Development of bifunctional CRBN-SOS1 degraders for treatment of mutant KRAS cancers

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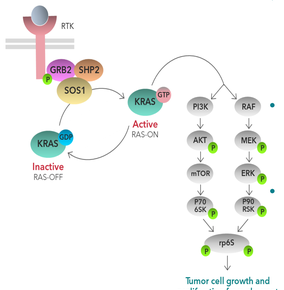
BACKGROUND



- SOS1 is a GEF which converts inactive GDP-loaded RAS proteins into the active GTP-loaded RAS.[1] SOS1 acts as the primary GEF for KRAS.
- KRAS is mutated in ~30% of all cancer with heavy implications in lung, colorectal and pancreatic cancers.[2]
- While mutant alleles of KRAS can shift the equilibrium to favor the GTP-loaded state, it's been shown that mutant KRAS proteins still depend on upstream nucleotide exchange for activation.[3-5]

- SOS1's role in GTP-loading of RAS proteins as well as its ability to mitigate upstream MAPK pathway reactivation highlights its potential as an attractive therapeutic target to treat KRAS-driven cancers irrespective of mutant alleles.[6]
- Thus, we sought out to develop SOS1 bifunctional degraders for single agent and combination approaches for mutant KRAS cancers.

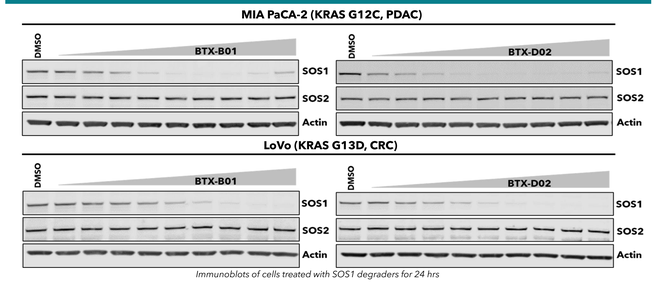
METHODS

- PRODEGY platform was utilized to develop a series of CRBN-based SOS1 degraders which resulted in **BTX-B01** and **BTX-D02**.
- Western Blots under adherent cell culture conditions (2D) were used to determine SOS1 degradation (DRC, CRBN- and Proteasomal-dependence) and active RAS levels.
- Western Blots under ultra-low attachment cell culture conditions (3D) were used to determine SOS1 degradation and inhibition of downstream signaling markers (pERK and pS6).
- Knockout cell lines were generated via nucleofection of Cas9-gRNA complexes.
- 3D proliferation assays were performed to measure functional activity using CellTiter-Glo 3D assay.
- Vehicle, BTX-B01, AMG510 and Trametinib were used in female BALB/c nude mice xenograft models.

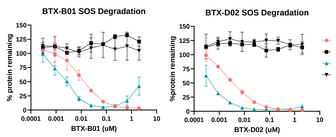
RESULTS

- BTX-B01 and BTX-D02 exhibit ≤10nM DC_{50} values SOS1 degradation with max degradation reaching >95% in several mutant KRAS cell lines in less than 6 hours.
- Co-treatment of SOS1 bifunctional degraders with CRBN competitors or proteasomal inhibitors rescue SOS1 degradation showcasing their dependence on CRBN and the proteasome.
- BTX-B01 and BTX-D02 downregulate active RAS (KRAS, HRAS, and MRAS) levels and downstream signaling markers, pERK and pS6.
- SOS1 bifunctional degraders inhibit cell proliferation in multiple KRAS mutant (G12A, G12C, G12V, G12S, G13D) cell lines with >100nM IC_{50} values and synergizes with KRAS G12C (AMG510) and G12D (MRTX1133) inhibition as well as MEK inhibition (Trametinib).
- Consistent with *in vitro* data, BTX-B01 inhibited tumor growth in KRAS G12C MIA PaCa-2 and NCI-H358 xenograft models. Coupling SOS1 degradation (BTX-B01) with KRAS G12C inhibition or MEK inhibition produced greater tumor growth inhibition.

