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Targeting the Catalytic HAT Domain of CBP/p300 for the Treatment of Hormone-Dependent Breast and Prostate Cancers

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INTRODUCTION

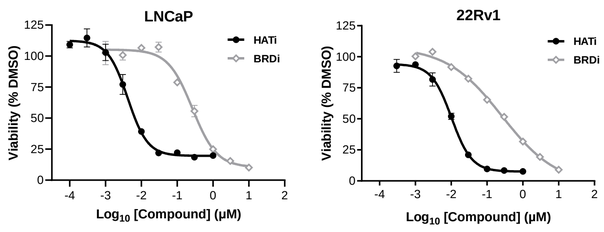
The lysine acetyltransferase paralogues CREBBP (CBP) and EP300 (p300) are transcriptional coactivators that regulate gene expression by directing chromatin accessibility and acetylation of specific lysine residues on histone H3. CBP/p300 are implicated in both solid and hematological cancers as transcriptional dysregulation can lead to oncogenic gene expression and signalling.

Androgen and estrogen hormone receptors (AR and ER, respectively) are ligand-dependent transcription factors which rely on the coactivator activity of CBP/p300. As aberrant activity of these receptors can drive oncogenic transcriptional programs to promote cancer growth, inhibiting CBP/p300 may be an orthogonal approach to target these hormone-dependent cancers, alone or in combination with other agents.

Here, we describe the effects of CBP/p300 histone acetyltransferase (HAT) domain inhibition in preclinical models of AR- and ER-driven cancers. We also describe the characterisation of a novel fragment-derived HAT domain inhibitor, ASTX528, alone and in combination with CDK4/6 inhibitors and ER degraders.

RESULTS: TARGETED EFFECT IN AR-DEPENDENT PROSTATE CANCER

AR-dependent prostate cancer cells are sensitive to CBP/p300 HATi

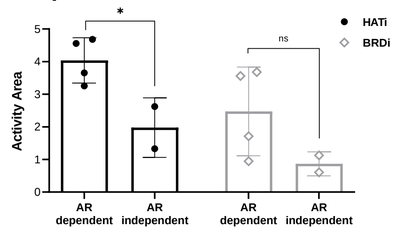


Cell line	AR expression	Mean EC_{50} (μM)	
		HATi	BRDi
LNCaP	Full length	0.0022	0.23
22R1	Full length, AR-V7	0.0082	0.26

Cells were incubated with an inhibitor of CBP/p300 HAT domain (HATi, CPI-1612[1]) or bromodomain inhibitor (BRDi, inobrodib[2]) for 6 days and viability determined by CellTiter-Glo® (above). Representative curves shown.

EC_{50} is expressed as the mean of 2 independent experiments and activity areas (area under the inhibition curve) were calculated from the dose-response curves generated from prostate cancer cell lines (right). *, *P* <0.05; ns, not significant.

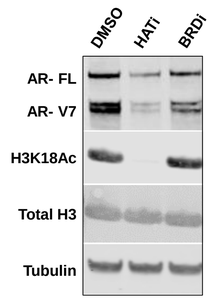
Sensitivity of HATi and BRDi in prostate cancer cell lines



- HATi potently reduces viability of AR^+ prostate cancer cells *in vitro*
- No significant sensitivity window observed for BRDi

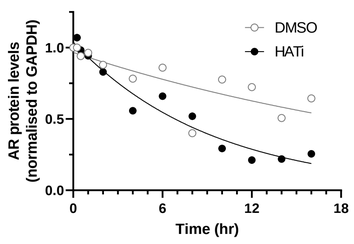
HATi, but not BRDi, impacts the stability of AR and AR-V7, the key drivers of prostate cancer

Protein levels in 22Rv1



22Rv1 cells were incubated with 1 μM HATi, BRDi or DMSO for 24 hours and proteins detected by Western blot.

