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Drug Discovery Service

A Comprehensive CRISPR/Cas9-Based RAS Knock-In Cell Line Platform (50+ Models) for Assessing Efficacy of KRAS-Targeted Inhibitors in RAS-Mutant Malignancies

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Abstract

The rat sarcoma gene (RAS) was the first discovered human proto-oncogene. Its product, the RAS protein, is a small guanine nucleotide-binding protein with intrinsic guanosine triphosphatase (GTPase) activity. Currently, three subtypes are known in the RAS family: KRAS, NRAS, and HRAS.

RAS is a key mediator of the MAPK pathway. Activated RAS proteins bind GTP, while inactivated RAS proteins bind GDP; thus, the function of RAS proteins primarily depends on their GTP/GDP ratio. Missense mutations in the RAS protein alter the GDP/GTP homeostasis and lead to sustained activation of signaling pathways by reducing GTP hydrolysis (G12, Q61), increasing GTP loading rates (G13, K117), or affecting nucleotide exchange (A146). This uncontrolled signaling promotes unregulated cell proliferation and ultimately leads to carcinogenesis. RAS mutations occur most frequently in the KRAS subtype (>80%), followed by NRAS and HRAS. Notably, KRAS mutations are found in 97.7% of pancreatic ductal adenocarcinomas. In colorectal adenocarcinoma, multiple myeloma, lung adenocarcinoma, and cutaneous melanoma, the overall prevalence of RAS mutations is 52.2%, 42.6%, 32.2%, and 29.4%, respectively.

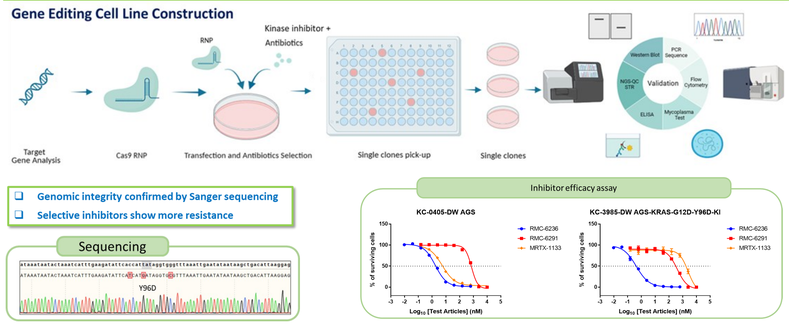
For decades, RAS was notoriously considered an "undruggable" target, posing significant challenges for drug development. The approval of AMG510 (sotorasib) broke this barrier, proving that direct targeting of RAS is achievable. Since then, the field of RAS-targeted therapy has advanced remarkably, moving the "undruggable" label into the past. With deepening understanding, targeting RAS is poised to drive the next wave of breakthroughs in cancer treatment.

Using CRISPR/Cas9 technology, we have developed over 50 knock-in cell line models with KRAS and NRAS mutations for evaluating KRAS-targeted inhibitors. These include common mutations such as G12C, G12D, and G12V, as well as various double and triple mutation combinations. This Ras knock-in mutant cell line platform stands as a powerful tool for developing and evaluating the next generation of RAS inhibitors.

