Preclinical studies of RP04340: a potent and orally available PROTAC compound targeting KRAS G12C/D/V mutant tumors

AACR2025 #1662

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ABSTRACT

Mutations in the RAS oncogene are highly prevalent in pancreatic ductal adenocarcinoma (PDAC), colorectal cancer (CRC), and lung adenocarcinoma (LUAD), with codon 12 of the *KRAS* gene being the most frequently altered site. Substitutions of glycine at codon 12 to cysteine (G12C), glutamate (G12D), or valine (G12V) occur in 66.3%, 23.7%, and 24.4% of PDAC, CRC, and LUAD patients, respectively. While recently approved drugs targeting KRAS G12C mutation have shown clinical benefit, there remains a significant unmet need for therapies addressing the highly prevalent G12D and G12V mutations.

Proteolysis-targeting chimeras (PROTACs) represent a novel therapeutic strategy for the targeted degradation of oncogenic proteins, potentially offering superior efficacy and mitigating resistance. In this study, we present RP04340, a potent and orally bioavailable PROTAC compound that selectively degrades KRAS G12C/D/V proteins and demonstrates robust anti-tumor activity.

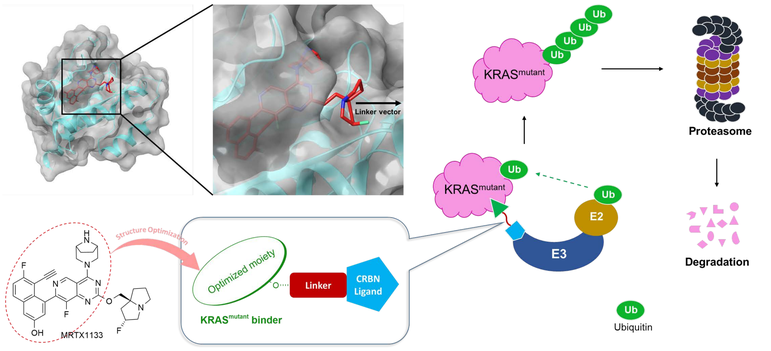
RP04340 induced significant degradation of KRAS G12D, KRAS G12C, and KRAS G12V proteins in multiple cancer cell lines harboring these mutations, with a 50% degradation concentration (DC_{50}) in the low nanomolar range. Within 12 hours of treatment, RP04340 reduced KRAS G12C/D/V protein levels by over 80% and effectively suppressed downstream signaling pathways, including pERK and DUSP6, followed with a significant suppression of cell proliferation.

In vivo studies using mouse xenograft models demonstrated significant tumor growth inhibition following daily oral administration of RP04340 at doses between 6.25 and 100 mg/kg, with a clear dose-response relationship. Pharmacokinetic analyses revealed that RP04340 accumulated preferentially in tumor tissues, achieving high intratumoral concentrations with repeated dosing. Furthermore, tissue distribution studies in mouse showed significant accumulation of RP04340 in the lungs and pancreas after consecutive dosing, supporting its suitability for treating cancers in these organs.

RESULTS

1. Targeting KRAS protein via PROTACs

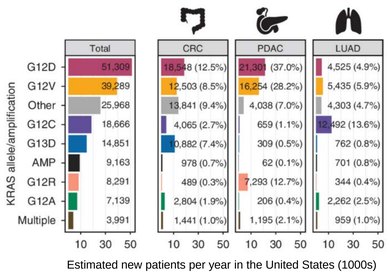
Design of KRAS$^{G12C/D/V}$ PROTACs and MOA of PROTAC mediated degradation



Advantages of KRAS PROTAC

- PROTACs function catalytically, enabling substoichiometric degradation of target proteins.
- Achieving KRAS degradation may translate to long-lasting suppression of oncogenic signaling, resulting in durable therapeutic benefits.
- KRAS degradation can disrupt critical multi-protein complexes on the cell membrane, essential for oncogenic signaling and cellular function.
- The degradation of oncogenic KRAS could lead to favorable alterations in the tumor microenvironment, fostering a more immune-permissive state.

