B004

Preclinical activity of an orally bioavailable PROTAC pan-KRAS degrader versus inhibitors in mutant KRAS models

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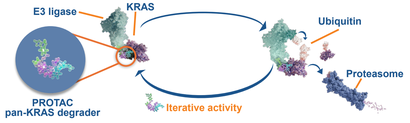
Background

- KRAS is a member of the small GTPase family of enzymes that regulate key processes in the cell by cycling between an "ON" state (bound to GTP) and an "OFF" state (bound to GDP)[1]
- *KRAS* is the most frequently mutated oncogene and is altered in 20–25% of all cancers[2,3]
- The PROTAC mechanism of action may offer advantages for targeting KRAS over traditional small-molecule inhibitors, including potential to:
  - Induce degradation and elimination of both the ON and OFF forms of KRAS
  - Selectively target and degrade KRAS, which may broaden the therapeutic window while preserving efficacy
  - Overcome *KRAS* upregulation commonly observed upon inhibitor treatment through the PROTAC's iterative activity

Mechanism of action of a PROTAC pan-KRAS degrader[a]



[a]General PROTAC protein degrader is shown.

Objective

- To evaluate the activity and selectivity of a tool PROTAC pan-KRAS degrader and compare it with pan-RAS (ON) and pan-KRAS inhibitors

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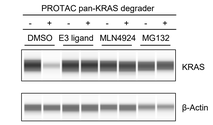
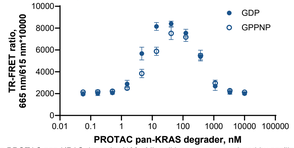
Figure 1: PROTAC pan-KRAS degrader mechanism of action

(A) KRAS degradation



(B) Ternary complex formation

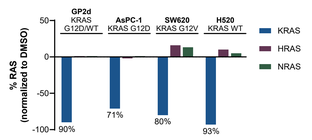


(**A**) SW620 cells were treated for 6 h with the indicated molecules ± a PROTAC pan-KRAS degrader (100 nM); cell lysates were analyzed by capillary electrophoresis followed by immunoblotting. (**B**) Equimolar biotin–Avi–tagged KRAS(1–169) G12D and His-tagged E3 ubiquitin ligase were incubated with serial dilutions of a PROTAC pan-KRAS degrader in the presence of excess GDP or GPPNP (nonhydrolyzable GTP analog); ternary complex formation was measured by TR-FRET.

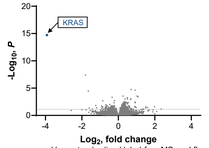
- Degradation of KRAS by the PROTAC pan-KRAS degrader in vitro was dependent on E3 ligase and proteasome activity (Figure 1A)
- The PROTAC pan-KRAS degrader formed ternary complexes with both OFF (GDP-bound) and ON (GTP-bound) KRAS (Figure 1B)

Figure 2: Selectivity of PROTAC pan-KRAS degrader

Changes in RAS protein levels



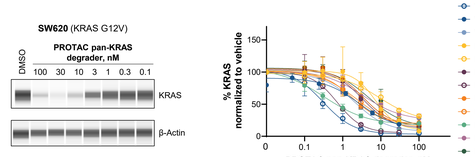
Proteomic changes



Spheroids were treated for 24 h with a PROTAC pan-KRAS degrader or controls. Cell lysates were prepared by a standardized label-free MS workflow and peptides analyzed by reversed-phase LC-MS/MS on an Orbitrap Astral system with data-independent acquisition. Data were normalized and statistically analyzed in FragPipe-Analyst using label-free quantification, t-tests, Benjamini–Hochberg false discovery rate correction, and defined significance cutoffs.

- Selective targeting of KRAS over HRAS and NRAS by the PROTAC pan-KRAS degrader was observed across 4 cell lines and confirmed by data-independent acquisition proteomics

Figure 3: KRAS degradation in panel of cells

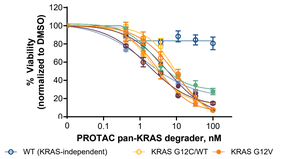


SW620 (KRAS G12V) and a panel of cancer cell lines carrying WT and KRAS mutants were treated for 24 h with serial dilutions of a PROTAC pan-KRAS degrader; lysates were analyzed by capillary electrophoresis followed by immunoblotting.

