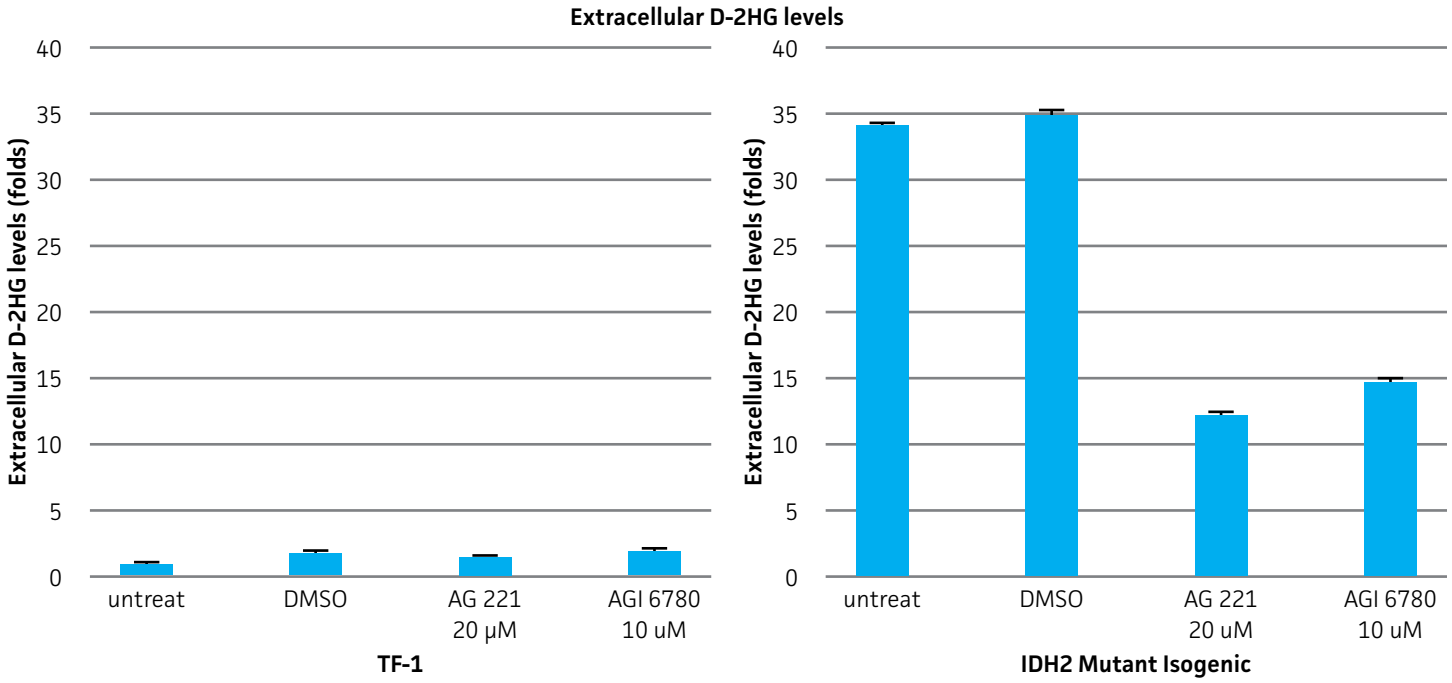


Figure 6. IDH2 mutant TF-1 D-2HG levels are blocked by IDH2 specific inhibitors. To determine the response of the IDH2-mutant Isogenic Cell Lines, we challenged the parental and the IDH2 mutant line with AGI-6780 and AG-221. Parental and IDH2 isogenic cell lines were cultured with or without IDH2-specific inhibitors for 3 days. Pico-Probe D-2HG assay kit was used to detect D-2HG levels seven days post drug treatment showing several fold reduction in extracellular D-2HG levels.

REFERENCES:

1. Enuameh, MS, et al. The generation of an EML4-ALK fusion NSCLC isogenic cell line relevant for drug discovery and development. Application Note Number 25, 2016.
2. Tian F, Volpe L. New isogenic cell models created by CRISPR genome editing for drug discovery. ATCC Excellence in Research Webinar, broadcast on April 13, 2017.

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