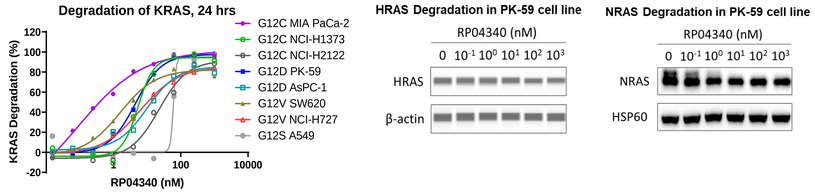
Prevalence of KRAS mutation in cancers

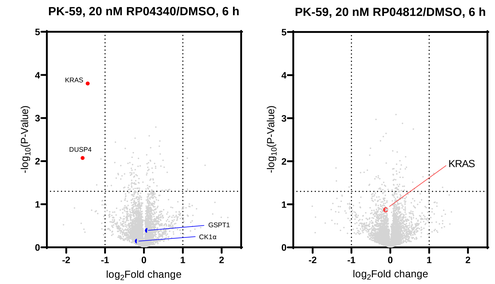


Cancer Discov. 2022 Apr 1;12(4):924-937.

2. RP04340 is a potent KRAS$^{G12C/D/V}$ PROTAC

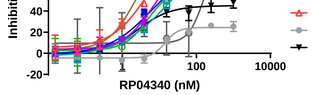
RP04340 is a KRAS degrader with no activity against HRAS or NRAS





- RP04340 exhibits selective degradation of KRAS, leading to significant inhibition of the downstream effector DUSP4. Notably, it does not promote the degradation of prevalent CRBN-recruited neo-substrates, including GSPT1 and CK1*α*.
- RP04812 features an *N*-methylation on the CRBN ligand structure of RP04340. This modification abolishes RP04812's ability to recruit CRBN, consequently preventing KRAS degradation.

High degradation activity results in potent inhibition of tumor cell proliferation

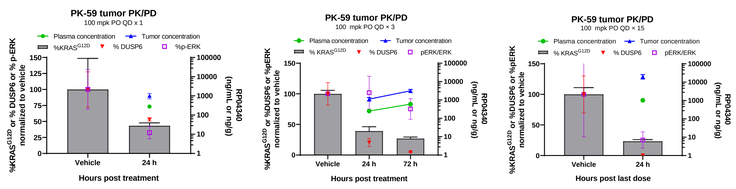


Cell Line	KRAS Mutation	Histology	RP04340		
			DC_{50} (nM)	D_{max} (%)	IC_{50} (nM)
PK-59	G12D	Pancreas	4.7	96.4	11.5
AsPC-1	G12D	Pancreas	13.5	89.5	20.9
MIA PaCa-2	G12C	Pancreas	2	97	8.8
NCI-H1373	G12C	Lung	5.9	98	13.6
NCI-H2122	G12C	Lung	30.4	88.3	154
SW620	G12V	Colon	2.9	83.2	2.3
NCI-H727	G12V	Lung	13.1	82.6	3.7
NCI-H1573	G12A	Lung	NT	NT	>1000
PSN-1	G12R	Pancreas	NT	NT	>1000
A549	G12S	Lung	61.5	98	>1000
TOV-21G	G13C	Ovary	NT	NT	>1000
HCT-116	G13D	Colon	NT	NT	>1000
NCI-H460	Q61H	Lung	NT	NT	>1000
MKN1	WT	Gastric	0.3	98.5	>1000

