Targeting KRAS^{G12D} mutant tumors with the PROTAC degrader RP03707

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Abstract #: 6050

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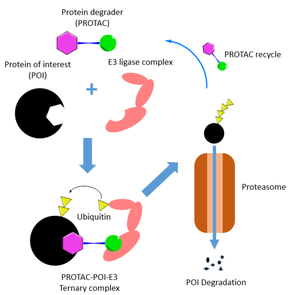
ABSTRACT

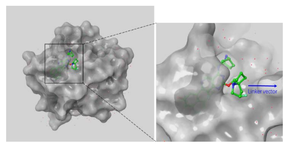
RAS oncogene mutations are prevalent in approximately 19% of cancer patients, with the most frequent alteration occurring in codon 12 of the KRAS gene, resulting in a variety of G12X oncoproteins. Recent advances in drug discovery have yielded several KRAS^{G12C} inhibitors currently in clinical use, benefiting a subset of patients. However, addressing the high-prevalence G12D mutation remains a substantial unmet medical need. Targeted protein degradation using PROTAC molecules offers a promising approach for treating KRAS^{G12D}-associated tumors, potentially providing superior efficacy and mitigating the development of resistance, a challenge frequently observed with KRAS^{G12C} inhibitors in clinical settings. In the present study, we report a PROTAC compound RP03707 that efficiently induces the degradation of the KRAS^{G12D} mutant protein and inhibits tumor growth. Treatment of AsPC-1 cells with RP03707 results in significant degradation of the KRAS^{G12D} protein, with a DC50 value in the sub-nanomolar range. Within 24 hours, the compound eliminates over 90% of G12D proteins and effectively suppresses downstream cellular MAPK signaling. Additional *in vitro* experiments demonstrate that RP03707 inhibits cell proliferation in multiple KRAS^{G12D} mutant cell lines, surpassing the anti-tumor efficacy of enzyme inhibitors. In a mouse GP2d xenograft tumor model, a single intravenous administration of RP03707 at 10 mpk results in excellent compound penetration and retention in tumor tissues, followed by 90% reduction of G12D protein levels for 7 days. Profound inhibition of tumor growth is observed not only in mouse GP2d xenograft but also in other mouse KRAS^{G12D} tumor models, even when the compound is administered in low and infrequent doses. Moreover, RP03707 exhibits high selectivity for degrading the KRAS^{G12D} protein and possesses favorable drug-like properties. RP03707, therefore, meets the criteria for advancing into drug development and represents a valuable therapeutic option for treating KRAS^{G12D}-associated tumors.

Mechanism of Action and Results

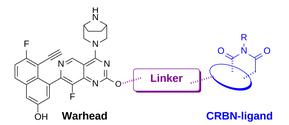
MoA of PROTAC mediated protein degradation



Design of RP03707 and RP03990



doi.org/10.1021/acs.jmedchem.1c01688

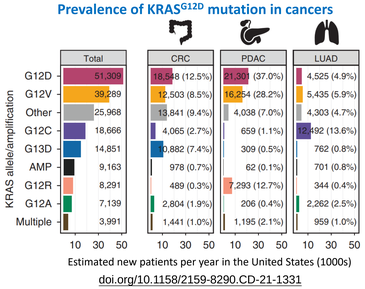


RP03707: R=H; **RP03990** (CRBN non-binder control): R=CH3

Advantages of PROTAC

- Is able to degrade the "undruggable" protein targets
- Has high potency due to the "catalytic" activity
- Ameliorates the development of drug resistance
- Eliminates both the enzymatic and scaffolding functions of pathogenic protein

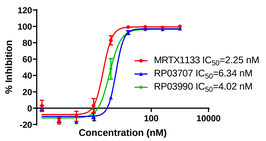
Prevalence of KRAS^{G12D} mutation in cancers



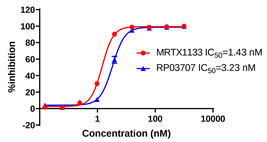
doi.org/10.1158/2159-8290.CD-21-1331

1. RP03707 binds to both the inactive and active forms of KRAS^{G12D} and inhibits downstream MAPK signaling

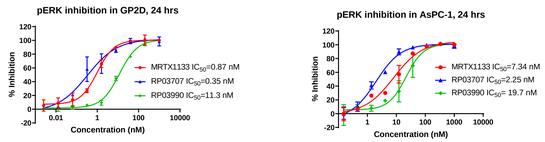
Binds KRAS^{G12D}-GDP and blocks GDP-GTP exchange



