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Presented at the

American Association for Cancer Research Annual Meeting 2024

San Diego, CA, USA • April 5-10, 2024

INCB161734: A Novel, Potent, and Orally Bioavailable KRAS G12D Selective Inhibitor Demonstrates Antitumor Activity in *KRAS G12D* Mutant Tumors

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Abstract

KRAS G12D (G12D) is one of the most frequent oncogenic driver mutations and is especially common in pancreatic ductal adenocarcinoma (PDAC) and colorectal cancers (CRC). Patients with *G12D*-mutated disease experience poor treatment outcomes, representing a significant unmet medical need. *G12D* mutation results in constitutively active signaling, including hyperactivation of the ERK and PI3K pathways, which drive cell proliferation and survival. Here, we describe INCB161734, a novel, potent, selective, and orally bioavailable small-molecule G12D inhibitor that demonstrates in vivo efficacy in *G12D*-bearing tumor models.

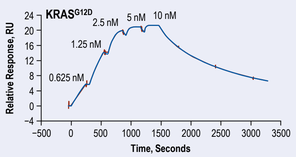
INCB161734 binds to both the GDP and GTP forms of the G12D mutant at the switch II pocket with picomolar affinity (K_D), and exhibits >80-fold selectivity over wildtype (WT) KRAS. INCB161734 potently inhibits SOS1-dependent GDP/GTP exchange activity in cell-free assays (IC_{50} <3 nM), and exhibits >40-fold selectivity for G12D vs WT KRAS. Additionally, INCB161734 demonstrates high selectivity for G12D over WT in various cellular assays using *G12D* mutant vs WT cancer-derived cell lines. INCB161734 potently inhibits ERK phosphorylation (a correlate for KRAS activity), with a mean IC_{50} of 14.3 nM (range: 1.9-45.2 nM) across 7 human and 3 mouse *G12D* cell lines; mean 21.5% inhibition was observed at 1 µM (maximum tested concentration) across 14 WT cell lines. Likewise, INCB161734 inhibits proliferation of *G12D* mutant cell lines, with a mean IC_{50} of 154 nM (range: 8.3-318 nM) across the same 7 human *G12D* cell lines; <30% inhibition (mean 13%) was observed at 1 µM across the same 14 WT cell lines. Treatment with INCB161734 induces caspase 3/7 cleavage in a PDAC cell line (*G12D* HPAC), with EC_{50} <100 nM; caspase 3/7 cleavage in a WT cell line (NCI-H838) was not induced at doses as high as 5 µM. In addition, INCB161734 induced cell-cycle arrest (S-phase inhibition) in the *G12D* HPAC cell line, with IC_{50} 12.8 nM; S-phase inhibition in the WT NCI-H838 cell line only occurred at much higher doses (IC_{50} >3.3 µM).

INCB161734 demonstrates excellent oral bioavailability with good absorption, low clearance, and low metabolic turnover. Orally dosed INCB161734 clearly shows target engagement, generating continuous near-maximal KRAS inhibition in *G12D* HPAC mouse xenograft tumors for almost the entire dosing interval. INCB161734 is efficacious against multiple types of *G12D*-mutated tumors and xenografts, resulting in significant tumor growth inhibition, growth arrest and/or regression in multiple PDAC (HPAC, Panc 04.03, and 2838c3) and CRC (CT26, GP2D, and LS513) mouse tumor models.

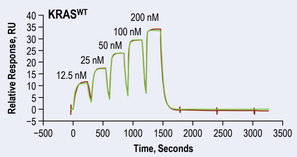
These preclinical results demonstrate that INCB161734 is a potent, selective, and orally bioavailable KRAS G12D inhibitor, strongly efficacious against *KRAS G12D* mutant tumors. The potential benefit of INCB161734 for patients with *KRAS G12D* mutant disease is under investigation in an ongoing clinical trial (NCT06179160).

