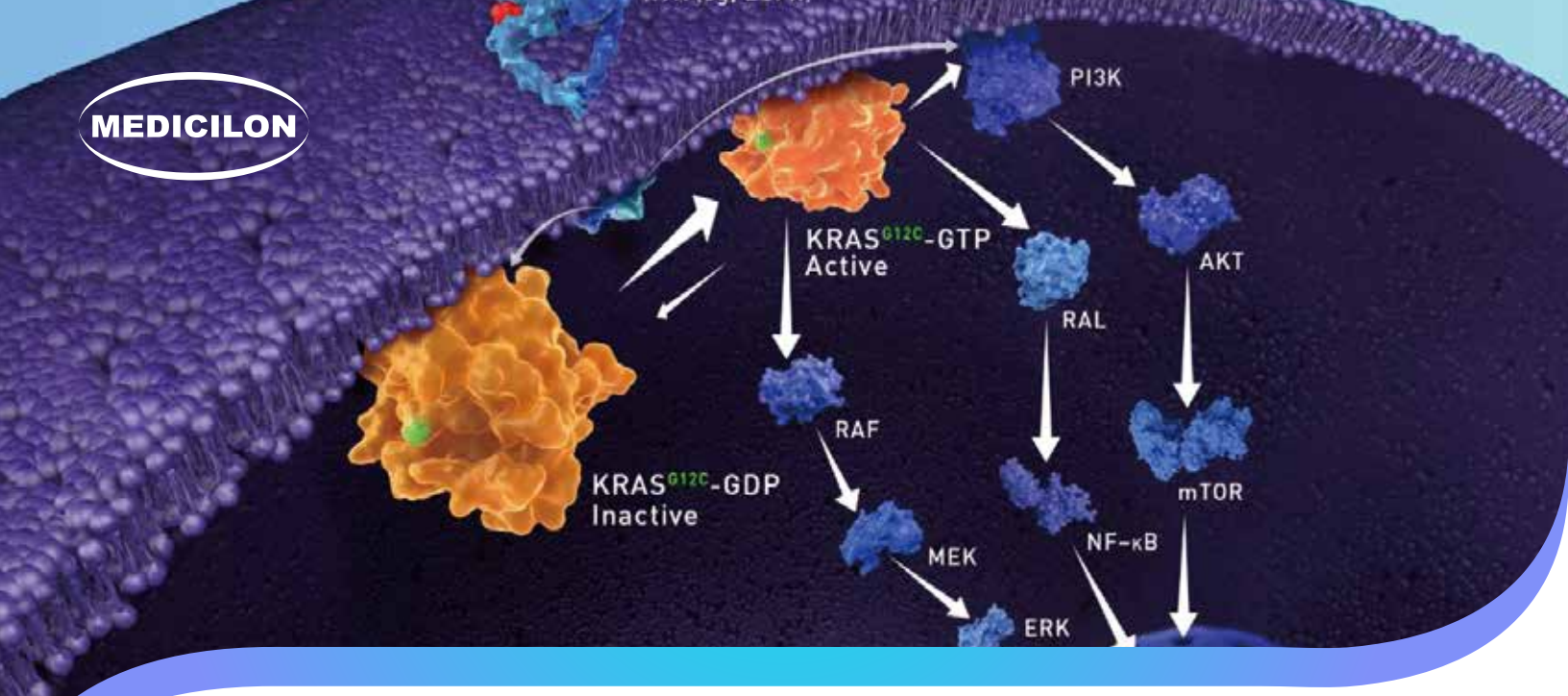
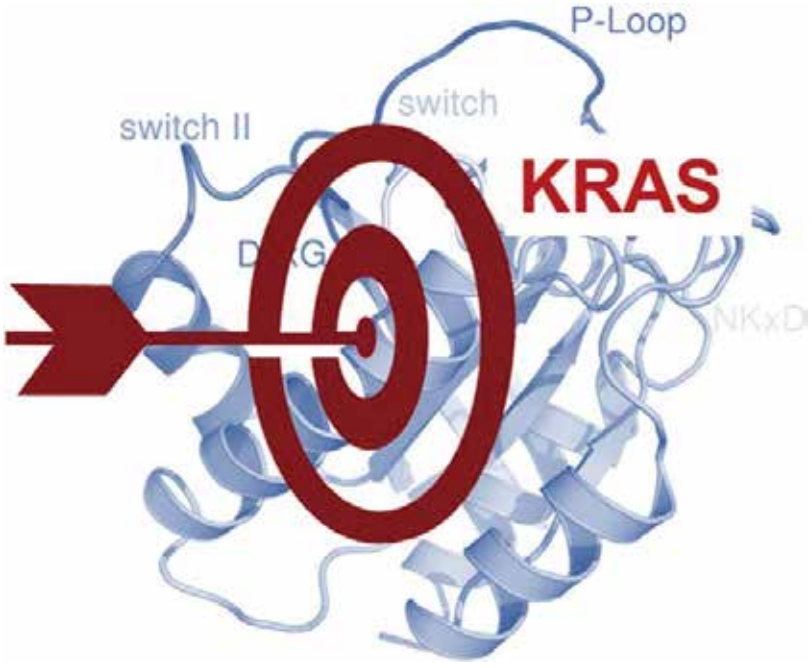


Medicilon KRAS-targeted Drugs R&D Service

RAS is one of the most frequently mutated oncogenes in human cancer. KRAS is the isoform most frequently mutated, which constitutes about 85% of RAS mutations. As the most frequently mutated RAS isoform, KRAS is intensively studied in the past years.

In the formulation of KRAS integrated research plan, Medicilon has in-depth communication with customers. The backbone of scientific research has combined the characteristics of each case with years of practical experience and technical accumulation, and carefully submitted high-quality experimental plans and results to customers. Medicilon provides KRAS-targeted drug discovery, CMC research (API + formulation), pharmacodynamics research, PK study, safety evaluation and other services.



