Preclinical investigation of orally bioavailable, potent KRAS^{Multi} inhibitor JAB-23425

Abstract #1660

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Background

- KRAS is the most frequently mutated oncogene and there has been an urgent and unmet need to target KRAS mutations in KRAS-driven cancer, such as KRAS^{G12D}, KRAS^{G12V} and KRAS^{G13D}.
- We have developed JAB-23425 as a highly potent and orally bioavailable KRAS^{Multi} inhibitor.
- JAB-23425 targets both “ON” and “OFF” states of KRAS, with good selectivity over HRAS and NRAS. JAB-23425 exhibited robust antitumor activities in preclinical models with multiple KRAS mutations or WT KRAS amplification, while sparing KRAS-independent cells.

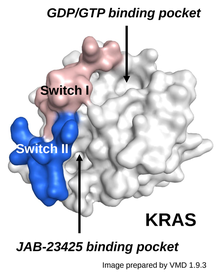


Figure 1. Binding surface of KRAS.

JAB-23425 is a potent and selective KRAS^{Multi} inhibitor

RAS	Nucleotide state	SPR, K_D (nM)	Biochemical assays, IC_{50} (nM)
KRAS G12C	GDP	ND	0.727
	GTP	ND	20.7
KRAS G12D	GDP	0.0220	0.501
	GTP	1.40	1.77
KRAS G12V	GDP	0.0141	0.344
	GTP	2.84	2.53
KRAS G13D	GDP	0.0374	0.756
	GTP	1.12	1.28
KRAS G12A	GDP	0.00997	0.281
	GTP	9.35	9.45
KRAS G12R	GDP	0.391	0.420
	GTP	3.52	1.84
KRAS Q61H	GDP	0.00869	0.602
	GTP	2.47	5.33
KRAS WT	GDP	0.0156	0.523
	GTP	4.66	6.94
HRAS WT	GDP	60.8	191
	GTP	>5000	>50000
NRAS WT	GDP	>40000	1004
	GTP	>40000	>50000

Table 1. JAB-23425 is a potent and selective KRAS^{Multi} inhibitor.
In the SPR assays, K_D values of JAB-23425 were determined by both “OFF” form of RAS (GDP state) and “ON” form of RAS (GTP state). In the biochemical assays, IC_{50} of JAB-23425 was determined by nucleotide exchange assays for “OFF” form of RAS (GDP state), and RAS::cRAF PPI assays for “ON” form of RAS (GTP state). We used a GTP analogue GppNp in both assays. ND, not detected.

JAB-23425 inhibits ERK phosphorylation and growth of KRAS-mutant cell lines

A

Cell line	Tissue of Origin	KRAS	JAB-23425 IC_{50} (nM)	
			pERK	3D Viability
GP2d	Colon	G12D	0.261	0.792
LS174T	Colon	G12D	0.630	35.4
LS513	Colon	G12D	0.848	5.34
A-427	Lung	G12D	0.814	7.62
Panc04.03	Pancreas	G12D	0.745	21.8
AsPC-1	Pancreas	G12D	0.918	3.82*
HPAF-II	Pancreas	G12D	0.896	5.39
KP-4	Pancreas	G12D	0.439	4.06
SW1990	Pancreas	G12D	0.549	0.934
HPAC	Pancreas	G12D	0.915	33.4
AGS	Stomach	G12D	0.335	0.497*
SW620	Colon	G12V	1.21	0.459*
SW403	Colon	G12V	1.39	37.0

Cell line	Tissue of Origin	KRAS	JAB-23425 IC_{50} (nM)	
			pERK	3D Viability
SW480	Colon	G12V	1.53	41.4
NCI-H441	Lung	G12V	1.62	5.92
Panc03.27	Pancreas	G12V	2.05	16.4
YAPC	Pancreas	G12V	1.07	9.11
Capan-1	Pancreas	G12V	4.42	11.3
Capan-2	Pancreas	G12V	2.01	3.11
RKN	Soft Tissue	G12V	2.94	0.755
NCI-H358	Lung	G12C	0.642	1.48
NCI-H1373	Lung	G12C	0.605	1.12
NCI-H1792	Lung	G12C	3.33	2.29
SW1573	Lung	G12C	0.340	14.8
NCI-H747	Colon	G13D	0.162	0.813
DLD-1	Colon	G13D	0.601	17.6
HCT-116	Colon	G13D	2.22	45.8
LoVo	Colon	G13D	0.918	1.62
NCI-H1944	Lung	G13D	1.40	15.5
MM.1S	Blood	G12A	ND	20.9
RERF-LC-Ad1	Lung	G12A	0.539	3.83
TCC-PAN2	Pancreas	G12R	12.8	74.0
A549	Lung	G12S	1.92	4.89
NCI-H460	Lung	Q61H	1.21	21.8
Calu-6	Lung	Q61K	28.3	82.1

