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A Novel Small-Molecule CBP/p300 HAT Domain Inhibitor Demonstrates Potent *In Vivo* Activity and a Favourable Safety Profile in Preclinical Species

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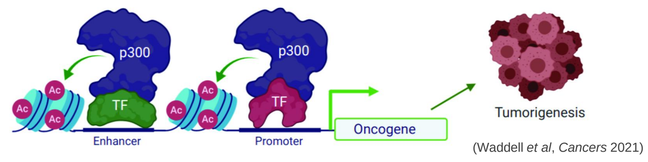
INTRODUCTION

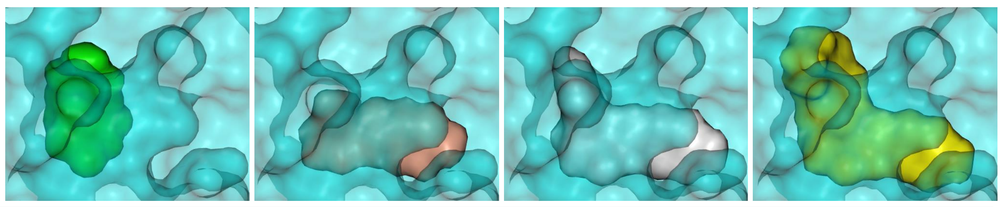
CREB binding protein (CBP) and its paralog, EP300 (p300), are highly homologous lysine acetyltransferases and transcriptional cofactors implicated in human cancers. Dose-limiting tolerability issues have been observed with dual CBP/p300 bromodomain (BRD) inhibitors, which may limit their clinical utility. We hypothesised that a dual inhibitor targeting the histone acetyltransferase (HAT) domain may improve the therapeutic window. Here we describe the characterisation of ASTX528, a potent, fragment-derived CBP/p300 HAT inhibitor with a differentiated safety profile from BRD inhibitors.



(Waddell *et al*, *Cancers* 2021)

RESULTS: *IN VITRO*

Fragment-based discovery of a novel inhibitor of CBP/p300 HAT domain



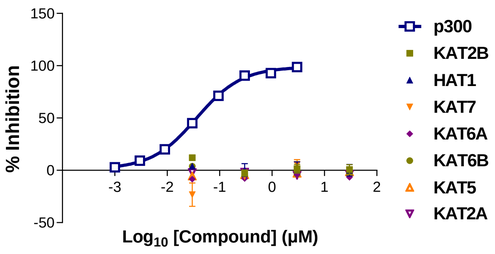
	Fragment 1	Fragment 2	Lead	ASTX528
p300 IC_{50} (μM)	59% at 1000	> 1000	<0.020	<0.010
Ligand Efficiency	< 0.24	< 0.24	0.40	0.35

Fluorescence-based assays against full-length p300 was used to monitor enzyme activity

- A fragment screen was carried out and multiple structurally validated hits were obtained
- Structure-guided optimisation of the fragment hits led to the discovery of ASTX528 which inhibits p300 enzyme activity with an average IC_{50} of <10 nM

Selectivity and target engagement

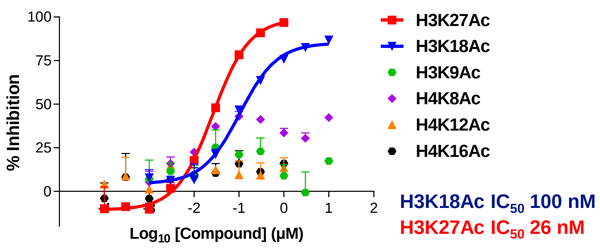
Biochemical HAT selectivity panel



- ASTX528 inhibits the HAT domain of p300 protein with >1000-fold selectivity over other HATs and bromodomains
- ASTX528 is not selective over CBP

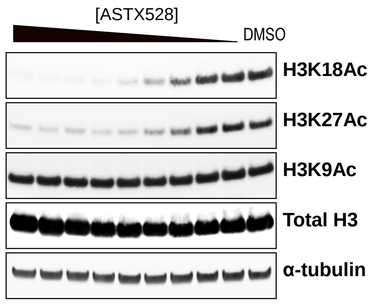
Biochemical assay data was generated at Eurofins against the HAT enzymes indicated

Cellular lysine selectivity panel LNCaP (AR+)



ASTX528-treated LNCaP cells were analysed by lysine-acetylation immunofluorescence assay using antibodies against histones H3 and H4 acetylated at the indicated lysines.

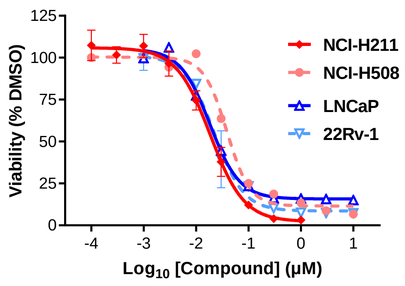
Cellular lysine panel NCI-H211 (CBP LoF)



Cells were treated with DMSO or ASTX528 at doses ranging from 0.001 - 10 μM for 4 hours then lysed for protein analysis by western blotting against indicated antibodies.

