Discovery of BTX-10908, a first-in-class, orally bioavailable SOS1 bifunctional degrader, for the treatment of RTK- and KRAS-driven tumors

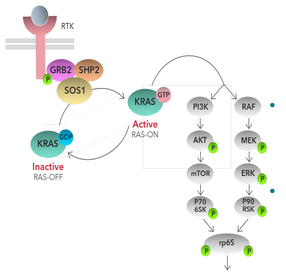
Kyle Begovich[1], Angela Schoolmeesters[1], Elena Martinez-Terroba[1] Navin Rajapakse[1], Qiao Liu[1], Arvind Shakya[1], Akinori Okano[1], Venkat Mali[1], Shenlin Huang[1], Aparajita Chourasia[1], and Leah Fung[1]

[1]Biotheryx, Inc., San Diego, CA

Contact: Kyle Begovich, kbegovich@biotheryx.com

Abstract #: 6056

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-10908**.
- Western Blots under adherent cell culture conditions (2D) were used to determine SOS1 degradation (Kinetics, Washout, CRBN- and Proteasomal-dependence) or under ultra-low attachment cell culture conditions (3D) were used to determine SOS1 degradation and inhibition of downstream signaling markers.
- 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.
- *In vivo* experiments were conducted in female BALB/c nude mice xenograft models.

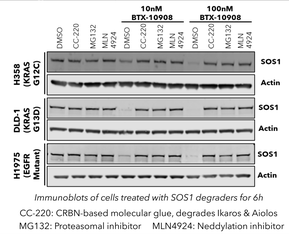
Identification of BTX-10908 as a D.C.

Compound	BTX-10908 (D.C. SOS1d)	10908 TBL TBL: Target-Binding Ligand	BTX-6654[7] (PoC SOS1d)	MRTX0902 (SOS1i)
H358 SOS1-HiBiT (DC_{50}, D_{max})	4.5nM, 90%	N/A	8.7nM, 87%	N/A
SOS1 Binding (IC_{50})	485nM	56nM	4nM	8nM
DLD-1 pERK AlphaLISA (IC_{50})	3nM	93nM	110nM	80nM
K562 WT Proliferation (IC_{50})	1nM	>2000nM	22nM	56nM
K562 CRBN KO Proliferation (IC_{50})	582nM	N/A	243nM	N/A
MIA PaCa-2 WT 3D Proliferation (IC_{50})	2nM	>2000nM	1nM	49nM
MIA PaCa-2 SOS1 KO 3D Proliferation (IC_{50})	>2000nM	N/A	>2000nM	>2000nM
F% (across species)	16-25%	N/A	0%	38-83%[8]

In Vitro Potency	*In Vivo* Potency	Physicochemical Properties	Safety/ Tolerability	Higher Species PK	Developability
✓	✓	✓	✓	✓	✓

BTX-10908 demonstrates rapid, selective and mechanism-dependent degradation of SOS1

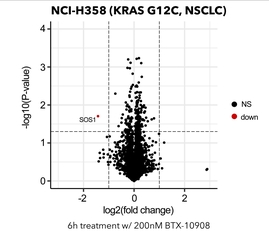
SOS1 degradation requires CRBN & the proteasome



Immunoblots of cells treated with SOS1 degraders for 6h

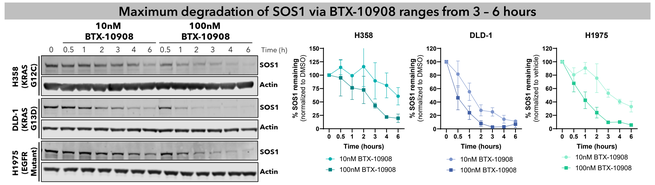
CC-220: CRBN-based molecular glue, degrades Ikaros & Aiolos
MG132: Proteasomal inhibitor MLN4924: Neddylation inhibitor

BTX-10908 is highly selective for SOS1

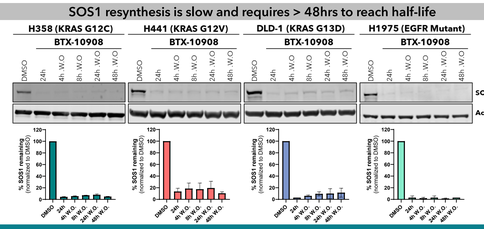


6h treatment w/ 200nM BTX-10908

Maximum degradation of SOS1 via BTX-10908 ranges from 3 – 6 hours

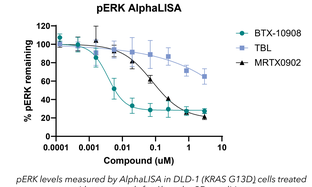


SOS1 resynthesis is slow and requires > 48hrs to reach half-life



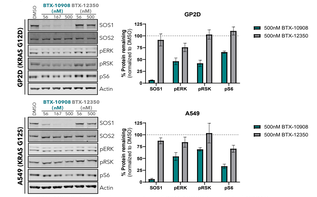
BTX-10908 inhibits downstream RAS-MAPK signaling