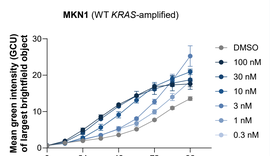
- The PROTAC pan-KRAS degrader had potent activity (DC_{50}: 0.3–9 nM) across a broad set of *KRAS* alterations

Figure 4: Antiproliferative activity and apoptosis induction in vitro

(A) Cell viability



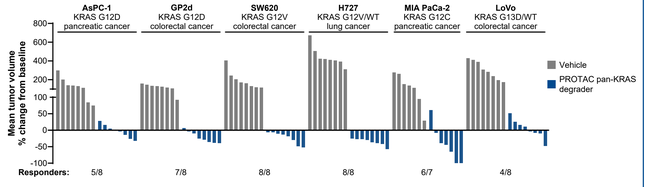
(B) Caspase activity



(**A**) 3D spheroids from cell lines were treated for 5 days with serial dilutions of a PROTAC pan-KRAS degrader; viability was measured by CellTiter-Glo 3D. (**B**) MKN1 spheroids were treated with serial dilutions of a PROTAC pan-KRAS degrader, and caspase activity was measured by Incucyte.

- The PROTAC pan-KRAS degrader had antiproliferative activity in spheroids with various *KRAS* alterations (IC_{50}: 2–10 nM; Figure 4A) and induced apoptosis in multiple cell lines (WT *KRAS*-amplified as example in Figure 4B)

Figure 5: Antitumor activity in vivo

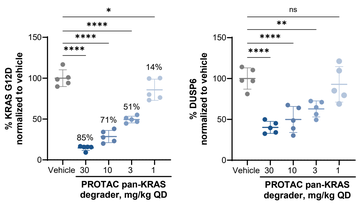


A PROTAC pan-KRAS degrader was administered PO 30 mg/kg QD, 30 mg/kg BID, or 60 mg/kg BID with vehicle controls to CB17 SCID or athymic nu female mice (~200 mm³ at dosing) bearing an AsPC1 CDX, a GP2d CDX, a SW620 CDX, an H727 CDX, a MIA PaCa-2 CDX, or a LoVo CDX; tumor volume was monitored and change from baseline was calculated for each mouse at study end.

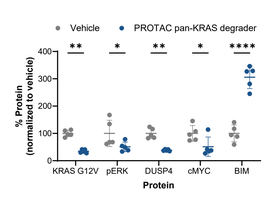
- QD or BID oral dosing of the PROTAC pan-KRAS degrader induced tumor regressions in CDX models of pancreatic, colorectal, and lung cancer

Figure 6: KRAS degradation and signaling suppression in vivo

(A) KRAS G12D and DUSP6 protein levels



(B) Protein levels

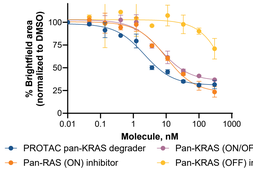


CB17 SCID female mice bearing (**A**) GP2d CDX or (**B**) SW620 CDX tumors were administered a PROTAC pan-KRAS degrader PO for 3 days; tumor lysates were analyzed by gel electrophoresis followed by immunoblotting quantification of KRAS G12D, DUSP6, pERK, cMYC and BIM protein expression levels. *P*-values were determined by a one-way ANOVA (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

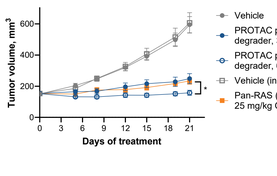
- Oral administration of the PROTAC pan-KRAS degrader led to degradation of KRAS G12D (Figure 6A) and KRAS G12V (Figure 6B) as well as MAPK inhibition, decreases in the proliferative marker cMYC, and increases in the pro-apoptotic marker BIM

Figure 7: PROTAC pan-KRAS degrader activity vs inhibitors in KRAS G13D model

(A) Cell viability



(B) Change in tumor volume

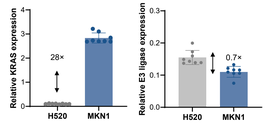


(**A**) Inhibition of proliferation of spheroids from LoVo cells after treatment for 5 days with serial dilutions of a PROTAC pan-KRAS degrader or inhibitors and measured by Incucyte assay. (**B**) TGI analysis of a PROTAC pan-KRAS degrader or pan-RAS (ON) inhibitor administered PO with vehicle controls to athymic nu female mice (~200 mm³ at dosing) bearing a LoVo CDX; tumor volume was measured. *P=0.01 Welch's t test.

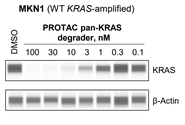
- The PROTAC pan-KRAS degrader showed stronger antiproliferative effects than pan-RAS (ON) or pan-KRAS inhibitors (Figure 7A) and greater TGI than a pan-RAS (ON) inhibitor (Figure 7B) in a KRAS G13D xenograft model

Figure 8: PROTAC pan-KRAS degrader activity vs inhibitors in WT *KRAS*-amplified model