$KRAS^{G12D}$ Activation Potently and Selectively Inhibited by INCB161734 Binding

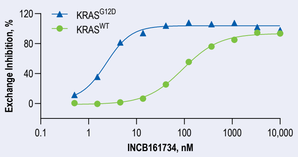
A KRAS^{G12D} SPR Assay: K_D 0.36 nM



B KRAS^{WT} SPR Assay: K_D 29 nM



C GDP/GTP Exchange Assay



D Biochemical Analysis

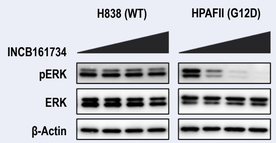
Assay	KRAS Protein	K_D, nM	Fold Selectivity vs WT
SPR	KRAS^{G12D} (GDP)	0.38	75×
	KRAS^{G12D} (GTP)	0.32	90×
	KRAS^{WT} (GDP)	28.7	-
Assay	**KRAS Protein**	**IC_{50}, nM**	**Fold Selectivity vs WT**
SOS1-dependent GDP/GTP exchange	KRAS^{G12D}	2.8	40×
	KRAS^{WT}	112	-

GDP, guanosine diphosphate; GTP, guanosine triphosphate; IC_{50}, half-maximal inhibitory concentration; K_D, equilibrium dissociation constant; RU, relative unit; SOS1, son of sevenless homolog 1; SPR, surface plasmon resonance; WT, wildtype.

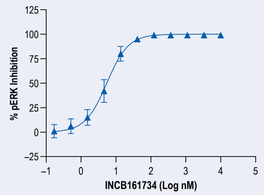
- INCB161734 is highly selective for KRAS^{G12D} over KRAS^{WT} in cell-free biochemical assays. **A** and **B)** INCB161734 demonstrates strong binding to KRAS^{G12D} but not KRAS^{WT} in SPR assay, with the relative binding constants (K_D) reflecting 80× selectivity for KRAS^{G12D} over KRAS^{WT}. **C)** INCB161734 potently inhibits SOS1-mediated GDP/GTP exchange of KRAS^{G12D} but not KRAS^{WT}, with the relative IC_{50} reflecting 40× selectivity for KRAS^{G12D} over KRAS^{WT}. **D)** INCB161734 potently binds to both the GTP-bound and GDP-bound forms of KRAS^{G12D}
- INCB161734 demonstrates strong and selective KRAS^{G12D} binding
- INCB161734 binding to KRAS^{G12D} strongly impairs GDP → GTP exchange, thereby inhibiting KRAS^{G12D} activity

INCB161734 Potently and Selectively Inhibits KRAS^{G12D} Downstream Signaling in Cells

A Selective ERK Signaling Inhibition



B pERK Inhibition in HPAFII (PDAC)



C INCB161734 pERK Inhibition in Cell Lines

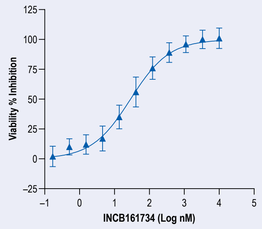
Cell Line	Origin	pERK (IC_{50}), nM
A427	Human NSCLC	23
AsPC1	Human PDAC	7
GP2D	Human CRC	2
HPAC	Human PDAC	10
HPAFII	Human PDAC	4
LS513	Human CRC	13
Panc 04.03	Human PDAC	19
CT26	Mouse CRC	45
KPCY 005	Mouse PDAC	9
KPCY 013	Mouse PDAC	11
KRAS^{WT} human cancer-derived cell line panel	pERK IC_{50} (median)	>1 µM
	Mean inhibition at 1 µM	21.57%

CRC, colorectal cancer; IC_{50}, half-maximal inhibitory concentration; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; pERK, phosphorylated ERK; WT, wildtype.

- INCB161734 potently inhibits ERK phosphorylation in *KRAS^{G12D}* but not *KRAS^{WT}* cells. **A)** H838 (*KRAS^{WT}*) and HPAFII (*KRAS^{G12D}*) cells were cultured in increasing concentrations of INCB161734 and pERK, ERK, and β-actin levels were assessed by immunoblot. **B)** Quantitative assessments of pERK expression and inhibition were conducted via HTRF (homogeneous time resolved fluorescence) assay. **C)** Summary data from analysis of pERK inhibition in 10 *KRAS^{G12D}* cancer-derived cell lines (7 human, 3 mouse) and 14 *KRAS^{WT}* human cancer-derived cell lines (*KRAS^{WT}* panel consisted of HepG2, Hs578T, Hs852T, KMM1, NCI-H661, NCI-H838, P31JUF, PA1, RD, RL952, SKMEL2, SW48, T24, and TYKNU cell lines)
- INCB161734 potently and selectively inhibits signaling downstream of KRAS^{G12D} but not KRAS^{WT} in cancer-derived cell lines in vitro