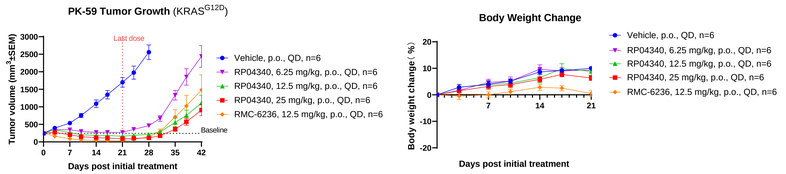
NT: not tested

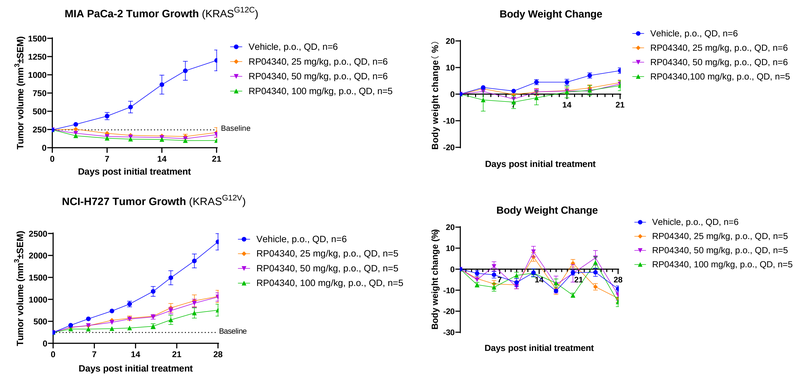
3. RP04340 exhibits tumor growth inhibition in preclinical models

Dose-dependent KRAS degradation by RP04340 *in vivo* leads to significant suppression of downstream signaling cascades



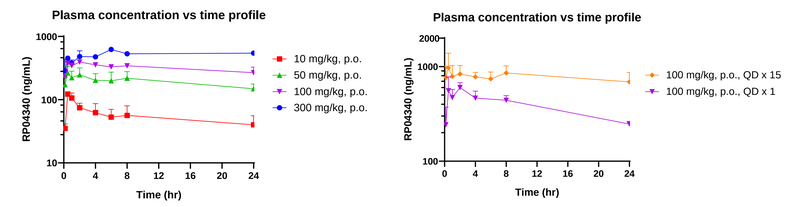
Oral RP04340 suppresses tumor growth and shows sustained anti-tumor efficacy



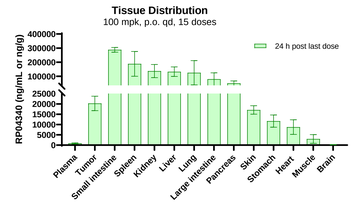


4. RP04340 possesses favorable drug-like properties

Oral RP04340 in mice shows dose- and frequency-dependent pharmacokinetic exposure



Preferential tumor tissue accumulation



Favorable drug-like properties

Assay	Value
PPB (%)	97.7 (m), 93.8 (h)
PS $T_{1/2}$ (min)	>289 (m), >289 (r)
MMS(CLint (liver) (mL/min/kg)	<37.9 (m), <17.2 (r), <9.56 (h)
CYP450 Inhibition @10 μM (%) 1A2/2B6/2C8/2C9/2C19/2D6/3A4-M/3A4-T	9.61/33.2/40.2/16.9/33.1/25.6/19.7/14.1
hERG Inhibiton @ 10 μM (%)	12.04

SUMMARY

- RP04340, a novel orally bioavailable PROTAC, selectively degrades KRAS G12C/D/V mutant proteins, achieving low nanomolar DC_{50} values in KRAS-mutant cell lines and degrading >80% of target proteins. RP04340 suppresses downstream signaling (pERK, DUSP6) and significantly inhibits tumor cell proliferation.
- In xenograft models, RP04340 demonstrates dose-dependent tumor growth inhibition (6.25 – 100 mg/kg, oral dosing) with significant efficacy across all tested cancer types. It exhibits preferential tumor tissue accumulation and achieves high intratumoral concentrations. RP04340 also shows enrichment in lung and pancreas tissues after repeated dosing — consistent with target organ tropism.
- RP04340 demonstrates robust preclinical efficacy and favorable pharmacokinetic features, positioning it as a promising therapeutic candidate for KRAS G12C/D/V-driven cancers.