Ras mutation knock in cell Panel

No.	ID	Cell Line name	No.	ID	Cell Line name
1	KC-3985	AGS-KRAS-G12D-Y96D-KI	25	KC-5594	CT26-KRAS-V8M-G12A-KI-4B3
2	KC-4388	AGS-KRAS-G12D-H95D-KI	26	KC-5533	CT26-KRAS-V8M-G12A-KI-1C4
3	KC-4415	AGS-KRAS-G12D-H95Q-KI	27	KC-5519	CT26-KRAS-V8M-G12A-G13D-KI-3A4
4	KC-4416	AGS-KRAS-G12D-H95Q-KI	28	KC-5559	CT26-KRAS-V8M-G12S-KI-1A4
5	KC-4631	AGS-KRAS-G12D-H95R-KI	29	KC-5596	CT26-KRAS-V8M-G12S-KI-1C3
6	KC-4632	AGS-KRAS-G12D-H95R-KI	30	KC-5558	CT26-KRAS-V8M-G12R-KI-2B1
7	KC-4844	GP2d-KRAS-G12D-Y96D-KI	31	KC-5595	CT26-KRAS-V8M-G12R-KI-2C3
8	KC-4902	GP2d-KRAS-G12D-Y96D-KI	32	KC-5637	CT26-KRAS-V8M-G12V-KI-3A1
9	KC-5104	GP2d-KRAS-E62Q-Y71H-KI	33	KC-5534	CT26-KRAS-V8M-G12V-KI-2C2
10	KC-5170	GP2D-KRAS-E62Q-KI	34	KC-5503	CT26-KRAS-V8M-G12C-KI-Abcb1a-KO-1C4
11	KC-4810	Aspc-1-KRAS-G12D-Y96D-KI	35	KC-5485	CT26-KRAS-V8M-G12C-KI-Abcb1a-KO-1B3
12	KC-4811	Aspc-1-KRAS-G12D-Y96D-KI(+/-)	36	KC-5635	CT26-KRAS-V8M-G12C-KI-Abcb1a-Abcb1a-KO-1B4
13	KC-4843	Aspc-1-KRAS-G12D-Y96D-KI	37	KC-5636	CT26-KRAS-V8M-G12C-KI-Abcb1a-Abcb1a-KO-1C2
14	KC-4911	NCI-H358-KRAS-G12C-H95C-KI	38	KC-5589	SW620-KRAS-G12V-A59G-KI-1A2
15	KC-4912	NCI-H358-KRAS-G12C-H95C-KI	39	KC-5590	SW620-KRAS-G12V-A59G-KI-1C3
16	KC-4970	NCI-H358-KRAS-G12C-R68S-KI	40	KC-5923	Capan-2-KRAS-G12V-A59G-R68M-KI-1C2
17	KC-4971	NCI-H358-KRAS-G12C-R68S-KI	41	KC-5879	Capan-2-KRAS-G12V-A59G-KI-2A3
18	KC-4973	NCI-H358-KRAS-Q61H-KI	42	KC-5880	Capan-2-KRAS-G12V/A59G-A59G/E62K-KI-2C2
19	KC-4720	MIAPaCa2-KRAS-G12C-H95C-KI	43	KC-5829	Capan1-KRAS-G12V-A59G-KI
20	KC-4721	MIAPaCa2-KRAS-G12C-H95C-KI	44	KC-5830	Capan1-KRAS-G12V-A59G-KI
21	KC-4722	MIAPaCa2-KRAS-G12C-R68S-KI	45	KC-5376	A375-NRAS-Q61K-KI-1B1
22	KC-4723	MIAPaCa2-KRAS-G12C-R68S-KI	46	KC-5377	A375-NRAS-Q61K-KI-5B1
23	KC-5274	CT26-KRAS-V8M-G12C-KI 1C1	47	KC-5766	A375-NRAS-Q61R-KI
24	KC-5273	CT26-KRAS-V8M-G12C-KI 3B1	48	KC-5767	A375-NRAS-Q61R-KI