INCB161734 Inhibits Cell Cycle and Proliferation, and Induces Caspase Cleavage in *KRAS^{G12D}* Cells

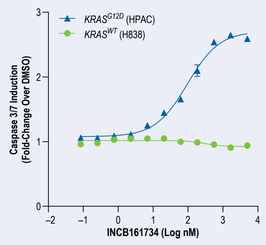
A Viability Inhibition in HPAFII



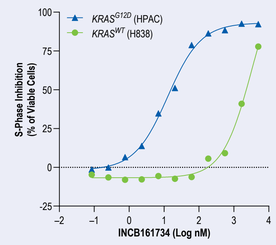
B Proliferation/Viability

Cell Line	Origin	Viability (IC_{50}), nM
A427	Human NSCLC	318
AsPC1	Human PDAC	97
GP2D	Human CRC	8
HPAC	Human PDAC	154
HPAFII	Human PDAC	150
LS513	Human CRC	156
Panc 04.03	Human PDAC	196
KRAS^{WT} human cancer-derived cell line panel	Viability IC_{50} (median)	>1 µM
	Mean inhibition at 1 µM	13.09%

C Caspase 3/7 Cleavage



D Cell-Cycle Disruption (S-Phase Inhibition)

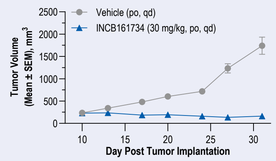


CRC, colorectal cancer; DMSO, dimethyl sulfoxide; IC_{50}, half-maximal inhibitory concentration; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; WT, wildtype.

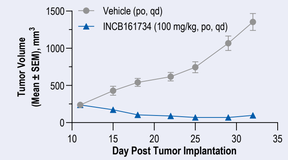
- INCB161734 potently inhibits cellular viability of *KRAS^{G12D}* but not *KRAS^{WT}* cells. **A)** Cell viability was assessed using CellTiter-Glo Luminescent assays. **B)** Summary data from analysis of cell viability inhibition in 7 *KRAS^{G12D}* human cancer-derived cell lines and 14 *KRAS^{WT}* human cancer-derived cell lines (*KRAS^{WT}* panel consisted of HepG2, Hs578T, Hs852T, KMM1, NCI-H661, NCI-H838, P31JUF, PA1, RD, RL952, SKMEL2, SW48, T24, and TYKNU cell lines). **C)** Caspase 3/7 cleavage was quantified using a luminescence-based assay. **D)** Cell-cycle changes were quantified using a flow cytometry-based assay
- INCB161734 potently decreases the viability of *KRAS^{G12D}* cancer cell lines while inducing caspase cleavage and cell-cycle arrest

Oral INCB161734 Demonstrates Strong Antitumor Activity in Preclinical Tumor Models

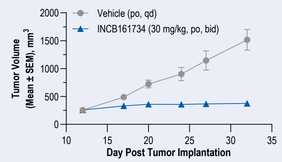
A Panc 04.03 *KRAS^{G12D}* (PDAC)



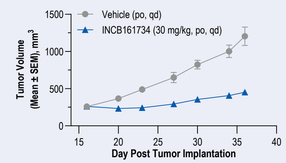
B LS513 *KRAS^{G12D}* (CRC)



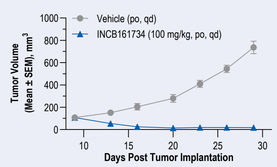
C HPAC *KRAS^{G12D}* (PDAC)



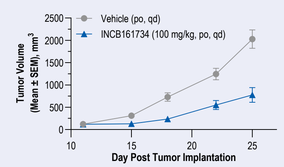
D GP2D *KRAS^{G12D}* (CRC)



E KPCY 2838c3 *KRAS^{G12D}* (PDAC)



F CT26 Clone 299 *KRAS^{G12D}* (CRC)

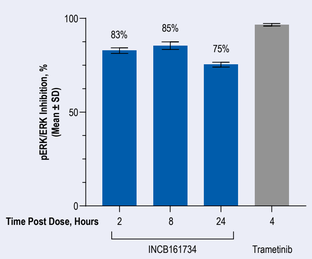


N=8 (A, D) or N=10 (B, C, E, F) mice per group. bid, twice daily; CRC, colorectal carcinoma; PDAC, pancreatic ductal adenocarcinoma; po, orally; qd, once daily; SEM, standard error of mean.