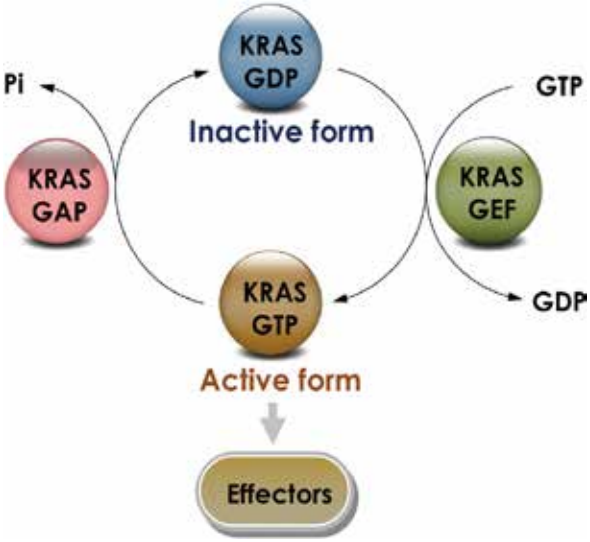
Targeting the untargetable KRAS[1]

Introduction of KRAS

RAS is a family of GTPase proto-oncogenes, comprising three closely related RAS isoforms: HRAS, KRAS and NRAS. From all of the RAS isoforms, KRAS is most frequently mutated, followed by NRAS and then HRAS. KRAS mutations are particularly frequent in the pancreatic, lung and colorectal cancers. In cancer, the most frequently mutated residues are G12, G13, and Q61. KRAS protein exists as two splice variants, KRAS4A and KRAS4B, in which KRAS4B is the dominant form in human cells.

KRAS (Kirsten rat sarcoma 2 viral oncogene homolog) gene is a proto-oncogene that encodes a GTP/GDP-binding protein that belongs to the GTPase RAS family.

The KRAS protein acts as molecular switchs that cycle between a GDP-bound inactive state and a GTP-bound active state. KRAS protein switches between an inactive to an active form via binding to GTP and GDP, respectively.

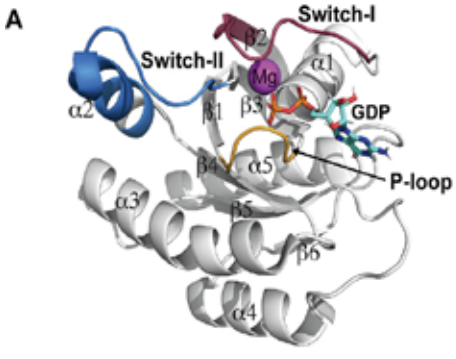


KRAS GTPase cycle[1]

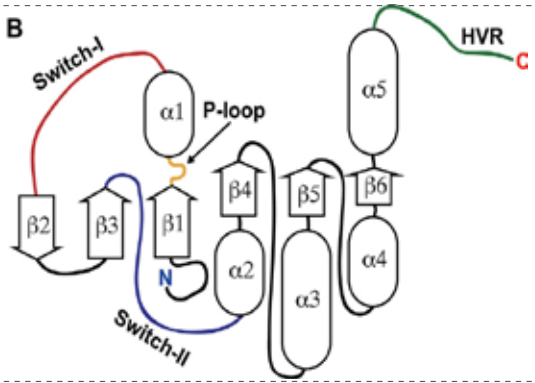
Although the KRAS protein harbors both intrinsic nucleotide exchange and GTP hydrolysis, its cellular signaling state arises from activation by guanine exchange factors (GEFs), such as son of sevenless (SOS) and Ras guanyl nucleotide-releasing protein, which catalyze GTP loading and deactivation by GTPase activating proteins (GAPs), such as p120GAP and neurofibromin (NF1), which stimulate GTP hydrolysis.

Structure of KRAS

KRAS protein contains four domains. The first domain at the N-terminus is identical in the three RAS forms, and the second domain exhibits relatively lower sequence identity. Both regions are important for the signaling function of the KRAS protein and jointly form the G-domain. KRAS protein has a molecular weight of 21 kDa, and is made up of six beta-strands (forming the protein core) and five alpha-helices, which form two major domains: the G-domain and the C-terminal. The G domain of KRAS, comprised of residues 1-166, includes the GTP-binding pocket, a region within which is essential for the interactions between the putative downstream effectors and GTPase-activating proteins (GAPs). The G domain is highly conserved and contains switch I and switch II loops, which are responsible for GDP-GTP exchange. The C-terminal, a hypervariable region including the CAAX (C=cysteine, A=any aliphatic amino acid, X=any amino acid) motif, guides posttranslational modifications and determines plasma membrane anchoring. This region plays an important role in the regulation of the biological activity of RAS protein.



Crystal structure KRAS[2]