BTX-10908 exhibits more potent inhibition of pERK compared to SOS1i's



pERK levels measured by AlphaLISA in DLD-1 (KRAS G13D) cells treated with compounds for 6h under 2D conditions

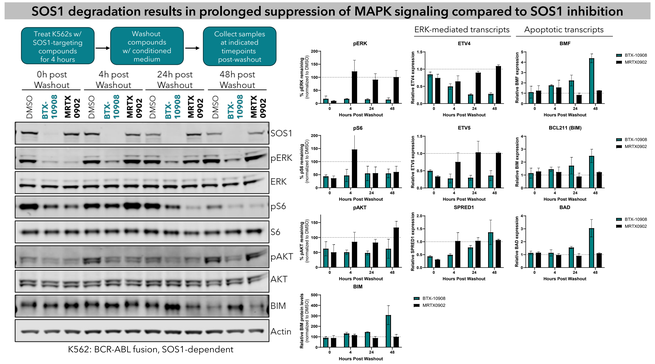
Inhibition of downstream signaling is driven heavily by SOS1 degradation rather than SOS1 inhibition



BTX-12350: N-methylated version of BTX-10908 (can't bind CRBN)

Immunoblots of cells treated w/ compounds for 4h (A549) or 6h (GP2D) under 3D conditions

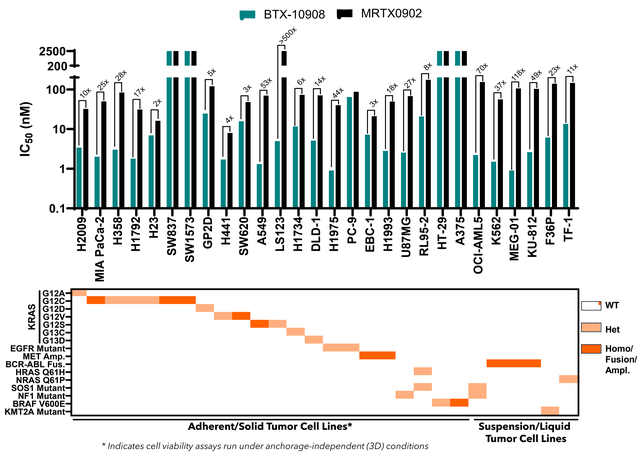
SOS1 degradation results in prolonged suppression of MAPK signaling compared to SOS1 inhibition



K562: BCR-ABL fusion, SOS1-dependent

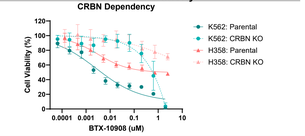
BTX-10908 exhibits antiproliferative activity *in vitro* and in xenograft models

BTX-10908 exhibits antiproliferative properties across various KRAS, RTK, and NF1 mutant cell lines

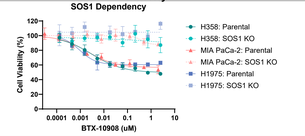


** Indicates cell viability assays run under anchorage-independent (3D) conditions*

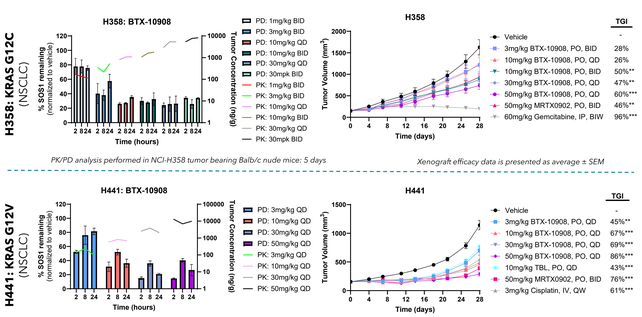
BTX-10908 demonstrates CRBN-dependent functional activity



BTX-10908 displays SOS1-dependent functional activity



BTX-10908 effectively degrades SOS1 and inhibits tumor growth in KRAS mutant xenograft models



PK/PD analysis performed in NCI-H358 tumor bearing Balb/c nude mice: 5 days

Xenograft efficacy data is presented as average ± SEM

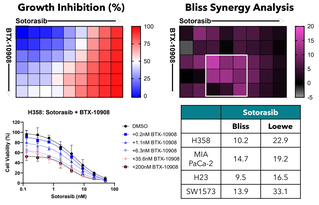
PK/PD analysis performed in NCI-H441 tumor bearing Balb/c nude mice: 28 days