B

Cell line	Tissue of Origin	KRAS	JAB-23425 IC_{50} (nM)	
			pERK	3D Viability
EBC-1	Lung	WT, Amplification	0.150	1.33
MKN-1	Stomach	WT, Amplification	1.83	3.17
SK-MEL-2	Skin	WT	>10000	>10000
KU-19-19	Bladder	WT	>10000	>10000
A375	Skin	WT	>10000	>10000
NCI-H2126	Lung	WT	456	587
NCI-H1666	Lung	WT	ND	4113
H9C2 (2-1)	Normal Rat Heart	WT	602	5937*
MRC-5	Normal Human Lung Fibroblast	WT	1763	9547*

Table 2. JAB-23425 inhibits the growth of KRAS-mutant/amplified cancer cells.
A. JAB-23425 IC_{50} data in KRAS-dependent cancer cell lines harboring multiple KRAS mutations, by pERK$^{T202/Y204}$ HTRF assays (2 h) and CTG viability assay (6 days). ND, not detected. * 2D CTG viability assay.
B. JAB-23425 IC_{50} data in KRAS WT cell lines with or without KRAS amplification, by pERK$^{T202/Y204}$ HTRF assay (2 h) and CTG viability assay (6 days). Viability of cancer cell lines having KRAS WT amplification (KRAS-dependent) was significantly inhibited by JAB-23425, while there is no obvious inhibitory effect of JAB-23425 on cancer or normal cell lines without KRAS mutation or amplification (KRAS-independent). SK-MEL-2 and KU-19-19 harbor NRAS^{Q61R} mutation, and A375 harbors BRAF^{V600E} mutation. ND, not detected. * 2D CTG viability assay.

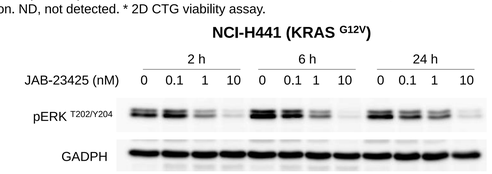
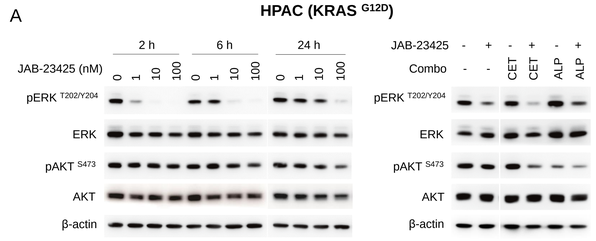


Figure 2. Western blot of NCI-H441 cell line. pERK$^{T202/Y204}$ was decreased by JAB-23425 treatment.

Combinational study of JAB-23425 *in vitro*



B

Cell line	KRAS	JAB-23425 Combination ZIP Synergy Scores			
		SOS1	EGFR	CDK4/6	PIK3CA
		BI-3406	cetuximab	palbociclib	alpelisib
LS513	G12D	11.7	14.0	4.05	7.05
HPAC	G12D	13.6	5.25	7.86	5.75
LS174T	G12D	13.6	9.71	7.79	8.65
AsPC-1	G12D	10.9	8.79	4.95	3.06
SW403	G12V	8.38	18.2	3.46	9.85
SW480	G12V	8.87	4.48	13.3	15.9
Capan-1	G12V	10.3	12.4	1.06	4.45
Panc03.27	G12V	7.64	12.4	ND	7.15
HCT-116	G13D	6.23	3.75	6.99	4.41
DLD-1	G13D	6.58	5.18	1.21	2.13

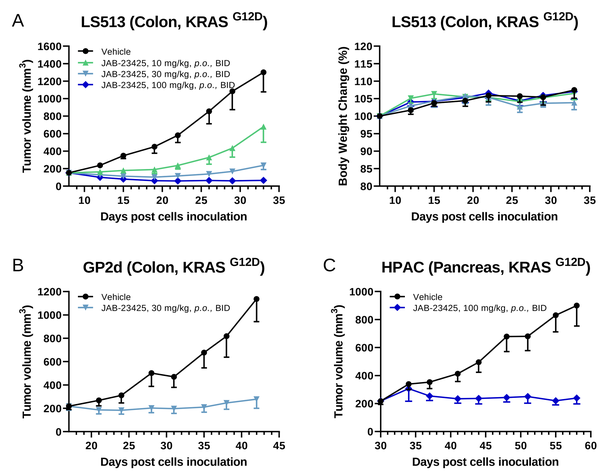
ZIP score	> 5	-5 to 5	Not Detected
	synergy	additivity	ND

Figure 3. *In vitro* efficacy of JAB-23435 in combination with other therapeutic drugs.
A. Western blot of HPAC cell line. Left panel shows inhibition of pERK$^{T202/Y204}$ by JAB-23425 at different time points. Right panel shows enhanced inhibition on pERK$^{T202/Y204}$ and/or pAKT^{S473} by combinational treatments with cetuximab or alpelisib for 24 h. The concentrations for JAB-23425, cetuximab (CET) and alpelisib (ALP) are 30 nM, 100 mg/L and 500 nM, respectively.
B. ZIP Synergy Scores of JAB-23425 combinational treatment *in vitro*. Cells were treated with either single agent or in combination for 6 days in 3D-format, then cell viability was determined by 3D CTG assay. ZIP synergy scores were generated by method described in reference 5. All inhibitors used for combination are purchased from commercial sources.