- ASTX528 potently inhibits the HAT domain of p300 and is highly selective
- ASTX528 specifically deacetylates its histone substrates, H3K18 and H3K27

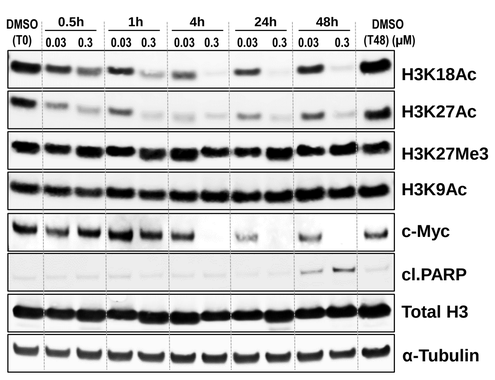
Effects on cell viability and signaling



Cell line	Tissue	Relevant Background	EC_{50} (nM)
NCI-H211	SCLC	CBP LoF	<25
NCI-H508	Colorectal	CBP LoF	<25
LNCaP	Prostate	Full length AR	<20
22Rv1	Prostate	Full length AR, AR-V7	<40

Cells were incubated with dose response of ASTX528 for 6 days and viability determined by CellTiter-Glo®. Representative curves shown.
Average EC_{50} from 2 or 3 experiments are shown.

NCI-H211



Cells were incubated with ASTX528 over a 48h time-course and cell lysates analysed for target engagement (H3K18Ac, H3K27Ac) and downstream signalling markers (H3K27Me3, c-Myc, cleaved PARP) analysed by western blotting. H3K9Ac, total histone H3 and α-tubulin were used as the loading controls.

- ASTX528 potently inhibits proliferation of cells with CBP loss-of-function mutation and AR^+ prostate cancer cell lines
- An increase in cleaved PARP, a marker of cell death, was observed in treated cells

RESULTS: *IN VIVO*

Pharmacokinetics

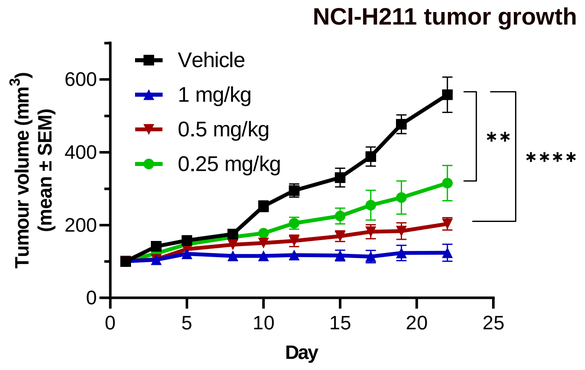
Species	Mouse	Rat	Dog	NHP
Clearance (mL/min/kg)	24	12	9.3	19
Vss (L/kg)	1.4	0.88	1.5	1.4
Bioavailability (%F)	48	19	92	17

Low dose cross-species IV/PO studies were performed and PK parameters derived

- ASTX528 is orally bioavailable in multiple preclinical species

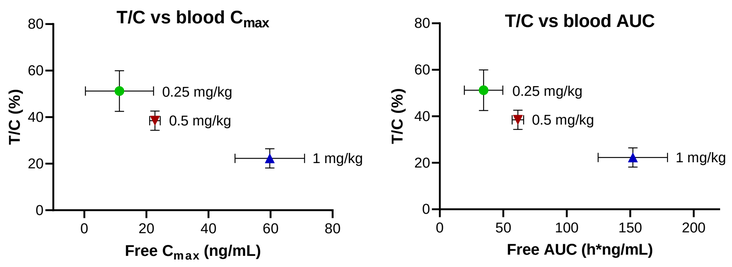
Target engagement and anti-tumor activity

NCI-H211 tumor growth inhibition



Tumor-bearing CB17 SCID mice were orally treated with the ASTX528 or vehicle (2% NMP, 0.5% HPMC) once a day. n=8. **, *P* <0.01; ****, *P* <0.0001.

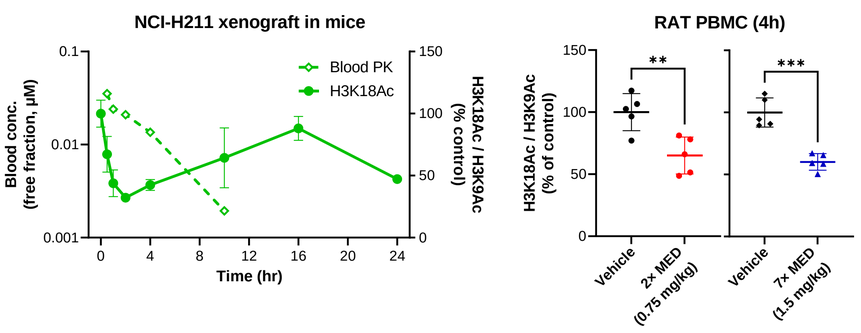
Efficacy- blood PK relationship was explored (below left and middle). T/C values were calculated from median RTV values. ASTX528 concentration in peripheral blood was determined on Day 22. Error bars represent SEM.