Schematic diagram for cell line generation



IC_{50} summary

KRAS-Knock in Lines and Benchmarking Data

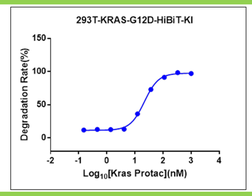
Cell ID	Cell line	Compound IC50(nM)				
		MRTX-1133	AMG510	MRTX-849	RMC6236	BI-2865
KC-0405-DW	AGS	4.24	6046	898.6	1.72	159
KC-3985-DW	AGS-KRAS-G12D-Y96D-KI	7453	10000	941	0.42	TBD
KC-4388	AGS-KRAS-G12D-H95D-KI	778.71	10000	783.43	2.22	TBD
KC-4415	AGS-KRAS-G12D-H95Q-KI	1539.69	10000	1088.98	2.38	TBD
KC-4416	AGS-KRAS-G12D-H95Q-KI	1365.05	10000	1034.26	1.51	TBD
KC-4631	AGS-KRAS-G12D-H95R-KI	729.63	10000	1164	2.36	10000
KC-4632	AGS-KRAS-G12D-H95R-KI	900.88	10000	1230	2.67	10000
KC-2053-DW	GP2d	4.08	10000	3192	1.55	352
KC-4844	GP2d-KRAS-G12D-Y96D-KI	4132	10000	3249	0.46	10000
KC-4902	GP2d-KRAS-G12D-Y96D-KI	3899	10000	2514	0.27	10000
KC-5104	GP2d-KRAS-E62Q-Y71H-KI	180.6	10000	2550	1.85	10000
KC-5170	GP2D-KRAS-E62Q-KI	1746	10000	2613	0.3	10000
KC-0166-DW	AsPC-1	44.56	10000	3839	3.01	1648
KC-4810	Aspc-1-KRAS-G12D-Y96D-KI	10000	10000	3546	0.19	10000
KC-4811	Aspc-1-KRAS-G12D-Y96D-KI(+/-)	10000	10000	3478	0.53	10000
KC-4843	Aspc-1-KRAS-G12D-Y96D-KI	10000	10000	4315	2.35	10000
KC-0509-DW	NCI-H358	405.3	6.82	12.16	0.64	387.4
KC-4911	NCI-H358-KRAS-G12C-H95C-KI	TBD	2.9	660	0.65	10000
KC-4912	NCI-H358-KRAS-G12C-H95C-KI	TBD	3.3	517.6	0.58	10000
KC-4970	NCI-H358-KRAS-G12C-R68S-KI	4172	202.7	375.8	7.39	10000
KC-4971	NCI-H358-KRAS-G12C-R68S-KI	4078	352.4	417.5	4.87	10000
KC-4973	NCI-H358-KRAS-Q61H-KI	309.5	10000	1433	2.09	104
KC-0302-DW	MIAPaCa2	533	9.49	12.42	0.41	201
KC-4720	MIAPaCa2-KRAS-G12C-H95C-KI	4942	7.92	1081	0.24	10000
KC-4721	MIAPaCa2-KRAS-G12C-H95C-KI	5505	5.19	1093	0.38	10000
KC-4722	MIAPaCa2-KRAS-G12C-R68S-KI	3716	2223.04	439.94	0.93	10000
KC-4723	MIAPaCa2-KRAS-G12C-R68S-KI	3926	418.37	295.16	1.27	10000
KC-0353	CT26	88.35	10000	1182	8.63	6811
	CT26-KRAS-M8V-KI 5A1	90.73	10000	1896	10.93	2067
KC-5274	CT26-KRAS-V8M-G12C-KI 1C1	9200	206.1	73.06	1.67	1685
KC-5273	CT26-KRAS-V8M-G12C-KI 3B1	5462	116.9	28.34	0.85	939.6
KC-5594	CT26-KRAS-V8M-G12A-KI-4B3	10000	10000	2173	21.79	2500
KC-5533	CT26-KRAS-V8M-G12A-KI-1C4	10000	10000	1750	23.64	2851
KC-5519	CT26-KRAS-V8M-G12A-G13D-KI-3A4	10000	10000	1146	26.16	4752
KC-5559	CT26-KRAS-V8M-G12S-KI-1A4	3072	10000	2176	7.81	2151
KC-5596	CT26-KRAS-V8M-G12S-KI-1C3	2656	10000	1329	6.05	1095
KC-5558	CT26-KRAS-V8M-G12R-KI-2B1	10000	10000	970	26.68	10000
KC-5595	CT26-KRAS-V8M-G12R-KI-2C3	10000	10000	1472	25.7	10000
KC-5637	CT26-KRAS-V8M-G12V-KI-3A1	10000	10000	2128	6.19	10000
KC-5534	CT26-KRAS-V8M-G12V-KI-2C2	10000	10000	809.8	4.59	10000
KC-5503	CT26-KRAS-V8M-G12C-KI-Abcb1a-KO-1C4	10000	171.7	66.85	1.91	10000
KC-5485	CT26-KRAS-V8M-G12C-KI-Abcb1a-KO-1B3	2148	56.58	10.72	0.59	594.2
KC-5635	CT26-KRAS-V8M-G12C-KI-Abcb1a-Abcb1a-KO-1B4	1833	89.5	34.57	0.95	1224
KC-5636	CT26-KRAS-V8M-G12C-KI-Abcb1a-Abcb1a-KO-1C2	635.3	40.05	19.01	0.92	602.9
KC-0314-DW	SW620	953.22	10000	TBD	0.35	250.77
KC-5589	SW620-KRAS-G12V-A59G-KI-1A2	1311.25	10000	TBD	12.7	10000
KC-5590	SW620-KRAS-G12V-A59G-KI-1C3	1316.04	10000	TBD	7.21	10000
KC-0185	Capan-2	1795	TBD	TBD	1.4	581
KC-5923	Capan-2-KRAS-G12V-A59G-R68M-KI-1C2	9180	TBD	TBD	15.88	10000
KC-5879	Capan-2-KRAS-G12V-A59G-KI-2A3	1592	TBD	TBD	23.26	875.4
KC-5880	Capan-2-KRAS-G12V/A59G-A59G/E62K-KI-2C2	10000	TBD	TBD	36.83	10000
KC-0335-DW	Capan1	2032	TBD	TBD	0.47	297
KC-5829	Capan1-KRAS-G12V-A59G-KI	1859	TBD	TBD	5.53	10000
KC-5830	Capan1-KRAS-G12V-A59G-KI	1918	TBD	TBD	7.45	10000