PK/PD correlation of JAB-23425

Figure 4. PK/PD correlation of JAB-23425.
A. JAB-23425 was administrated at single dose, 10, 30 and 100 mg/kg, *p.o.* in LS513 model. Plasma and tumor tissue were collected 2 hours post-dose to assess PD (pERK$^{T202/Y204}$) and PK (drug concentration).
B. JAB-23425 was administrated at single dose, 30 mg/kg, *p.o.* in LS513 model. Plasma and tumor tissue were collected at indicated time post-dose to assess PD (pERK$^{T202/Y204}$) and PK (drug concentration).

JAB-23425 has potent antitumor activity *in vivo*



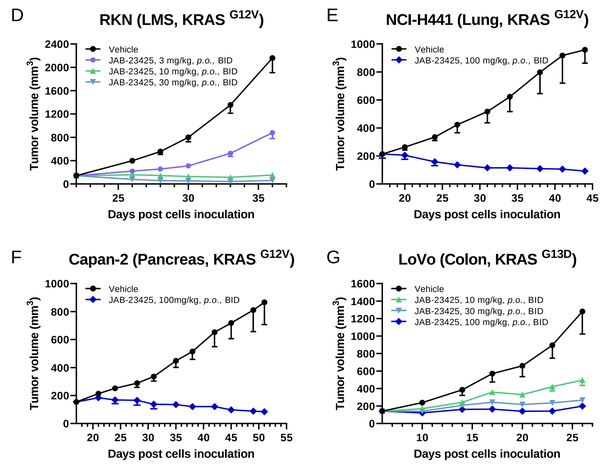


Figure 5. Antitumor activities of JAB-23425 as monotherapy *in vivo*.
A. Tumor volume (left panel) and body weight (right panel) change during the treatment of JAB-23425 at indicated doses, *p.o.*, BID in LS513 xenograft model.
B-G. Antitumor activity of JAB-23425 at indicated doses, *p.o.*, BID in multiple xenograft models bearing KRAS^{G12D}, KRAS^{G12V}, KRAS^{G13D}. 5-6 mice per group. LMS: Leiomyosarcoma.

JAB-23425 in combination with cetuximab results in enhanced anti-tumor effect

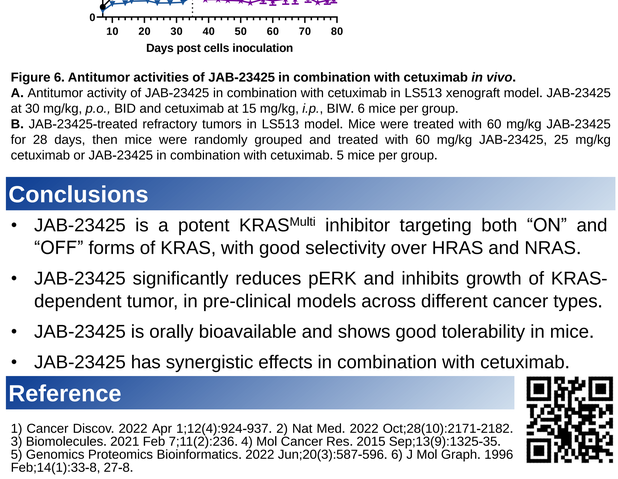


Figure 6. Antitumor activities of JAB-23425 in combination with cetuximab *in vivo*.
A. Antitumor activity of JAB-23425 in combination with cetuximab in LS513 xenograft model. JAB-23425 at 30 mg/kg, *p.o.*, BID and cetuximab at 15 mg/kg, *i.p.*, BIW. 6 mice per group.
B. JAB-23425-treated refractory tumors in LS513 model. Mice were treated with 60 mg/kg JAB-23425 for 28 days, then mice were randomly grouped and treated with 60 mg/kg JAB-23425, 25 mg/kg cetuximab or JAB-23425 in combination with cetuximab. 5 mice per group.

Conclusions

- JAB-23425 is a potent KRAS^{Multi} inhibitor targeting both “ON” and “OFF” forms of KRAS, with good selectivity over HRAS and NRAS.
- JAB-23425 significantly reduces pERK and inhibits growth of KRAS-dependent tumor, in pre-clinical models across different cancer types.
- JAB-23425 is orally bioavailable and shows good tolerability in mice.
- JAB-23425 has synergistic effects in combination with cetuximab.

Reference

1) Cancer Discov. 2022 Apr 1;12(4):924-937. 2) Nat Med. 2022 Oct;28(10):2171-2182. 3) Biomolecules. 2021 Feb 7;11(2):236. 4) Mol Cancer Res. 2015 Sep;13(9):1325-35. 5) Genomics Proteomics Bioinformatics. 2022 Jun;20(3):587-596. 6) J Mol Graph. 1996 Feb;14(1):33-8, 27-8.