AR protein decay in LNCaP



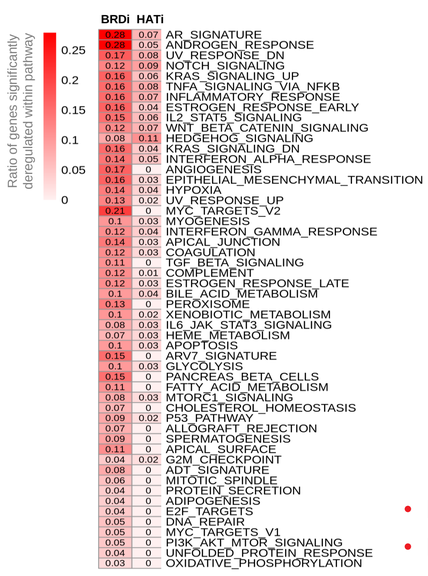
	DMSO	HATi	BRDi
AR half-life (hr)	17.4	6.8	15.4

LNCaP cells were treated with DMSO or 0.1 μM HATi or BRDi for 24 hours followed by protein synthesis inhibition with 10 μg/mL cycloheximide for up to 16 hours. AR protein levels were normalised to GAPDH, expressed as the fraction of the initial level (T_0) and half-life calculated.

- Protein levels of full-length AR and AR-V7 proteins are reduced after treatment with HATi but not with BRDi in 22Rv1
- HATi, but not BRDi, reduces the half-life of AR proteins in LNCaP cells

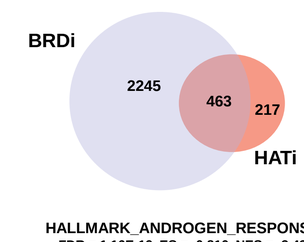
HATi has a smaller transcriptional perturbation footprint than BRDi

Pathway modulation

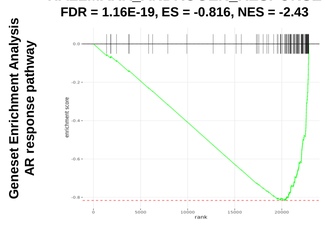


LNCaP cells were incubated with compound for 6 hours at 2x GI_{50} and transcriptomic analysis was conducted.

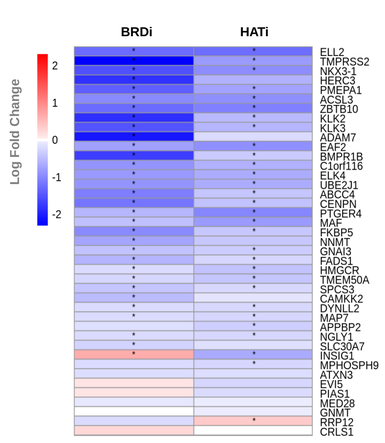
Number of Genes affected



HALLMARK_ANDROGEN_RESPONSE
FDR = 1.16E-19, ES = -0.816, NES = -2.43



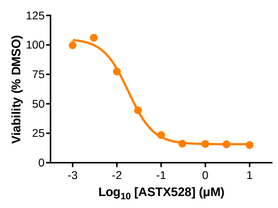
AR signature modulation



- Both HATi and BRDi treatment leads to AR pathway deregulation
- BRDi impacts a greater number of pathways and genes than HATi
- HATi may be a safe therapeutic option for AR-dependent prostate cancers

Potent *in vitro* and *in vivo* effects of the novel HAT domain inhibitor, ASTX528

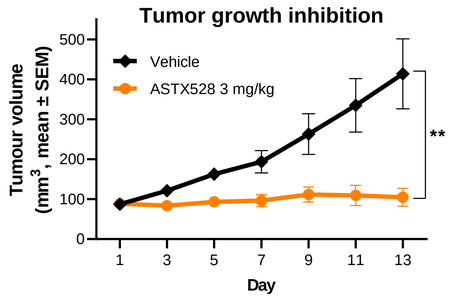
Cell growth inhibition (LNCaP)



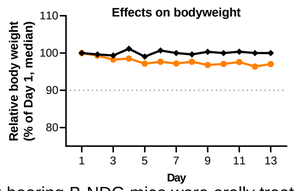
Cell viability was determined after a 6-day incubation with ASTX528 *in vitro*.