BTX-B01 & BTX-D02 are rapid and potent degraders of SOS1



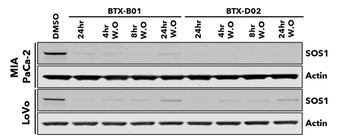
Immunoblots of cells treated with SOS1 degraders for 24 hrs

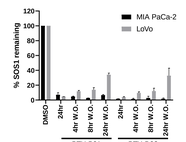


DC_{50} and D_{max} of BTX-B01 and BTX-D02

Cell Line	BTX-B01	BTX-D02
MIA PaCa-2	2nM, 96%	0.5nM, 96%
LoVo	10nM, 97%	4nM, 97%

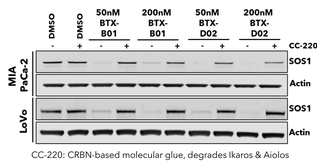
Resynthesis is slow and requires >24hrs to reach half-life





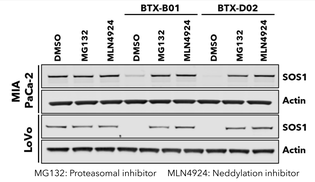
SOS1 degradation is dependent on CRBN and the proteasome

CRBN competition rescues SOS1 degradation



CC-220: CRBN-based molecular glue, degrades Ikaros & Aiolos

Proteasomal inhibition rescues SOS1 degradation

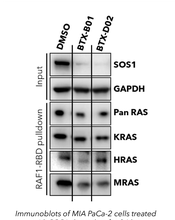


MG132: Proteasomal inhibitor MLN4924: Neddylation inhibitor

Immunoblots of cells treated with SOS1 degraders for 6 hrs

BTX-B01 & BTX-D02 inhibit downstream signaling

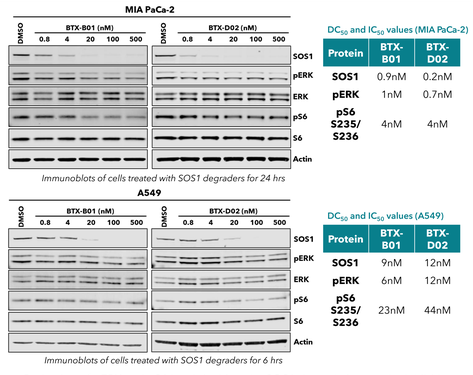
SOS1 degradation reduces active RAS levels along with pERK and pS6 levels



Immunoblots of MIA PaCa-2 cells treated with SOS1 degraders for 24 hrs

% reduction of protein

Protein	BTX-B01	BTX-D02
SOS1	90%	97%
Pan RAS	42%	30%
KRAS	26%	27%
HRAS	60%	20%
MRAS	62%	58%



Immunoblots of cells treated with SOS1 degraders for 24 hrs

DC_{50} and IC_{50} values (MIA PaCa-2)

Protein	BTX-B01	BTX-D02
SOS1	0.9nM	0.2nM
pERK	1nM	0.7nM
pS6 S235/S236	4nM	4nM

DC_{50} and IC_{50} values (A549)

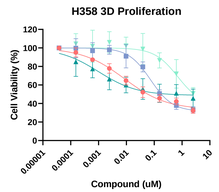
Protein	BTX-B01	BTX-D02
SOS1	9nM	12nM
pERK	6nM	12nM
pS6 S235/S236	23nM	44nM

Immunoblots of cells treated with SOS1 degraders for 6 hrs

Reduction of pERK and pS6 levels is driven by SOS1 degradation, not by inhibition

SOS1 degraders exhibit antiproliferative activity *in vitro* and in xenograft models

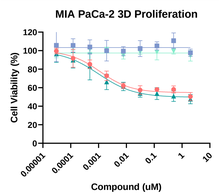
SOS1 degraders display CRBN-dependent functional activity