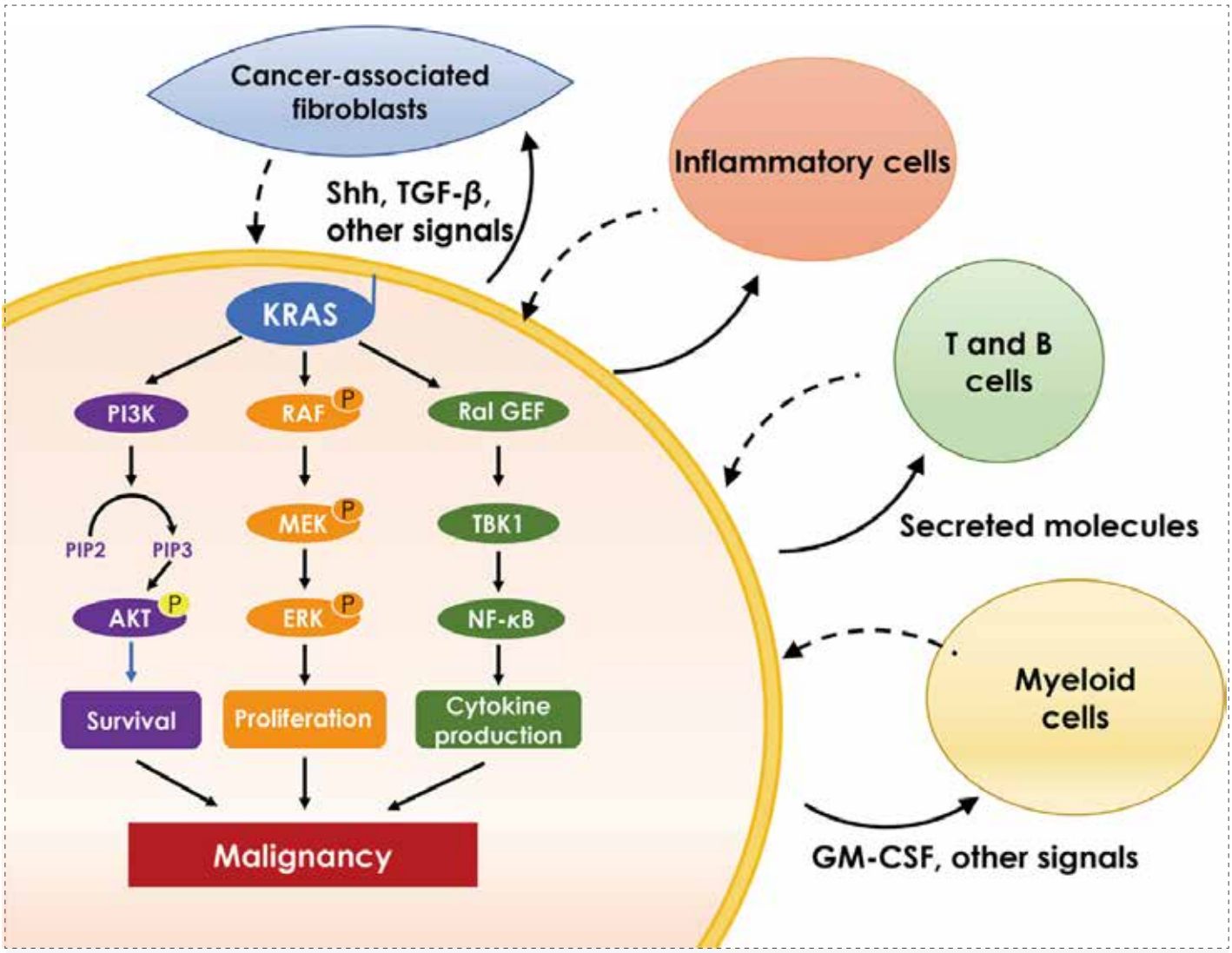
2D depiction of the secondary structure of KRAS[2]

Signaling Pathway of KRAS

KRAS is one of front-line sensors that initiate the activation of an array of signaling molecules, allowing the transmission of transducing signals from the cell surface to the nucleus, and affecting a range of essential cellular processes such as cell differentiation, growth, chemotaxis and apoptosis. In addition to the aforementioned GTP/GDP binding, the activation of KRAS signaling is now known as a multi-step process that requires proper KRAS post-translation, plasma membrane-localization and interaction with effector proteins.

The signal transduction of the KRAS protein does not exclusively occur at the plasma membrane. Activation of downstream signaling pathways by KRAS can also be triggered by signals from subcellular compartments, such as the endoplasmatic reticulum and the Golgi apparatus.

In response to extracellular stimuli, the conversion from inactive RAS-GDP to active RAS-GTP further promotes the activation of various signaling pathways, which includes MAPK pathway, PI3K pathway and the Ral-GEFs pathway, among them the MAPK pathway is the best characterized. It is known that RAS-GTP directly binds to RAF protein, recruiting RAF kinase family from cytoplasm to membranes, where they dimerize and become active. The activated RAF subsequently carries out a chain of phosphorylation reactions to its downstream substrates, namely MEK and ERK, and propagates the growth signal.



The major KRAS effector pathways[1]

Crystallization Studies of KRAS Protein

High Throughput Screen of Crystallization

More than 1,000 screen conditions





Hampton research HT Kits



QIAGEN HT Classics Suite



Mosquito

Shanghai Synchrotron Radiation Facility

Medicilon is involved in the design, construction and management of Shanghai Synchrotron Radiation Facility, an industrial beamline for macromolecular crystallography. Macromolecular beamline was open on July 2009.

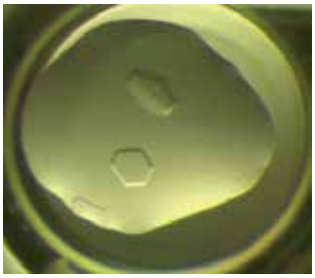


Macromolecular Crystallography for Industrial Use

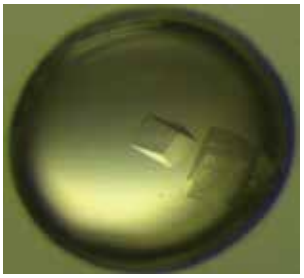
- Superior beamline and service
- Lower costs
- 3.5 GeV storage ring
- Year - round operation
- Very close to Zhangjiang High-Tech Park and Pudong airport.

Case study

KRAS-G12D



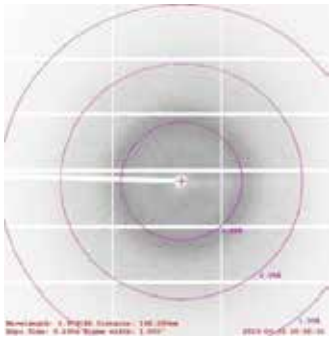
0.17 M Ammonium acetate
0.085 M Sodium acetate pH 4.6
15% (v/v) Glycerol
25.5% (w/v) PEG 4000