Binds KRAS^{G12D}-GTP and inhibits the interaction with Raf

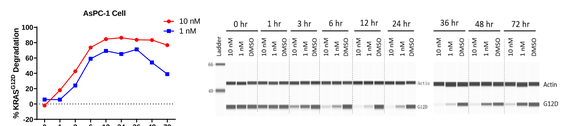


MAPK pathway in GP2D and AsPC-1 cells is potently suppressed by RP03707

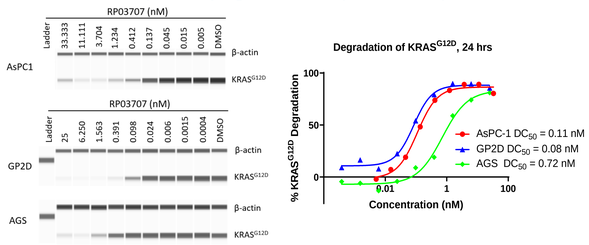


2. RP03707 induces the degradation of KRAS^{G12D} in a proteasome function dependent manner

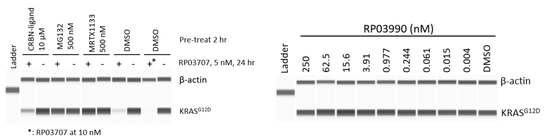
RP03707 induces a quick degradation of KRAS^{G12D} protein in AsPC1 cells



RP03707 profoundly degrades KRAS^{G12D} protein in multiple cell lines



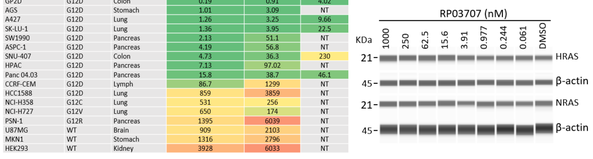
Induced degradation of KRAS^{G12D} is mediated by cellular UPS system



3. RP03707 preferentially inhibits the growth of tumor cells harboring KRAS^{G12D} mutation

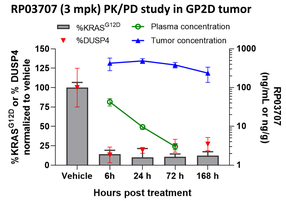
Cell Line	KRAS Mutation	Histology	2D 3 Day Proliferation IC_{50} (nM)		
			RP03707	MRTX1133	RP03990
GP2D	G12D	Colon	0.19	0.91	4.02
AGS	G12D	Stomach	1.01	3.09	NT
A427	G12D	Lung	1.26	3.25	9.66
SK-LU-1	G12D	Lung	1.36	3.95	22.5
SW1990	G12D	Pancreas	2.13	51.1	NT
ASPC-1	G12D	Pancreas	4.19	56.8	NT
SNU-407	G12D	Colon	4.73	36.3	230
HPAC	G12D	Pancreas	7.13	97.02	NT
Panc 04.03	G12D	Pancreas	15.8	38.7	46.1
CORE-CEM	G12D	Lymph	86.7	1299	NT
HCC1588	G12D	Lung	859	3859	NT
NCI-H358	G12C	Lung	531	256	NT
NCI-H727	G12V	Lung	650	174	NT
PSN-1	G12R	Pancreas	1395	6039	NT
U87MG	WT	Brain	909	2103	NT
MKN1	WT	Stomach	1316	3796	NT
HEK293	WT	Kidney	3928	6033	NT

RP03707 does not cause the degradation of HRAS and NRAS

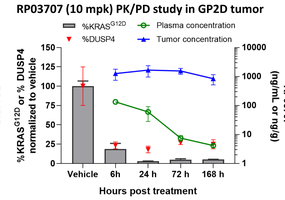


4. Single dosing of RP03707 results in drug accumulation in tumor tissues for more than 7 days

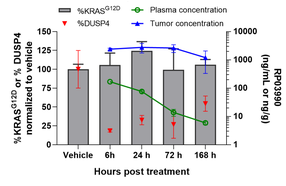
RP03707 (3 mpk) PK/PD study in GP2D tumor



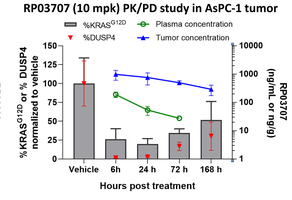
RP03707 (10 mpk) PK/PD study in GP2D tumor



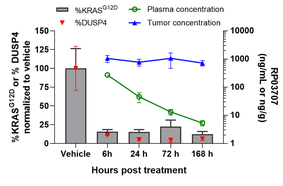
RP03990 (10 mpk) PK/PD study in GP2D tumor



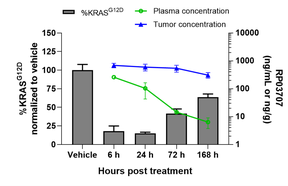
RP03707 (10 mpk) PK/PD study in AsPC-1 tumor