IC_{50} values

Genotype	BTX-B01	BTX-D02
Parental	8nM	2nM
CRBN KO	90nM	452nM

SOS1 degraders display SOS1-dependent functional activity



IC_{50} values

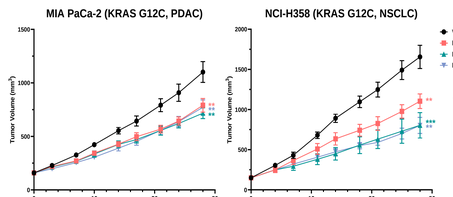
Genotype	BTX-B01	BTX-D02
Parental	1nM	2nM
CRBN KO	>2000nM	>2000nM

IC_{50} values for KRAS cell panel *(in vitro)*

Cell Line	Cell Type	Mutation	BTX-B01	BTX-D02
NCI-H2009	Lung	KRAS G12A	23	5.2
SW837	Colon	KRAS G12C	>2500	>2500
NCI-H358	Lung	KRAS G12C	8	2
NCI-H1792	Lung	KRAS G12C	60	22
NCI-H23	Lung	KRAS G12C	34	16
NCI-H2122	Lung	KRAS G12C	52	6
NCI-H2030	Lung	KRAS G12C	>2500	>2500
MIA PaCa-2	Pancreas	KRAS G12C	1	2
A427	Lung	KRAS G12D	>2500	>2500
GP2D	Colon	KRAS G12D	>2500	>2500
NCI-H441	Lung	KRAS G12V	14	13
NCI-H727	Lung	KRAS G12V	182	72
LCLC-97TM1	Lung	KRAS G12V	22	26
SHP-77	Lung	KRAS G12V	>2500	>2500
SW620	Colon	KRAS G12V	74	13
A549	Lung	KRAS G12S	1	8
HCT116	Colon	KRAS G13D	>2500	>2500
DLD-1	Colon	KRAS G13D	17	33
LoVo	Colon	KRAS G13D	70	31
MKN-1	Stomach	KRAS Amplified	26	5
K562	Heme	-	24	1

BTX-B01 and BTX-D02 demonstrate >1000-fold IC50 values in primary (NHLF) and nontumorigenic (HEK293) cell lines

BTX-B01 reduces tumor growth in KRAS G12C xenograft models: H358 & MIA PaCa-2

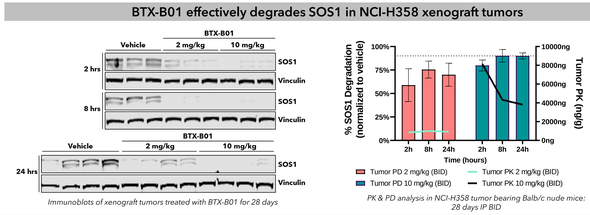


Tumor growth inhibition compared to Vehicle

Xenograft model	2mg/kg BTX-B01	10mg/kg BTX-B01
MIA PaCa-2	33%	41%
NCI-H358	37%	57%

Data is presented as average ± SEM

BTX-B01 effectively degrades SOS1 in NCI-H358 xenograft tumors

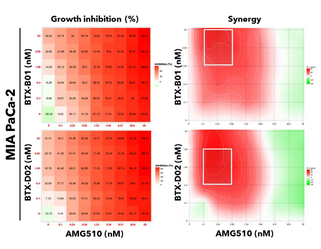


Immunoblots of xenograft tumors treated with BTX-B01 for 28 days

PK & PD analysis in NCI-H358 tumor bearing Balb/c nude mice: 28 days IP BID

SOS1 degraders synergize with KRAS inhibitors and MEK inhibitors *in vitro* and in xenograft models

BTX-B01 and BTX-D02 synergize with KRAS G12C inhibitor, AMG510 (3D Proliferation)