***In vivo* activity on LNCaP tumor-bearing mice**

Tumor growth inhibition



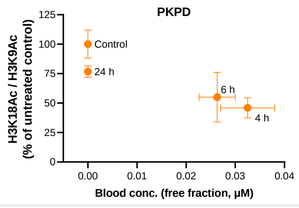
Effects on bodyweight



Tumor-bearing B-NDG mice were orally treated once a day with ASTX528 or vehicle (2% NMP, 0.5% HPMC). n=11. **, *P* <0.01.

PKPD were demonstrated after a single dose at 3 mg/kg (below). Sampling timepoints are indicated.

PKPD



- ASTX528, a fragment-derived small-molecule inhibitor of CBP/p300 HAT domain, reduces LNCaP cell viability *in vitro*
- ASTX528 treatment leads to stasis of LNCaP tumors

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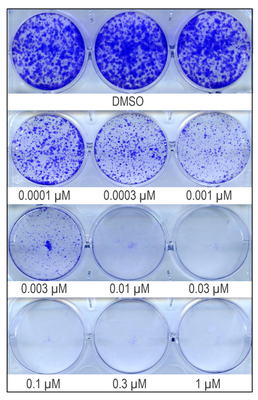
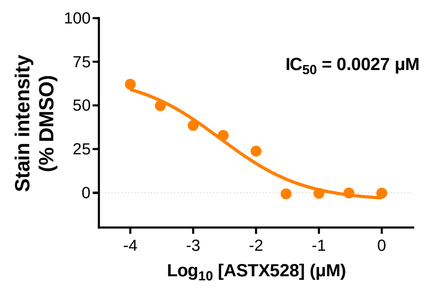
RESULTS: COMBINATIONS IN ER-DEPENDENT BREAST CANCER

ER+ breast cancer cells are sensitive to ASTX528 and synergises with CDK4/6i

ASTX528 colony formation assay

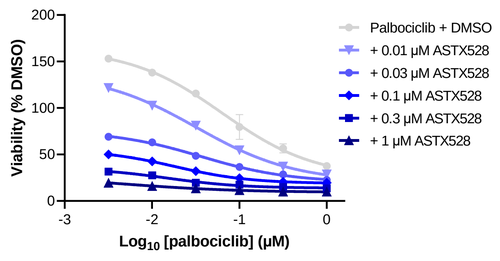




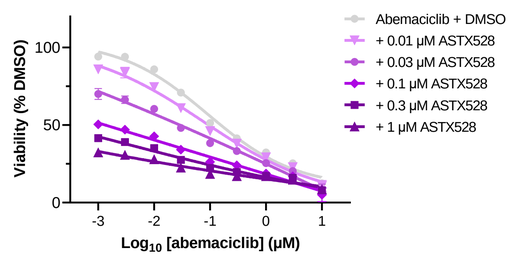
Six-well plates seeded with MCF7 cells were incubated with ASTX528 for 12 days then colonies stained with crystal violet. The stain was dissolved and quantified by absorbance measurements.

ASTX528 + CDK4/6 inhibitor combination

ASTX528 + Palbociclib



ASTX528 + Abemaciclib



Palbociclib (μM)

ASTX528 (μM)	0.003	0.01	0.03	0.1	0.3	1
0.003	-47	-33	-9	32	10	6
0.01	-28	-9	12	39	19	9
0.03	7	13	27	39	28	15
0.1	-5	2	13	20	25	18
0.3	-9	-4	4	6	8	10
1	-3	0	3	4	6	6

Abemaciclib (μM)

ASTX528 (μM)	0.01	0.03	0.1	0.3	10
0.003	1	5	7	2	2
0.01	7	12	12	6	5
0.03	10	23	20	14	10
0.1	10	18	28	19	14
0.3	4	9	18	22	16
1	-1	3	10	12	11