0.2 M Lithium sulfate
0.1 M Tris·HCl pH 8.5
30% (w/v) PEG 4000

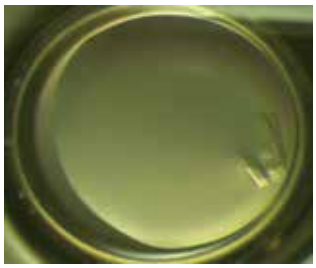
Screening of KRAS^{G12D}



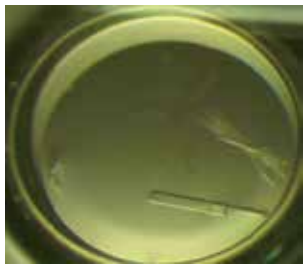


Synchrotron X-ray diffraction data of KRAS^{G12D}

KRAS-G12D with MRTX1133

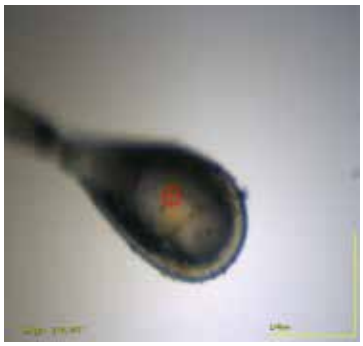


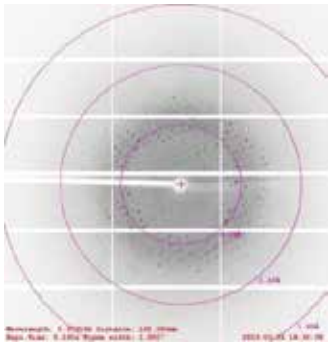
0.17 M Ammonium sulfate
15% (v/v) Glycerol
25.5% (w/v) PEG 4000



0.2 M Ammonium sulfate
0.1 M Sodium acetate pH 4.6
25 % (w/v) PEG 4000

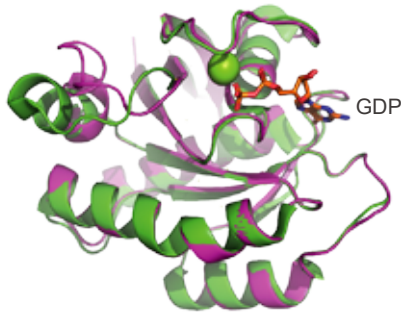
Screening of the co-crystallization





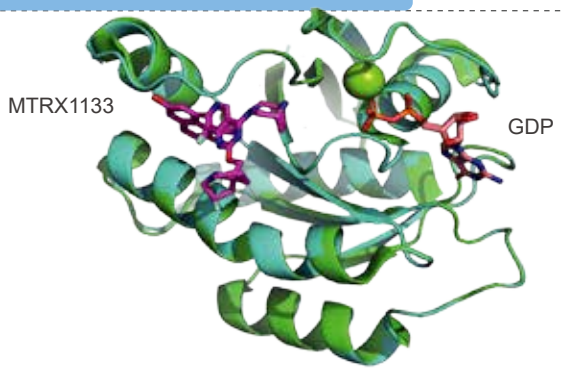
Synchrotron X-ray diffraction data of the co-crystallization

KRAS-G12D



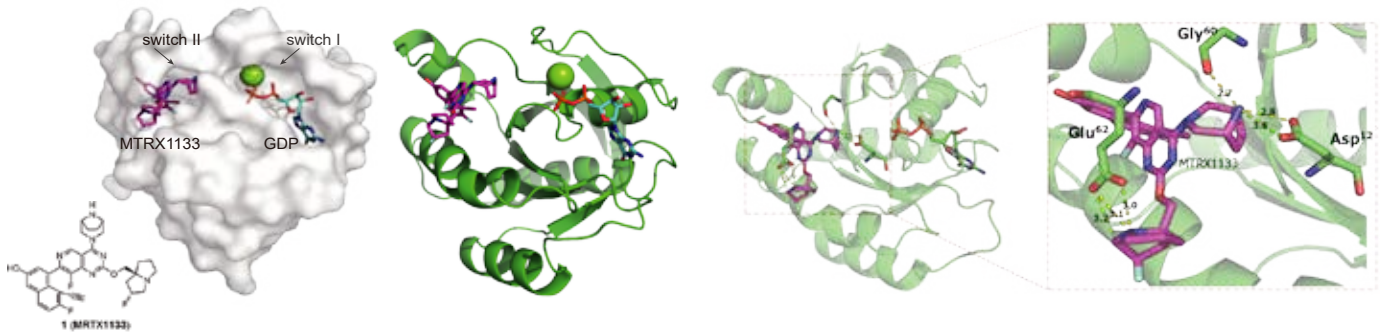
Compare the Structure of KRAS^{G12D} with 7RPZ, the green is PDB ID 7RPZ and the pink is the data from **Medicilon**. The data are very correlated.

KRAS-G12D with MRTX1133



Compare the Structure of KRAS^{G12D} co-crystallization with MRTX1133 (7RPZ, PDB), the green strucuture is PDB ID 7RPZ and the cyan is data from **Medicilon**. The data are very correlated.

KRAS-G12D with MRTX1133



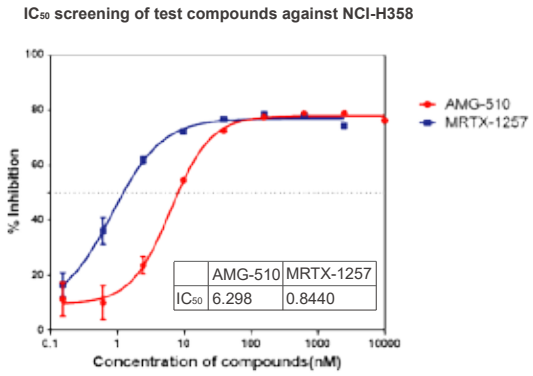
Structure of KRAS^{G12D} co-crystallized with MRTX1133 with GDP-bound

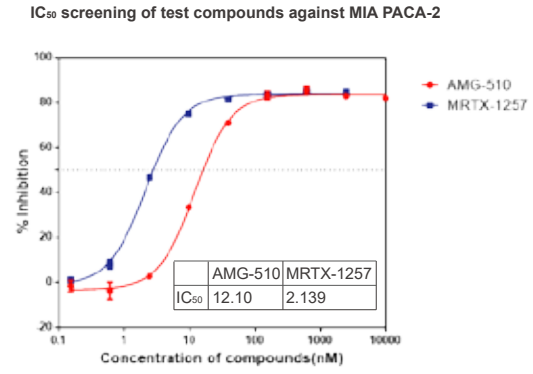
In Vitro Studies of KRAS-targeted Drugs

In vitro functional assays are crucial for the practical evaluation of a candidate KRAS-targeted drug in the initial stages of research and development. These assays offer scientific evidence for validating KRAS-targeted drug activity, and providing preliminary evidence that supports therapeutic efficacy. As such, they play a key role in the decision-making process in KRAS-targeted drug candidate selection.

