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

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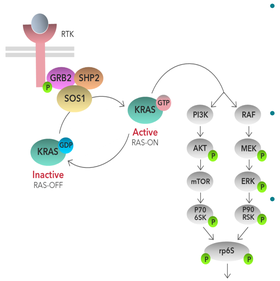
Abstract #: 3151

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

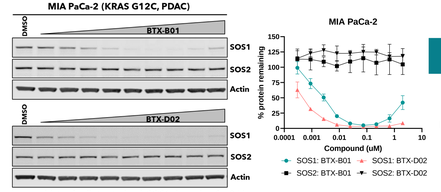
- PRODEGY platform was utilized to develop a series of CRBN-based SOS1 degraders which resulted in **BTX-B01** and **BTX-D02**.
- Western Blots under adherent cell culture conditions (2D) were used to determine SOS1 degradation (DRC, CRBN- and Proteasomal-dependence) and active RAS levels.
- Western 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 >85% 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 <150nM IC_{50} values and synergizes with KRAS G12C (AMG510) and G12D (MRTX1133) inhibition as well as MEK (Trametinib) and EGFR (Afatinib) inhibition.
- 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

SOS1 degraders exhibit low nM DC_{50} and >85% D_{max} in mutant KRAS cell lines



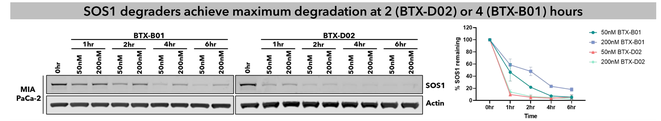
Immunoblots of cells treated with SOS1 degraders for 24 hrs

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

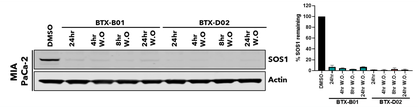
Cell Line	BTX-B01	BTX-D02
MIA PaCa-2 (G12C)	2nM, 96%	0.5nM, 96%
NCI-H358 (G12C)	7nM, 87%	2nM, 92%
LoVo (G13D)	10nM, 97%	4nM, 97%

Known SOS1-complex interactors (EGFR, SHP2, GRB2, KRAS) were not affected with SOS1 degraders

SOS1 degraders achieve maximum degradation at 2 (BTX-D02) or 4 (BTX-B01) hours

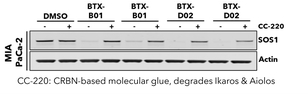


Resynthesis is slow and requires >24hrs to reach half-life



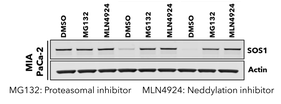
SOS1 degradation is dependent on CRBN, proteasome, & SOS1 binding site

CRBN competition rescues SOS1 degradation



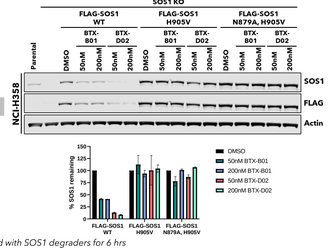
CC-220: CRBN-based molecular glue, degrades Ikaros & Aiolos

Proteasomal inhibition rescues SOS1 degradation



MG132: Proteasomal inhibitor MLN4924: Neddylation inhibitor

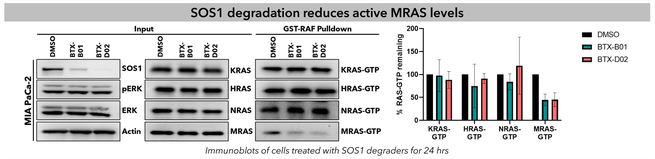
Mutations in ligand-binding site prevent SOS1 degradation



Immunoblots of cells treated with SOS1 degraders for 6 hrs

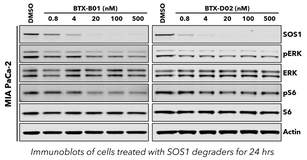
BTX-B01 & BTX-D02 inhibit downstream RAS-MAPK signaling

SOS1 degradation reduces active MRAS levels



Immunoblots of cells treated with SOS1 degraders for 24 hrs

SOS1 degraders reduces pERK and pS6 levels more potently than their respective ligands



Immunoblots of cells treated with SOS1 degraders for 24 hrs

DC_{50} and IC_{50} values

	MIA PaCa-2 (G12C)		A549 (G12S)		LoVo (G13D)	
Protein	BTX-B01	BTX-D02	BTX-B01	BTX-D02	BTX-B01	BTX-D02
SOS1	0.9nM	0.2nM	9nM	12nM	9nM	6nM
pERK	1nM	0.7nM	6nM	12nM	31nM	26nM
pS6 S235/ S236	4nM	4nM	23nM	44nM	22nM	9nM

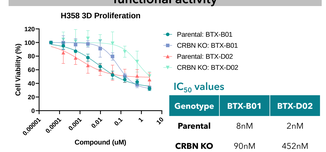
IC_{50} values

MIA PaCa-2	BTX-B01	B01 ligand	BTX-DO2	D02 ligand
pERK	1nM	6nM	0.7nM	11nM
pS6 S235/ S236	5nM	51nM	4nM	66nM
SOS1 Binding Assay (HTRF)	4nM	4nM	8nM	4nM

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

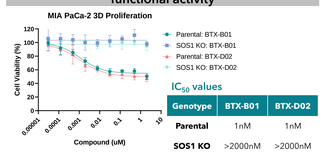
SOS1 degraders demonstrate CRBN-dependent functional activity