Xenograft efficacy data is presented as average ± SEM

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

BTX-10908 synergizes with KRAS G12Ci, Sotorasib

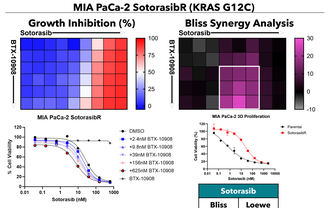
H358 (KRAS G12C)



	Sotorasib	
	Bliss	Loewe
H358	10.2	22.9
MIA PaCa-2	14.7	19.2
H23	9.5	16.5
SW1573	13.9	33.1

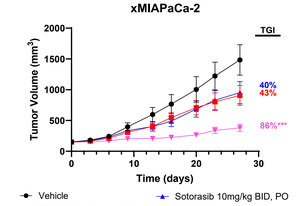
Synergy also observed w/ Adagrasib (KRAS G12Ci) & BI-2865 (pan-KRASi)

BTX-10908 resensitizes Sotorasib-Resistant MIA PaCa-2 3D model



	Sotorasib	
	Bliss	Loewe
18.8	21.9	

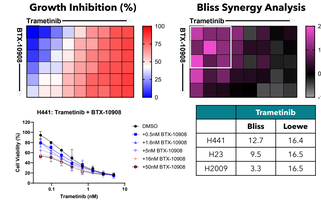
PoC SOS1 degrader BTX-6654 combined with Sotorasib results in enhanced TGI



Data is presented as average ± SEM

BTX-10908 synergizes with MEKi, Trametinib

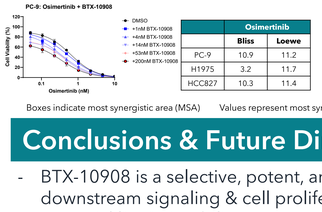
H441 (KRAS G12V)



	Trametinib	
	Bliss	Loewe
H441	12.7	16.4
H23	9.5	16.5
H2009	3.3	16.5

BTX-10908 synergizes with EGFRi, Osimertinib

PC-9 (EGFR Mutant)

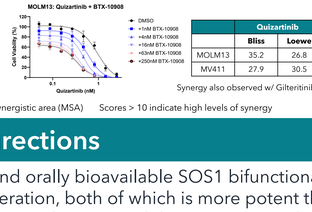


	Osimertinib	
	Bliss	Loewe
PC-9	10.9	11.2
H1975	3.2	11.7
HCC827	10.3	11.4

Boxes indicate most synergistic area (MSA)

BTX-10908 synergizes with FLT3i, Quizartinib

MOLM13 (FLT3-ITD)



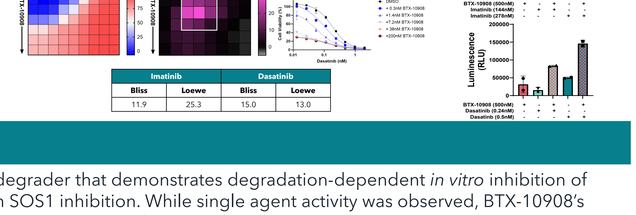
	Quizartinib	
	Bliss	Loewe
MOLM13	35.2	26.8
MV411	27.9	30.5

Synergy also observed w/ Gilteritinib

Values represent most synergistic area (MSA)

BTX-10908 is synergistic with BCR-ABL inhibitors, Imatinib and Dasatinib

K562 (BCR-ABL fusion)



Imatinib		Dasatinib	
Bliss	Loewe	Bliss	Loewe
11.9	25.3	15.0	13.0

Scores > 10 indicate high levels of synergy

Conclusions & Future Directions

- BTX-10908 is a selective, potent, and orally bioavailable SOS1 bifunctional degrader that demonstrates degradation-dependent *in vitro* inhibition of downstream signaling & cell proliferation, both of which is more potent than SOS1 inhibition. While single agent activity was observed, BTX-10908's potential lies in its ability to synergize with various inhibitors targeting the RTK-RAS-MAPK pathway.
- BTX-10908 displayed prolonged and sufficient exposure which allows for QD dosing compared to BID dosing commonly used for SOS1 inhibitors.
- BTX-10908 is currently undergoing further characterization (including additional xenograft studies) and preparing for IND-enabling studies.

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). **(7)** Begovich, K. et al. *Mol Cancer Ther* OF1-OF14 (2024). **(8)** Ketcham, J.M. et al. *J Med Chem* 65, 9678-9690 (2022)

http://www.biotheryx.com

biotheryx