KRAS Cellular Assay

Medicilon have validated cytotoxicity assays for KRAS mutant cell lines, both 2D and 3D assays could be used for evaluation of KRAS inhibitors.





Cell lines	Tissue	KRAS Mutant
AsPC-1	pancreas	G12D
Capan-1	pancreas	G12V
Capan-2	pancreas	G12V
CFPAC-1	pancreas	G12V
HPAF-II	pancreas	G12D
MIAPaCa-2	pancreas	G12C
Panc 10.05	pancreas	G12D
SU.86.86	pancreas	G12D
HCT116	large_intestine	G13D
HCT15	large_intestine	G13D
LoVo	large_intestine	G13D
T84	large_intestine	G13D
Calu-1	lung	G12C
NCI-H2122	lung	G12C
NCI-H23	lung	G12C
NCI-H358	lung	G12C
NCI-H441	lung	G12V
SW1463	large_intestine	G12C
SW480	large_intestine	G12V
SW620	large_intestine	G12V
SW837	large_intestine	G12C

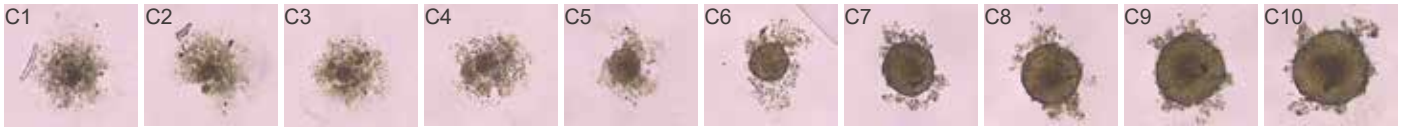
2D cell proliferation assays detected through CellTiter-Glo

Cell Cytotoxity Assay (3D)

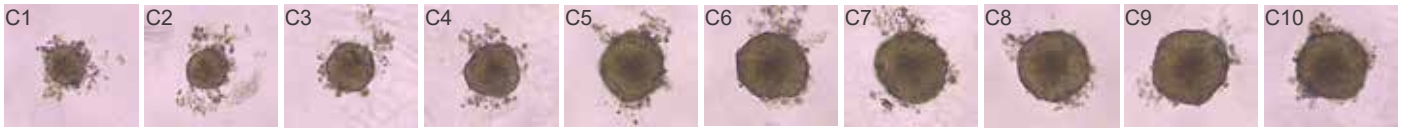
NCI-H358 (Lung, KRAS^{G12C}) Cell Cytotoxity Assay (3D)

Group	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Concentration (nM)	1000	333.3	111.1	37.04	12.35	4.115	1.372	0.457	0.152	0

Compound	Cell line	Incubation time (Day)	Start conc	Dilution fold
AMG510	NCI-H358	12	1 μM	3-fold diluted

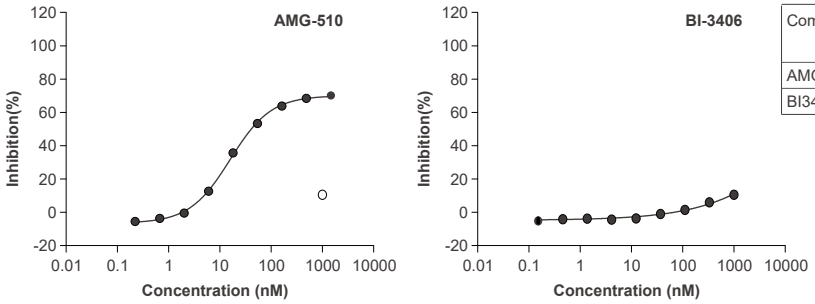


Compound	Cell line	Incubation time (Day)	Start conc	Dilution fold
BI3406	NCI-H358	12	1 μM	3-fold diluted



Cytotoxicity determined by photomicrography after 288 h treatment with drugs in NCI-H358 cells at concentrations starting from 1 μM and 1:3 serial dilution.

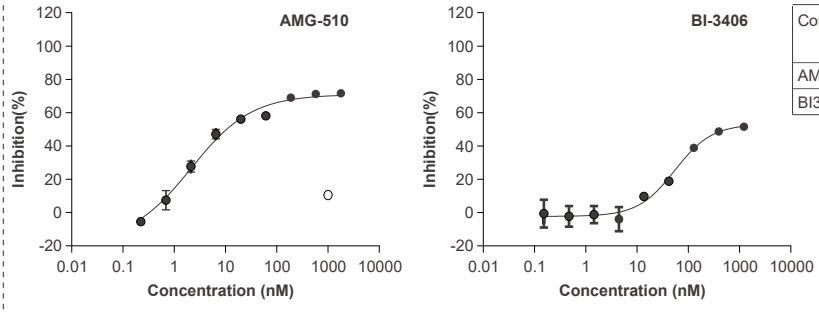
NCI-H358 (Lung, KRAS^{G12C}) Cell Cytotoxity CTG Assay (2D; 3 days)



Compound	CTG-IC$_{50}$s on NCI-H358 cells (nM)	Top Inhibition(%)	Start conc	Dilution fold
AMG510	10.89	86.86	1 μM	3-fold diluted
BI3406	>1000	13.78	1 μM	3-fold diluted

Cytotoxicity determined by CTG after 72 h treatment with drugs in NCI-H358 cells at concentrations starting from 1 μM and 1:3 serial dilution.