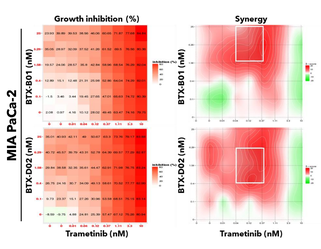


Synergy calculated using Loewe Analysis

Cell Line	AMG510 + BTX-B01 Overall Synergy Score	AMG510 + BTX-B01 Most Synergistic Area	AMG510 + BTX-D02 Overall Synergy Score	AMG510 + BTX-D02 Most Synergistic Area
MIA PaCa-2	11.6	28	7.6	24
NCI-H23	8.1	22.5	8.7	17.6
NCI-H358	5.9	14.1	9.3	16.9

Scores > 10 indicate high levels of synergy
Boxes indicate most synergistic area

BTX-B01 and BTX-D02 synergize with MEK inhibitor, Trametinib (3D Proliferation)

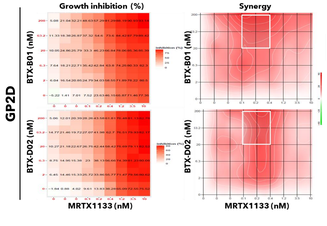


Synergy calculated using Loewe Analysis

Cell Line	Trametinib + BTX-B01 Overall Synergy Score	Trametinib + BTX-B01 Most Synergistic Area	Trametinib + BTX-D02 Overall Synergy Score	Trametinib + BTX-D02 Most Synergistic Area
MIA PaCa-2	4	11.6	4.8	11.8
NCI-H23	7.7	18.2	14.9	28.9
LoVo	N/D	N/D	5.4	13.8

Scores > 10 indicate high levels of synergy
Boxes indicate most synergistic area
N/D = Not determined/tested

BTX-B01 and BTX-D02 synergize with KRAS G12D inhibitor, MRTX1133 (3D Proliferation)

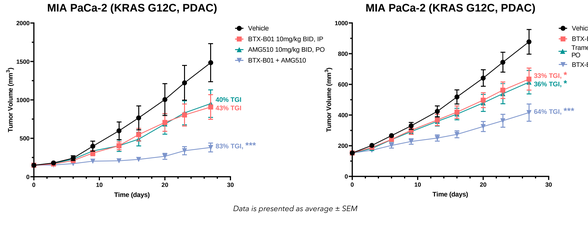


Synergy calculated using Bliss Analysis

Cell Line	MRTX1133 + BTX-B01 Overall Synergy Score	MRTX1133 + BTX-B01 Most Synergistic Area	MRTX1133 + BTX-D02 Overall Synergy Score	MRTX1133 + BTX-D02 Most Synergistic Area
GP2D	15.9	26.8	11.6	19

Scores > 10 indicate high levels of synergy
Boxes indicate most synergistic area

BTX-B01 with AMG510 or Trametinib results in better efficacy in MIA PaCa-2 xenograft model



Data is presented as average ± SEM

CONCLUSIONS

These preclinical data demonstrate the potential for SOS1 degraders as a promising modality for targeting SOS1 alone and in combination with other RAS-MAPK pathway inhibitors. SOS1 exhibits a long half-life (>24hrs) which makes it an ideal target protein to develop degraders against. SOS1 degraders display greater potency over inhibitors in *in vitro* assays, which can be seen with some other bifunctional degraders.

REFERENCES

(1) Boriack-Sjodin, P.A., et al. Nature 394, 337-343 (1998). **(2)** Prior, I.A., et al. *Cancer Res 80*, 2969-2974 (2020). **(3)** Lito, P., et al. *Science* 351, 604-608 (2016) **(4)** Nichols, R.J., et al. *Nat Cell Biol* 20, 1064-1073 (2018). **(5)** Hofmann, M.H., et al. *Cancer Discov* 11, 142-157 (2021). **(6)** Lito, P., et al. *Nat Med* 19, 1401-1409 (2013).

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