RP03707 (10 mpk) PK/PD study in PK59 tumor

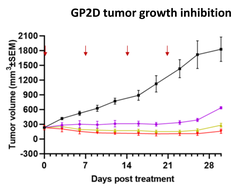


RP03707 (10 mpk) PK/PD study in SNU407 tumor

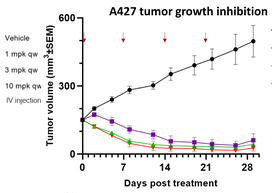


5. Infrequent iv injection of RP03707 suppresses tumor growth in multiple KRAS^{G12D} CDX models

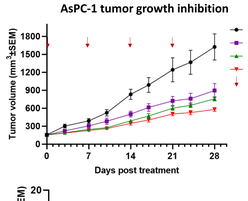
GP2D tumor growth inhibition



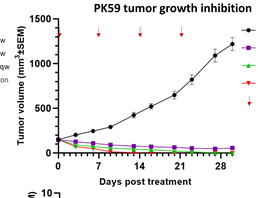
A427 tumor growth inhibition



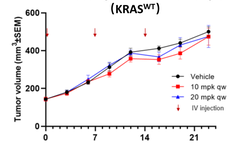
AsPC-1 tumor growth inhibition



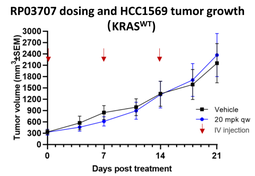
PK59 tumor growth inhibition



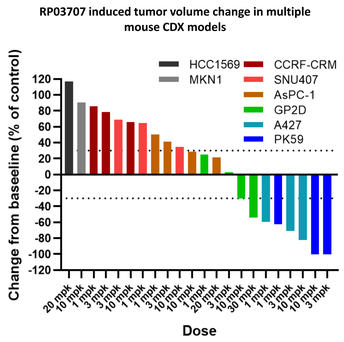
RP03707 dosing and MKN1 tumor growth (KRAS^{WT})



RP03707 dosing and HCC1569 tumor growth (KRAS^{WT})

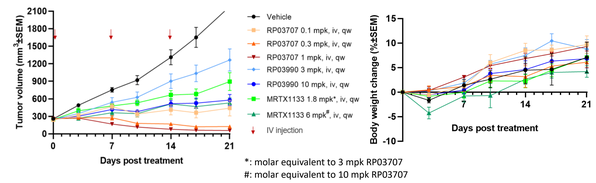


RP03707 induced tumor volume change in multiple mouse CDX models



6. RP03707, as a KRAS^{G12D} degrader, demonstrates superior efficacy over enzymatic inhibitors in suppressing tumor growth

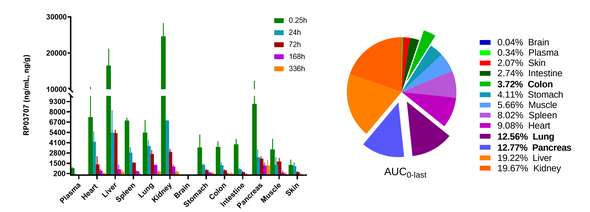
Efficacy comparison between RP03707, RP03990 and MRTX1133 in PK59 tumor



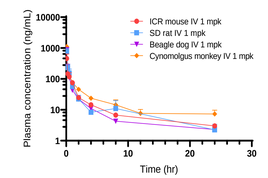
*: molar equivalent to 3 mpk RP03707
#: molar equivalent to 10 mpk RP03707

7. PK and ADME studies of RP03707 support the notion of drug development

Tissue distribution of RP03707 in mouse



PK in mouse, rat, dog and monkey



Drug like properties

Physicochemical / DMPK / Safety	RP03707
Aq. Solubility (μg/ml)	100.3
PPB% (m/r/d/cy/h)	95.3/95.7/95.0/95.3/97.8
Plasma stability ($T_{1/2}$ min, m/r/d/cy/h)	533/770/433/770/990
Microsomal stability ($T_{1/2}$ min, m/r/d/cy/h)	433/151/433/433/154
Clint (liver, ml/min/kg, m/r/d/cy/h)	12.7/16.6/4.61/4.32/8.10
CYP (CYP1A2/2B6/2C9/2C19/2D6/3A4)	> 10 μM
hERG(IC_{50})	> 10 μM
TDI	Low risk
PXR @1 and 10 μM	Low risk
Off-target degradations (IKZF1/3, GSPT1)	Low risk

Summary

- RP03707 selectively eliminates the KRAS^{G12D} protein and inhibits tumor cell proliferation both *in vitro* and *in vivo*
- The degradation of the oncogenic protein KRAS^{G12D} with the PROTAC molecule RP03707 exhibits a superior inhibitory effect on tumor growth compared to MRTX1133, an enzymatic inhibitor
- RP03707 demonstrates an attractive tissue distribution profile and drug-like properties that infrequent dosing results in prolonged drug accumulation in tumor tissue and inactivation of the KRAS-MARK pathway