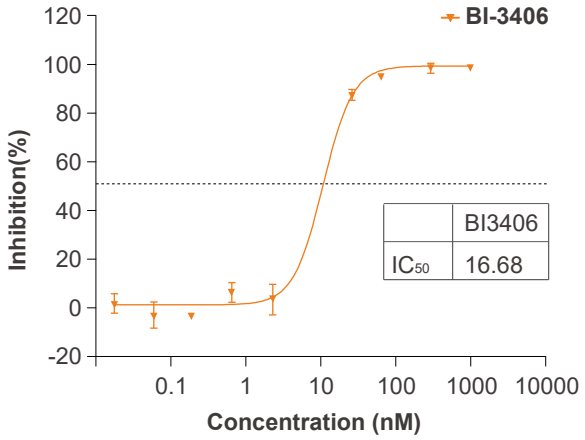
NCI-H358 (Lung, KRAS^{G12C}) Cell Cytotoxity CTG Assay (3D;12 days)

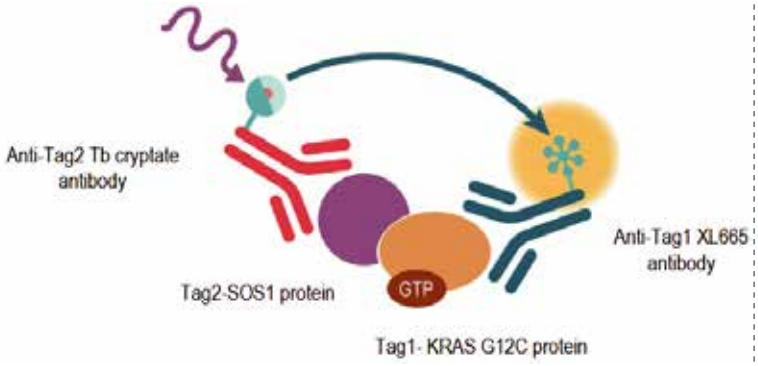


Compound	CTG-IC$_{50}$s on NCI-H358 cells (nM)	Top Inhibition(%)	Start conc	Dilution fold
AMG510	1.386	99.52	1 μM	3-fold diluted
BI3406	48.23	71.53	1 μM	3-fold diluted

Cytotoxicity determined by photomicrography after 288 h treatment with drugs in NCI-H358 cells at concentrations starting from 1 μM and 1:3 serial dilution.

Protein-based Assay

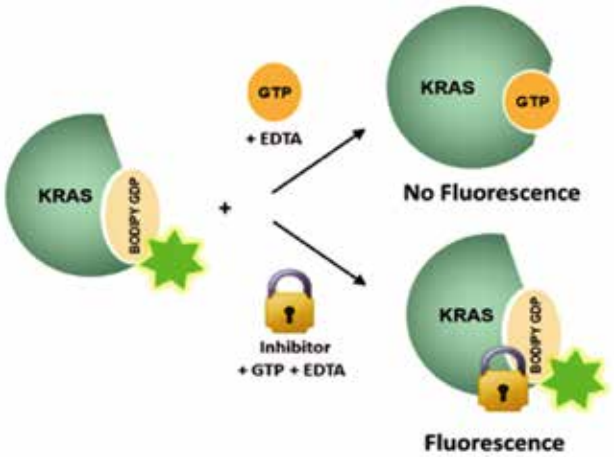




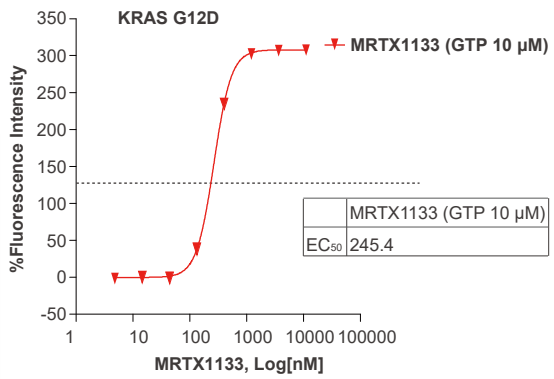
IC_{50} screening of test compounds on Kras G12C-SOS1 Binding

Guanine Nucleotide Exchange Assay

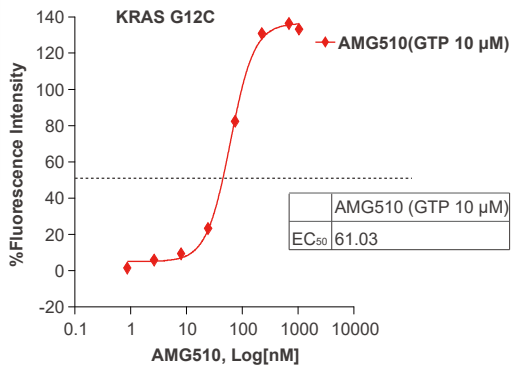
A



B



C



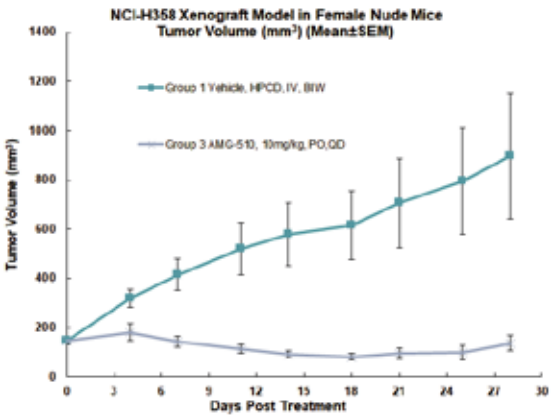
- KRAS was incubated in a solution containing 1 mM BODIPY FL-GDP, 20 mM HEPES pH 7.6, 10 mM EDTA, 20 mM ammonium sulfate and 1 mM DTT for 48 hours at 4°C.
- The reaction was stopped with the addition of 20 mM $MgCl_2$.
- The BODIPY-FL-GDP loaded KRAS protein is concentrated to remove BODIPY-FL-GDP.
- Panel A: MoA for the assay