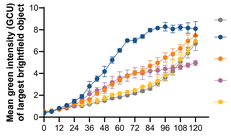
(A) KRAS and E3 ligase expression



(B) KRAS degradation



(C) Caspase activity

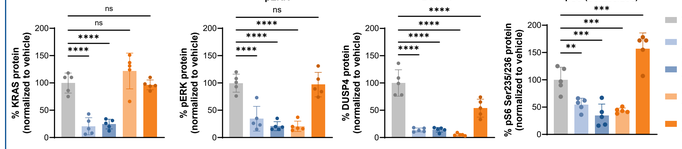


(**A**) H520 and MKN1 spheroids cell lysates were analyzed by capillary electrophoresis followed by immunoblotting. (**B**) MKN1 cells were treated for 24 h with serial dilutions of a PROTAC pan-KRAS degrader; lysates were analyzed by capillary electrophoresis followed by immunoblotting. (**C**) Spheroids from MKN1 cells were treated for 5 days with 5 nM of a PROTAC pan-KRAS degrader, inhibitors, or DMSO control; caspase activity was measured by Incucyte.

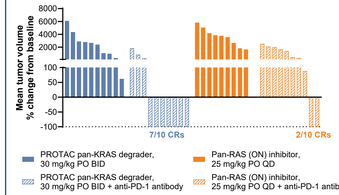
- In the MKN1 WT *KRAS*-amplified model with 28-fold higher KRAS protein levels than a WT *KRAS* model (H520) but comparable E3 ligase levels (Figure 8A), PROTAC pan-KRAS degrader treatment led to complete KRAS degradation (Figure 8B) and showed greater caspase activity than pan-RAS (ON) or pan-KRAS inhibitors (Figure 8C), as well as stronger antiproliferative effects (data not shown)

Figure 9: PROTAC pan-KRAS degrader activity vs pan-RAS (ON) inhibitor in CT26 syngeneic model

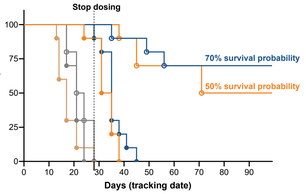
(A) Levels of KRAS and MAPK pathway markers



(B) Change in tumor volume



(C) Survival



(**A**) CT26 tumors were implanted subcutaneously in BALB/c female mice and either PROTAC pan-KRAS degrader (30 mg/kg BID) or pan-RAS ON inhibitor (25 mg/kg QD) was dosed PO for 3 days; tumor lysates were analyzed by gel electrophoresis followed by immunoblotting. *P*-values were determined by a one-way ANOVA (ns is not significant; **P<0.01; ***P<0.001; ****P<0.0001). (**B-C**) Efficacy studies were initiated when tumor size reached a mean volume of ~20 mm³; PROTAC pan-KRAS degrader (30 mg/kg BID) or pan-RAS (ON) inhibitor (25 mg/kg QD) were dosed PO alone or with anti–PD-1 antibody (10 mg/kg BIW) or IgG control by IP injection. Survival was monitored up to 72 days after dosing was stopped. Figure 9B was previously presented.[4]

- The PROTAC pan-KRAS degrader led to more durable MAPK pathway inhibition (Figure 9A) and enhanced combinatorial efficacy (complete responses and prolonged survival) with immune checkpoint blockade (Figure 9B-C) compared with a pan-RAS (ON) inhibitor in the mutant KRAS CT26 syngeneic mouse model

Conclusions

- Targeted KRAS degradation with an oral PROTAC pan-KRAS degrader resulted in broad activity across KRAS alterations with selectivity over other RAS isoforms
- PROTAC pan-KRAS degrader activity translated into differentiated preclinical activity, robust antitumor efficacy, and enhanced immune checkpoint blockade combination potential compared with pan-RAS inhibition

Abbreviations

ANOVA=analysis of variance
BID=twice daily
BIM=Bcl-2–interacting mediator of cell death
BIW=twice weekly
CDX=cell line–derived xenograft
C_{min}=minimum plasma concentration
cMYC=cellular MYC proto-oncogene
CN=copy number
CR=complete response
DC_{50}=half-maximal degradation concentration
DMSO=dimethyl sulfoxide
DUSP4/6=dual specificity phosphatase 4/6
GCU=generic caspase units
HRAS=Harvey rat sarcoma viral oncogene homolog
IC_{50}=half-maximal inhibitory concentration
IgG=immunoglobulin
IP=intraperitoneal
KRAS=Kirsten rat sarcoma viral oncogene homolog
LC-MS/MS=liquid chromatography–tandem mass spectrometry
MAPK=mitogen-activated protein kinase
NRAS=neuroblastoma rat sarcoma viral oncogene homolog
PD-1=programmed cell death protein 1
pERK=phosphorylated extracellular signal-regulated kinase
PO=orally
PROTAC=PROteolysis TArgeting Chimera
pS6 (Ser235/236)=ribosomal protein S6 phosphorylated at serine 235 and 236
QD=once daily
SCID=severe combined immunodeficiency
TGI=tumor growth inhibition
TR-FRET=time-resolved Förster resonance energy transfer
WT=wild-type

References

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Acknowledgments

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