Dose (mg/kg)	Day 22 PD: H3K18Ac / H3K9Ac (% control at 4h-6h)
1	15 - 23%
0.5	44 - 55%
0.25	67 - 76%

Tumor lysates were analysed by Meso Scale Discovery (MSD) assays (above). H3K18Ac levels were normalised to H3K9Ac in and expressed relative to the mean of untreated controls.

Target engagement after a single-dose



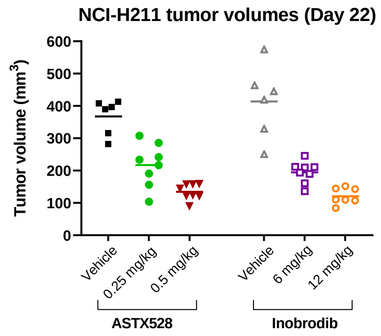
Left: NCI-H211 tumor-bearing mice were dosed once with ASTX528 and tumors analysed by MSD. n=3 per timepoint.

Right: PBMCs were isolated from rats 4 hours after a single dose of ASTX528, lysed and analysed by MSD. PK was determined and fold-over-MED in SCID mice is indicated for each dose n=5. **, *P* <0.01; ***. *P* <0.001.

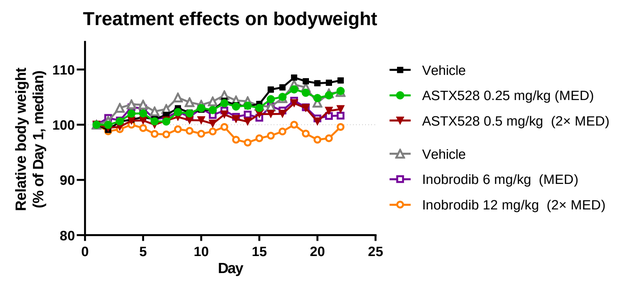
- ASTX528 dose-dependently reduces tumor growth, with 0.25 mg/kg deemed as the minimum effective dose (MED)
- A single-dose causes rapid, dose-dependent deacetylation of H3K18 in NCI-H211 mouse tumor tissues and rat PBMCs

ASTX528 is well tolerated at multiple-fold MED

NCI-H211 tumor volumes (Day 22)

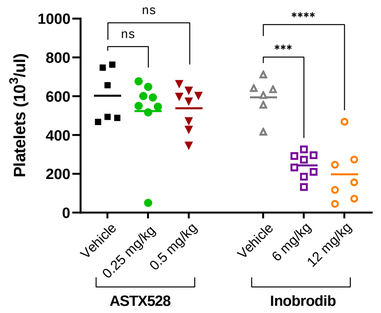


Treatment effects on bodyweight

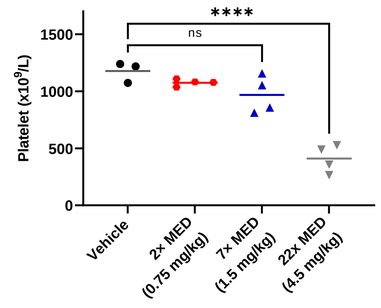


NCI-H211 tumor-bearing mice were treated with ASTX528 or inobrodib[2] or vehicle once a day. MED and 2x MED were established. Bodyweights and peripheral blood platelet counts were compared. n=6-8. ns, not significant; ***, *P* <0.001; ****, *P* <0.0001.

Platelets in mice
(NCI-H211 tumor-bearing SCID mice, Day 14)



Platelets in rats



Male rats were orally treated with ASTX528 at 0.75 - 4.5 mg/kg QD for 14 days. n=3-4. ns, not significant; ****, *P* <0.0001.

- Treatment with ASTX528 at 2-fold MED causes no significant haematological changes in NCI-H211 tumor-bearing mice while BRD inhibitor at its MED significantly reduces platelet counts
- Platelets and other haematological parameters are unaltered in rats after a 14-day treatment with ASTX528 up to 7-fold MED

Astex is committed to the ethical use of animals and adheres to the principles of 3Rs (see www.astx.com)

CONCLUSION

- **The novel CBP/p300 HAT inhibitor ASTX528 is potent and highly selective**
- **ASTX528 inhibits proliferation of cancer cells *in vitro* and significantly reduces tumor growth at low dose levels *in vivo***
- **The safety profile shows targeting the HAT domain with ASTX528 may improve the therapeutic window over current BRD inhibitors**

Reference: (1) Waddell *et al*, (2021) *Cancers* 13:2872; (2) Welti J et al, (2021) *Cancer Discov.* 11:1118-1137

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