Pharmacology Evaluation of KRAS-targeted Drugs

KRAS Mutation - CDX Model

Cancer Type	Cell Lines	Cancer Type	Cell Lines
KRAS G12C	MIA PaCa-2, NCI-H358, UM-UC-3, Calu-1	KRAS G12V	SW480, CAPAN-1, NCI-H727
KRAS G12D	GP2D, SW1990, AsPC-1	KRAS G13D	LoVo, HCT-116, HT15

Medicilon Case: CDX - KRAS Mutation (G12C)



- **Animals:** Female BALB/c Nude mice
- **Cells:** NCI-H358
- **Model Establishment:** Right flank, SC

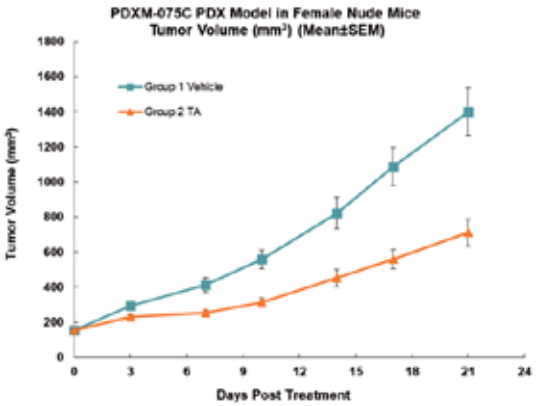
PDX Key Mutation/Overexpression/Resistance

GENE	PDX ID	GENE	PDX ID
KRAS Mutation	PDXM-060C (p.G12V), PDXM-069C (p.G12V), PDXM-075C (p.G12D), PDXM-076C (p.G13D), PDXM-212Li (p.G12D)	BCR-ABL Fusion	PDXM-242Le
		ERBB2 Overexpression	PDXM-069C, PDXM-016C, PDXM-060C, PDXM-087C, PDXM-104C…
TP53 Mutation	PDXM-060C (p.R273H), PDXM-072C(p.Y234H)	…	…
PIK3CA Mutation	PDXM-075C (p.H1047L), PDXM-092Ga (p.E545G)		

Resistance*	PDX ID	Resistance*	PDX ID
Docetaxel + Cisplatin	PDXM-271O (Ovarian cancer)	Radiation	PDXM-311(H&N)
VDLP + MA + CVAD	PDXM-293Le (Leukemia)	…	…

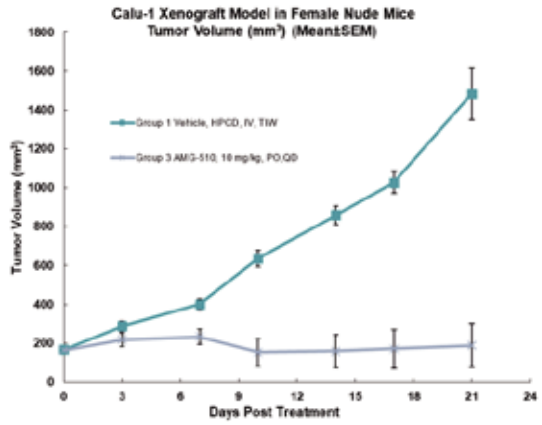
** note: these resistance models are not related to KRAS mutation*

Medicilon Case: PDX - KRAS Mutation (G12D)

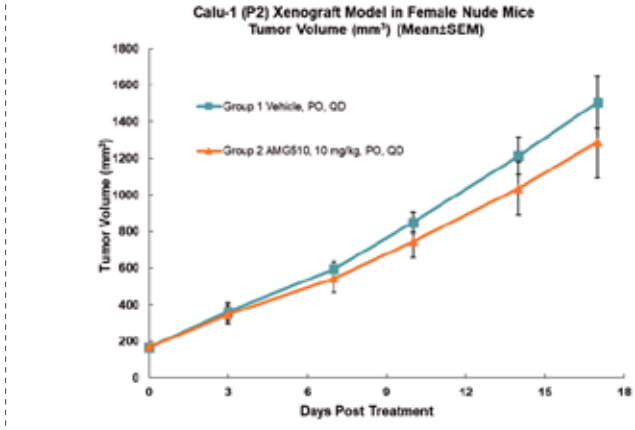


- **Animals:** Female BALB/c Nude mice
- **Tissue:** PDX Colon Cancer
- **Model Establishment:** Right flank SC Trocar

Medicilon Case: AMG-510 Resistant Model - Calu-1 (G12C)



Wild Type Lung Cancer Model



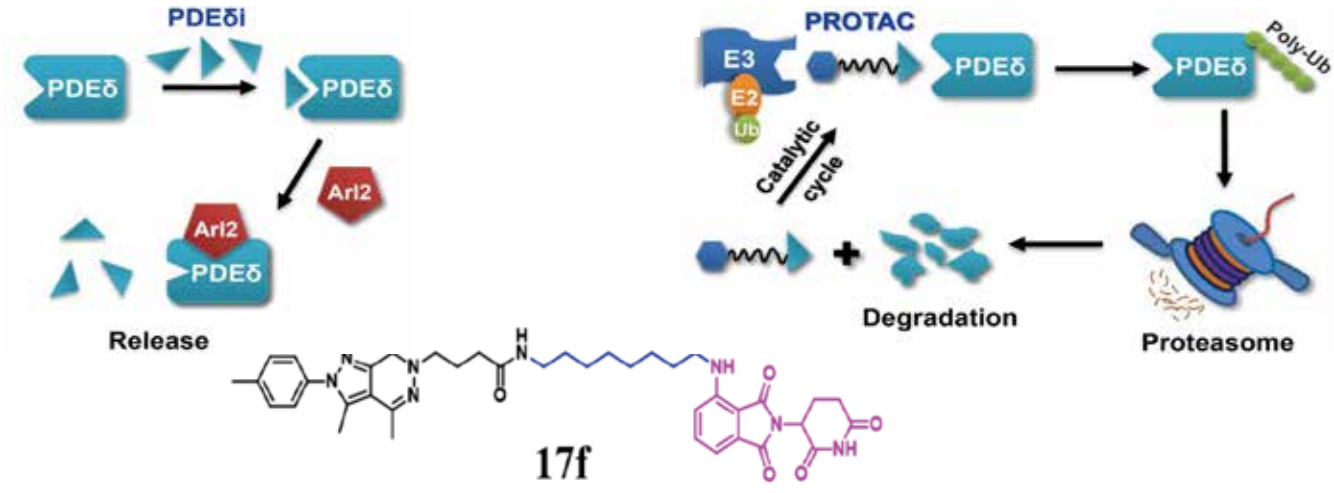
Resistant Lung Cancer Model (established through *in vivo* treatment cycle twice (P2), two months each cycle. Show here is the P3 results.

