Novel small molecule "ON" state inhibitor for GTP bound pan-KRAS (G12) mutations

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Summary

Introduction: KRAS is part of the RAS family of small GTPases. They act as molecular switches by cycling between an active GTP-bound (ON) state and an inactive GDP-bound (OFF) state in response to extracellular signals. KRAS is frequently mutated in nearly 30% of cancers, with the G12, G13, and Q61 hotspots being the most common [1]. Among these, G12 mutations account for 83% of KRAS mutations, being one of the most prevalent drivers of cancers [2]. Active KRAS triggers effector pathways like MAPK and PI3K, promoting cellular proliferation and survival. Oncogenic KRAS remains constitutively active due to defective GTP hydrolysis [3]. Therefore, inhibitors that target the "ON" state can effectively interrupt the signalling pathways that promote cancer cell growth and survival. Recently pan-KRAS tricomplex inhibitor, which inhibits KRAS ON state, has entered the clinic. Therefore, targeting the KRAS in ON state could be of high clinical value in cancers with defects in G12 mutations and in overcoming resistance. In this regard, we are developing a series of novel small molecule pan-KRAS ON state inhibitors.

Methods: Structure-based drug design followed by screening in the biochemical assays identified novel pan-KRAS (G12) mutant "ON" state inhibitors. Over 20 scaffolds were synthesized and screened in various assays. In vitro potency and structure-activity relationships were evaluated using AlphaLISA and Nucleotide exchange assays. Cell-based activity was measured by p-ERK levels and 2D/3D cell proliferation assays. PK-PD assessment was conducted in A427 lung cancer and AGS gastric cancer xenograft models in mice.

Results: In vitro Screening of JTX compounds

Sl. No	JTX Number	AlphaLISA IC_{50} (μM) G12D	G12C	G12R	WT	3D Viability, IC_{50} (μM) (72h, AGS)
1	JTX-101	5.2	5.1	4.6	ND	6.7
2	JTX-102	2.3	2.3	2.8	2.0	1.8
3	JTX-103	4.0	6.7	4.4	6.9	0.5
4	JTX-104	2.8	2.7	2.5	3.4	1.5
5	JTX-105	2.6	1.9	2.5	1.9	1.0
6	JTX-106	4.3	11.4	6.6	5.1	1.3
7	JTX-107	3.6	8.0	11.8	ND	ND
8	JTX-108	4.7	11.0	5.6	4.2	1.80
9	JTX-109	1.9	ND	ND	2.3	ND

Compounds were screened in RAS : cRAF PPI AlphaLISA assays for "ON" form of RAS (GTP-bound state). We used a GTP analogue GppNp in the assay; 3D CTG viability assays (72h) were performed in cancer cell lines harbouring multiple KRAS mutations – Data shown here is for AGS cell line (G12D); ND, not determined

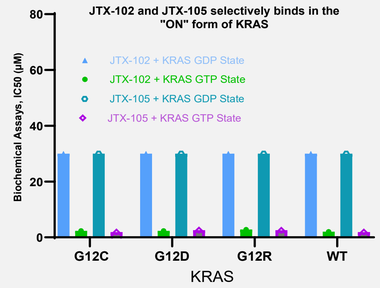
JTX compounds bind to the "ON" state of KRAS and effectively interrupt signalling pathways that promote cancer cell growth and survival.

JTX-102/JTX-105 selectively bind in the GTP state of KRAS

JTX compounds Bind to GTP state

RAS	Nucleotide State	Biochemical Assays, IC_{50} (μM) JTX-102	JTX-105	G12Di
KRAS G12C	GDP	>30	>30	0.040
	GTP	2.3	1.9	0.08
KRAS G12D	GDP	>30	>30	0.005
	GTP	2.3	2.6	0.03
KRAS G12R	GDP	>30	>30	0.010
	GTP	2.8	2.5	0.03
KRAS WT	GDP	>30	>30	0.007
	GTP	2.0	1.9	0.13

Graphical representation of GTP vs GDP – IC_{50}

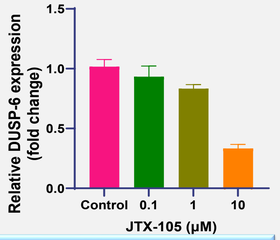


In the biochemical assays, IC_{50}s of JTX-102 and JTX-105 were determined by nucleotide exchange assays for "OFF" form of RAS (GDP state), and RAS: cRAF PPI AlphaLISA assays for "ON" form of RAS (GTP state). We used a GTP analogue GppNp in both assays. ND, not determined.