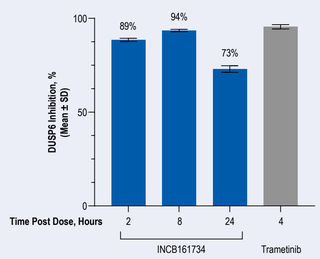
- INCB161734 demonstrates strong antitumor activity when orally administered to immunocompromised mice bearing *KRAS^{G12D}* xenografts (A-D) or syngeneic *KRAS^{G12D}* murine tumors (E-F). **A)** Panc 04.03 xenograft, **B)** LS513 xenograft (CRC), **C)** HPAC xenograft (PDAC), **D)** GP2D xenograft (CRC), **E)** KPCY 2838c3 syngeneic tumor (PDAC), **F)** CT26 clone 299 syngeneic tumor (CRC)
- Orally dosed INCB161734 is efficacious against *KRAS^{G12D}*-mutated tumors and xenografts in preclinical mouse models
- INCB161734 was well tolerated at all tested doses in these in vivo studies

A Single Oral Dose of INCB161734 Inhibits KRAS Signaling in *KRAS^{G12D}* Tumors Through 24 Hours

A ERK Phosphorylation



B *DUSP6* mRNA Expression



N=4 mice per group. *DUSP6*, dual specificity phosphatase 6; pERK, phosphorylated ERK; SD, standard deviation.

- INCB161734 potently inhibits ERK pathway activity in tumor tissue from mice bearing *KRAS^{G12D}* xenografts. Tumor-bearing animals were given a single oral dose of INCB161734 (100 mg/kg) or vehicle control. The MEK inhibitor trametinib (6 mg/kg) was used as a positive control. Tumor tissue was collected over the following 24 hours to assess **A)** ERK phosphorylation (via Meso-Scale Discovery ERK Activation Assay) and **B)** *DUSP6* mRNA expression (via TaqMan qPCR)
- One oral dose of INCB161734 generates near-maximal inhibition of KRAS^{G12D}-mediated ERK signaling over a 24-hour assessment period post dose

Conclusions

- ***KRAS^{G12D}* represents one of the most frequent driver mutations in cancer. Effective treatments for this mutation represent a significant unmet need, particularly in pancreatic and colorectal cancer**
- **INCB161734 is a novel, potent, selective, and orally bioavailable *KRAS^{G12D}* small-molecule inhibitor**
  - **Demonstrates a substantial selectivity over KRAS^{WT} in biochemical and cellular assays**
  - **Potent binding to KRAS^{G12D} in biochemical (cell-free) assays**
  - **Inhibits KRAS^{G12D} signaling in cancer-derived cells in vitro**
  - **Disrupts the cell cycle and induces caspase cleavage in *KRAS^{G12D}* but not *KRAS^{WT}* cancer cell lines**
  - **Potent and efficacious in preclinical animal models as an oral agent**
    - **Inhibits KRAS signaling in *KRAS^{G12D}* xenograft tumors**
    - **Generates substantial antitumor activity in *KRAS^{G12D}* xenograft and syngeneic tumors**
- **INCB161734 demonstrates excellent oral bioavailability in higher preclinical species**
- **A phase 1 clinical trial testing INCB161734 for patients with advanced or metastatic *KRAS^{G12D}* mutant solid tumors is currently ongoing (NCT06179160)**

Disclosures

Farren, Roman, Gallion, Allali-Hassani, Sokolsky, Kong, Smith, Wang, Correa, Deller, Epling, Procak, Zhang, Pecko, Kennedy, Boer, Kurzeja-Lipinski, Covington, Chen, Wallower, Rocha, Pan, Perry, Yuska, Wang, Macarron, Kim: Employment and stock ownership – *Incyte Corporation*.

Acknowledgments

All experiments carried out with funding by Incyte Corporation, Wilmington, DE, USA. Authors would like to thank all colleagues who contributed to this research effort. Editorial and graphics support was provided by Envision Pharma Group (Fairfield, CT, USA) and funded by Incyte Corporation.

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