Pharmacokinetic (PK) Studies of KRAS-targeted Drugs

Medcilon provides high quality quantification assays for key parameters in KRAS-targeted drugs PK study, presenting accurate results.

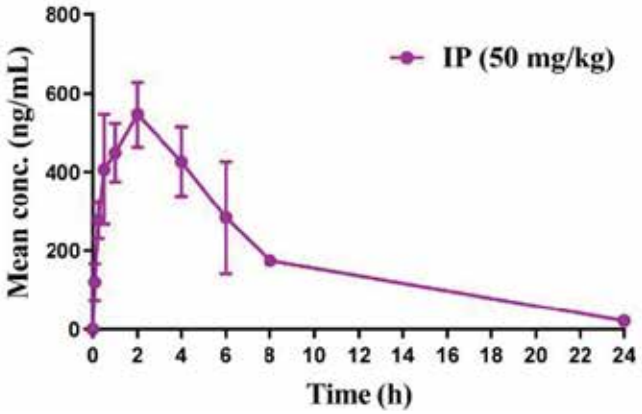
Medicilon Case: Pharmacokinetics of KRAS-PDEδ Inhibitors

KRAS-PDEδ protein-protein interaction represents an appealing target for cancer therapy. A series of potent PROTAC PDEδ degraders were designed and synthesized. The most promising Compound 17f is a PROTAC PDEδ degrader for the treatment of KRAS mutant colorectal cancer. Compound 17f provided a new chemical tool or lead compound for navigating the druggablility of KRAS-PDEδ interaction. Compound 17f achieved significant tumor growth inhibition in the SW480 colorectal cancer xenograft model. This proof-of-concept study provided a new strategy to validate the druggability of KRAS-PDEδ interaction and offered an effective lead compound for the treatment of KRAS mutant cancer.



PROTAC strategy and KRAS-PDEδ inhibitor Compound 17f[3]

Pharmacokinetic (PK) studies of Compound 17f were evaluated in Sprague-Dawley (SD) rats. After ip administration dosing at 50 mg/kg, the concentrations of Compound 17f in plasma were analyzed. These assays were conducted by **Medicilon.** The half-life of 17f was approximately 5.1 h and the peak concentration C_{max} was 564 ng/mL. Despite its relatively large size (MW = 723), Compound 17f could be effectively absorbed and achieved a sufficient plasma exposure in rats, with the area under the curve (AUC) value of 4710 h·ng/mL.



Parameter	Value
$T_{1/2}$ (h)	5.11 ± 0.66
T_{max} (h)	1.67 ± 0.58
C_{max} (ng/mL)	563.78 ± 54.36
$AUC_{(0-\infty)}$ (h*ng/mL)	4710.02 ± 676.70
CL (mL/h/kg)	10763.17 ± 1509.38

PK parameters of Compound 17f in rats[3]

Medicilon Assisted Projects

XNW14010

In May 2022, the State Drug Administration approved the clinical application of XNW14010, a new class 1 anti-tumor drug from Evopoint Biosciences, which is intended for the treatment of patients with advanced solid tumors with KRAS G12C mutation. XNW14010 is a highly selective small molecule KRAS G12C protein covalent binding inhibitor. **Medicilon provided comprehensive preclinical research services (including pharmacokinetics and safety evaluation) for the development of XNW14010, providing strong support for the project's clinical approval.**

GFH925/IBI351

In September 2024, Genfleet Therapeutics launched its KRAS G12C inhibitor, Dabote® (Fuzerelisib Tablets, GFH925/I-BI351). This product, as a monotherapy, is indicated for adult patients with KRAS G12C-mutant advanced NSCLC who have received at least one prior systemic therapy. **Medicilon leveraged its well-established pharmacokinetics research platform to provide early-stage animal pharmacokinetic services for Fuzerelisib.**

References:

[1] Pingyu Liu, et al. Targeting the untargetable KRAS in cancer therapy. Acta Pharm Sin B. 2019 Sep;9(5):871-879. doi: 10.1016/j.apsb.2019.03.002.

[2] Tatu Pantsar. The current understanding of KRAS protein structure and dynamics. Comput Struct Biotechnol J. 2019 Dec 26:18:189-198. doi: 10.1016/j.csbj.2019.12.004.

[3]Junfei Cheng, et al. Discovery of Novel PDEδ Degraders for the Treatment of KRAS Mutant Colorectal Cancer. J Med Chem. 2020 Jul 23;63(14):7892-7905. doi: 10.1021/acs.jmedchem.0c00929.

[4]Gongmin Zhu, et al. Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer. Mol Cancer. 2021 Nov 6;20(1):143. doi: 10.1186/s12943-021-01441-4.

[5]Tamas Yelland, et al. Stabilization of the RAS:PDE6D Complex Is a Novel Strategy to Inhibit RAS Signaling. J Med Chem. 2022 Feb 10;65(3):1898-1914. doi: 10.1021/acs.jmedchem.1c01265.

[6]Timothy H Tran, et al. KRAS interaction with RAF1 RAS-binding domain and cysteine-rich domain provides insights into RAS-mediated RAF activation. Nat Commun. 2021 Feb 19;12(1):1176. doi: 10.1038/s41467-021-21422-x.

MEDICILON

Email: info@medicilon.com **Website:** www.medicilon.com

Address: ➢ 20 Maguire Road, Suite 103, Lexington, MA 02421, USA

➢ 585 Chuanda Road, Pudong, Shanghai, 201299, China