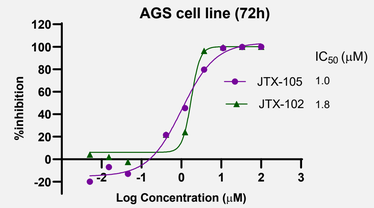
Anti-proliferative activity, p-ERK and DUSP6 inhibition

RAS	Cell line	Tissue Origin	3D Viability, IC_{50} (μM) JTX-102	JTX-105
KRAS G12C	Miapaca2	Pancreas	6.7	18.5
KRAS G12D	AGS	Stomach	1.8	1.0
KRAS G12R	PSN -1	Pancreas	16.9	15.1
KRAS WT	BXPC3	Pancreas	17.2	19.2

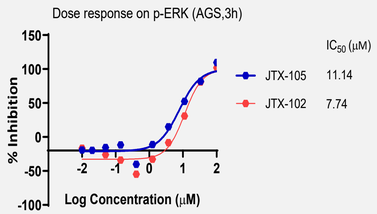
mRNA Modulation of DUSP6



3D cell viability assay



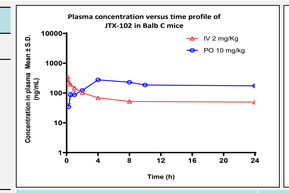
3D Spheroid p-ERK HTRF assay

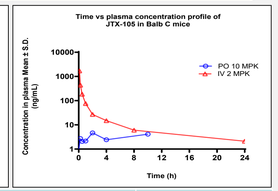


3D CTG viability assay (72h) were performed in KRAS-dependent cancer cell lines harbouring multiple KRAS mutations. The viability was assessed by Cell Titer Glo® ; AGS cells were treated with JTX-102 and JTX-105 at various doses for 3h and cellular p-ERK levels were measured using HTRF assays and mRNA levels of DUSP6* were assessed by qPCR. Data represented in all the graph is for AGS (G12D) cell line.
*JTX-102 data not shown

ADME and In vivo Pk Profiles of JTX-102 and JTX-105

Species	Compound	Mouse JTX-102	JTX-105
IV(1mg/kg)	t1/2 (h)	NRV	2.56
	Cmax (ng/mL)	348	1709
	AUC (h*ng/mL)	1579	947
	VZ(L/Kg)	28.9	23.3
	CL(mL/min/kg)	6.03	34.3
PO (5 mg/kg)	t1/2	NRV	NRV
	Cmax (ng/mL)	277	4.66
	AUC (h*ng/mL)	4516	32.1
	%F	57	0.68

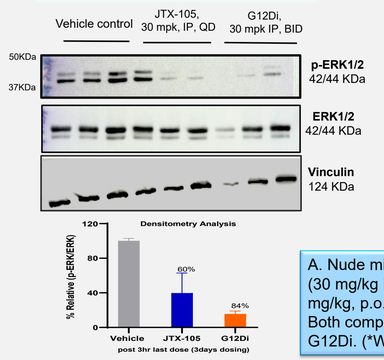


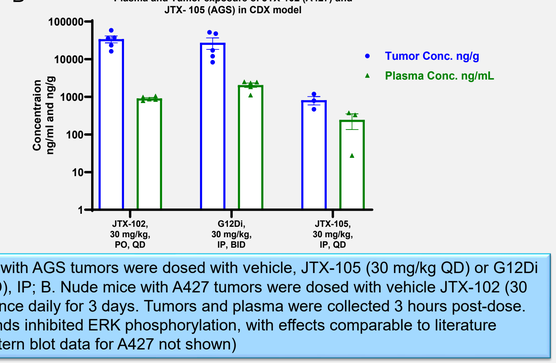


Liver Microsomes Stability (% remaining)	JTX-102	JTX-105
HLM	99	89
MLM	>99	90
RLM	98	ND

JTX- 102 and JTX-105 was formulated in 5:10:85 DMSO /solutol /saline for iv dosing and in 0.5% methylcellulose for po dosing. iv, *intravenous* dose ; po, *peros* dose. ND, not determined.

PK/PD of JTX-102 and JTX-105 in AGS/A427 Xenograft model





A. Nude mice with AGS tumors were dosed with vehicle, JTX-105 (30 mg/kg QD) or G12Di (30 mg/kg BID), IP; B. Nude mice with A427 tumors were dosed with vehicle JTX-102 (30 mg/kg, p.o.) once daily for 3 days. Tumors and plasma were collected 3 hours post-dose. Both compounds inhibited ERK phosphorylation, with effects comparable to literature G12Di. (*Western blot data for A427 not shown)

Conclusions

- JTX compounds inhibit wt and mutant K-RAS in RAS: cRAF PPI AlphaLISA assays in the "ON" form of K-RAS (GTP state).
- No inhibition is observed in the nucleotide exchange assays (NEA) in the "OFF" form of K-RAS (GDP state) up to concentrations of 30μM demonstrating highly selectivity binding towards the "ON" form of K-RAS
- These compounds exhibit good anti-proliferative activity in both K-RAS WT and K-RAS mutant cell lines and concentration-dependent reduction in p-ERK and DUSP6 levels in AGS (G12D) cells.
- JTX-102 and JTX-105 show strong inhibition of p-ERK level in tumor tissue in AGS xenograft model demonstrating pharmacodynamic effect
- JTX compounds show sustained exposure making it amenable for further in vivo profiling. Further Structure-activity relationship (SAR) exploration is on-going.

References & Acknowledgements

1. Vetter, I.R.; Wittinghofer, A. Science **2001**, 294, 1299–1304; Molina-Arcas, M.; Samani, A.; Downward, J. (2021). Genes **2021**, 12, 899; Moore, A.R.; et al. *Nature Reviews Drug Discovery* 2020;19: 533–52.

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