NRAS-Knock in Lines and Benchmarking Data

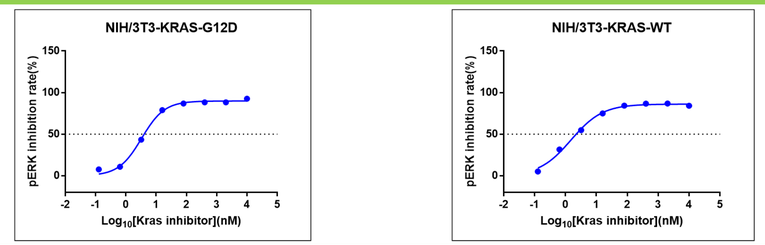
Cell ID	Cell line	Compound IC50(nM)						
		RMC-6236	BI-2865	AMG510	MRTX-849	Trametinib	Dabrafenib	Vemurafenib
KC-0158-DW	A375	10000	10000	10000	2484	0.69	2.58	96.26
KC-5376-DW	A375-NRAS-Q61K-KI-1B1	10000	10000	10000	2606	2.6	1350	10000
KC-5377-DW	A375-NRAS-Q61K-KI-5B1	10000	10000	10000	2521	1.81	533.2	10000
KC-5766	A375-NRAS-Q61R-KI	10000	10000	10000	1573	8.29	10000	10000
KC-5767	A375-NRAS-Q61R-KI	10000	10000	10000	1580	5.44	4957	10000

PROTAC degradation assay

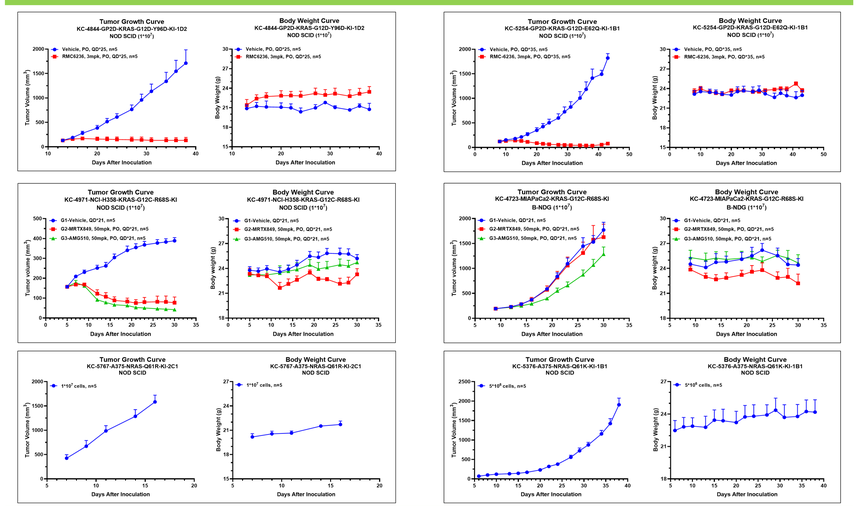
No.	ID	Cell Line name
1	KC-1049	293T-KRAS-G12D-HiBiT-KI
2	KC-1851	293T-KRAS-G12A-HiBiT-KI
3	KC-4021	HCT116-KRAS-G12D-HiBiT-KI
4	KC-4760	293T-KRAS-HiBiT-KI
5	KC-4022	HCT116-KRAS-G13D-HiBiT-KI
6	KC-1929	MIAPaCa2-KRAS-G12C-HiBiT-KI



Signal pathway assay by HTRF



In *vivo* growth Curve



Summary

- Ras mutation knock in cell lines that cover most hot kinases have been constructed, such as G12C and G12D mutation.
- Ras mutation knock in cell lines have been well validated by sequencing, and inhibitor tests.
- Ras mutation knock in cell lines are useful models for novel drug discovery *in vitro* and *in vivo*.

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