IC_{50} values

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

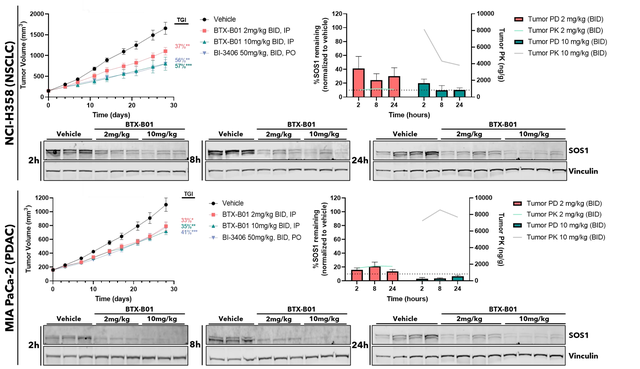
SOS1 degraders display SOS1-dependent functional activity



IC_{50} values

Genotype	BTX-B01	BTX-D02
Parental	1nM	1nM
SOS1 KO	>2000nM	>2000nM

BTX-B01 effectively degrades SOS1 and inhibits tumor growth in KRAS G12C xenograft models: NCI-H358 & MIA PaCa-2



Xenograft efficacy data is presented as average ± SEM

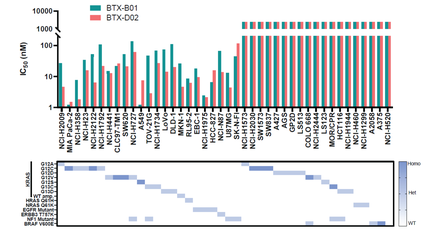
Immunoblots of xenograft tumors treated with BTX-B01 for 28 days *PK & PD analysis in NCI-H358 tumor bearing Balb/c nude mice: 28 days IP BID*

SOS1 degraders display lower IC_{50} values compared to ligands

IC_{50} values in 3D Proliferation

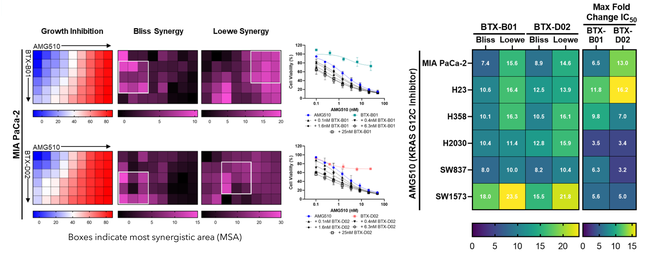
CMPD	MIA PaCa-2	NCI-H358	A549	LoVo
BTX-B01	1nM	8nM	1nM	74nM
B01 ligand	14nM	43nM	28nM	140nM
BTX-D02	1nM	2nM	7nM	14nM
D02 ligand	33nM	24nM	39nM	83nM

SOS1 degraders exhibit antiproliferative activities across various KRAS, RTK, and NF1 mutant cell lines



SOS1 degraders synergize with EGFR-RAS-MAPK inhibitors *in vitro* and in xenograft models

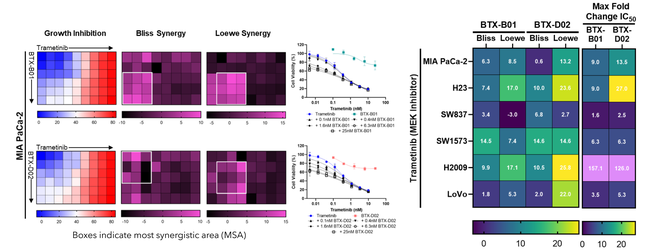
BTX-B01 and BTX-D02 synergize with KRAS G12C inhibitor, AMG510



Boxes indicate most synergistic area (MSA)

Values represent most synergistic area (MSA) Scores > 10 indicate high levels of synergy

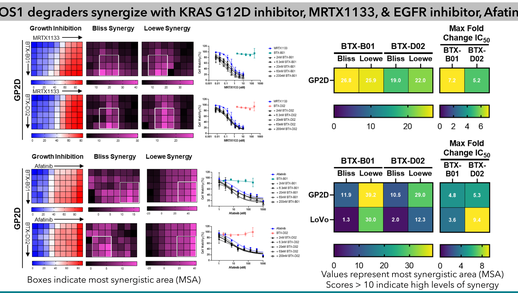
BTX-B01 and BTX-D02 synergize with MEK inhibitor, Trametinib



Boxes indicate most synergistic area (MSA)

Values represent most synergistic area (MSA) Scores > 10 indicate high levels of synergy

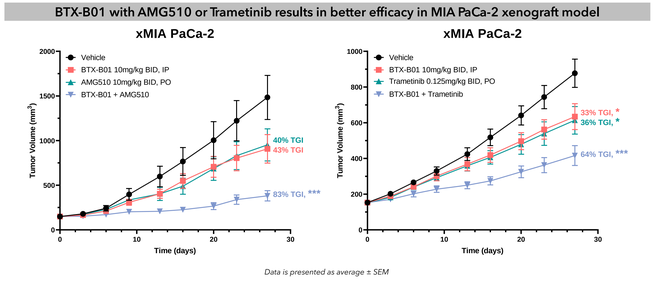
SOS1 degraders synergize with KRAS G12D inhibitor, MRTX1133, & EGFR inhibitor, Afatinib



Boxes indicate most synergistic area (MSA)

Values represent most synergistic area (MSA) Scores > 10 indicate high levels of synergy

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



Data is presented as average ± SEM

Conclusions & Future Directions

-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.

-Our program is currently in lead optimization with efforts to develop potent, orally bioavailable SOS1 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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