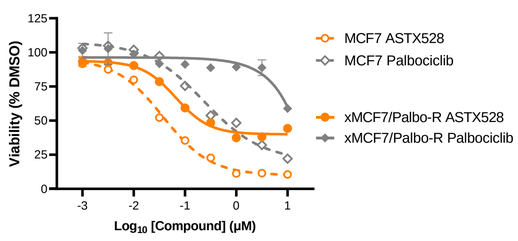
Synergy / 0 / Antagonism

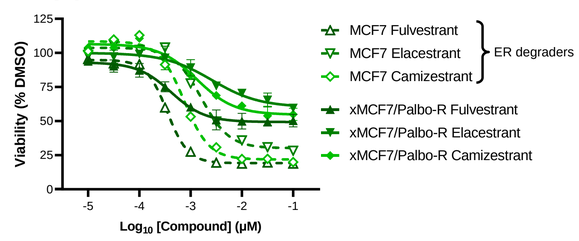
MCF7 cells were incubated with palbociclib (left, 7 days) or abemaciclib (right, 5 days) in the presence or absence of ASTX528 and the viability determined with CellTiter-Glo®. Synergy levels were assessed using Combenefit (HSA).

- ASTX528 alone is effective in reducing colony formation of MCF7 cells
- Combination of ASTX528 and CDK4/6i synergistically reduce MCF7 cell viability

Synergy in MCF7 cells with an acquired resistance to a CDK4/6 inhibitor

Single-agent sensitivity profile





Viability of MCF7 cells and a derivative with an acquired resistance to palbociclib (xMCF7/Palbo-R, WuXi Apptec) were assessed using CellTiter-Glo® after treatment with ASTX528, palbociclib or an ER degrader for 7 days.

Combinations with CDK4/6i and ER degrader

ASTX528 + fulvestrant

Fulvestrant (nM)

ASTX528 (μM)	0.03	0.1	0.3	1	3	10
0.003	-8	-4	15	5	3	6
0.01	0	5	19	8	2	5
0.03	7	7	17	14	6	6
0.1	8	5	13	19	15	17
0.3	2	2	3	3	6	8
1	3	2	3	2	4	5

ASTX528 + palbociclib + fulvestrant

Palbociclib (μM)

ASTX528 (μM)	0.003	0.01	0.03	0.1	0.3	1
0.003	17	18	23	24	21	16
0.01	19	19	26	26	23	18
0.03	12	14	15	15	21	20
0.1	10	7	9	10	13	13
0.3	2	1	3	6	5	4
1	8	2	2	3	3	5

Synergy / 0 / Antagonism

The effects of combining ASTX528 with fulvestrant and palbociclib in the presence of 0.3 nM fulvestrant on cell viability was assessed using Combenefit.

- ASTX528 reduces viability of parental and CDK4/6i-resistant MCF7 cells
- Partial sensitivity to ER degraders is observed in the CDK4/6i-resistant cells
- Combining ASTX528 with fulvestrant synergistically reduces the viability of CDK4/6i-resistant cells which is further enhanced by the addition of CDK4/6i

CONCLUSION

- CBP/p300 is critical for AR and ER signalling and here we show that AR-dependent prostate cancer and ER-dependent breast cancer cell lines are sensitive to CBP/p300 HAT domain inhibition both *in vitro* and *in vivo*
- Currently no CBP/p300 HAT domain inhibitors being evaluated in the clinic, hence, ASTX528 is a novel, potentially first-in-class oncology drug which may provide an orthogonal approach for targeting cancers dependent on these hormone receptors
- Our synergy data also support exploration of combination therapy strategies with hormone therapy and/or CDK4/6 inhibition in ER-driven breast cancer

Reference: (1) Wilson JE, et al (2020) *ACS Med. Chem. Lett.* 11:1324-1329; (2) Welti J et al, (2021) *Cancer Discov.* 11:1118-1137

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