Therapeutic targeting of the p300/CBP bromodomain for the treatment of castration resistant prostate cancer

CellCentric

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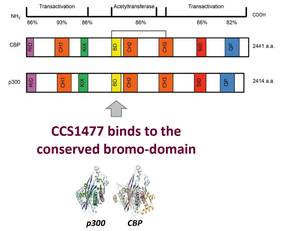
Introduction

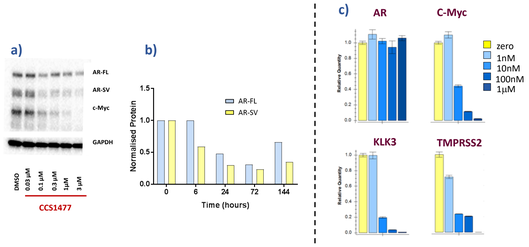
- Sustained androgen receptor pathway activation is the hallmark of castration resistant prostate cancer (CRPC).
- Therapeutic strategies for CRPC include targeted degradation of the androgen receptor (AR) and AR variants (ARV).
- E1A binding protein (p300) and CREB binding protein (CBP) are two closely related histone acetyl transferase proteins that are critical transcriptional regulators of the androgen receptor.
- We have developed CCS1477 which is a potent, selective and orally active small molecule inhibitor of the bromodomain of p300/CBP and have investigated its role in regulating androgen receptor expression and function.

1. CCS1477 is a potent and selective inhibitor of p300/CBP bromodomains



	CCS1477
p300/CBP Kd (nM)	1.3 / 1.7
BRD4 Kd (nM)	222
Selectivity	170
Bromoscan @ 1µM; 32 bromodomains (% cont)	BRD4 (18%); BRD1/2/3/T (15-43%) WDR (33%)
Kinome scan @10µM; 97 kinases	No significant activity
Cerep Safety Screen 44 @10µM	No significant activity

2. CCS1477 degrades AR-FL, AR-SV and c-Myc proteins and reduces expression of AR-target genes



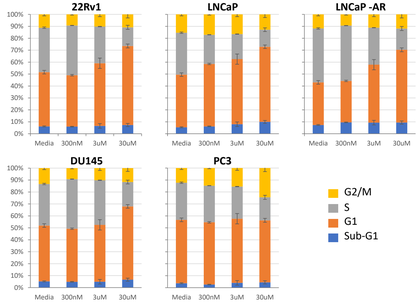
a) Representative Western analysis of AR-FL, AR-SV (V7) and c-Myc protein in 22Rv1 cells after 24h treatment with CCS1477
b) 22Rv1 cells were maintained in 10% charcoal stripped FCS and treated with 1µM (IC90) CCS1477 for the times indicated. Western analysis of AR protein expression quantified by densitometry.
c) qPCR analysis of AR and down-stream genes after treatment with increasing doses of CCS1477 for 72h.

3. CCS1477 preferentially inhibits AR driven prostate cancer cells

Cell Line	AR status	Model	CCS1477 Proliferation IC50 µM
LNCaP	AR-FL	Hormone responsive	0.230
LNCaP-AR	AR-FL over-expressed	CRPC	0.150
VCaP	AR-FL AR-SV	CRPC	0.049
22Rv1	AR-FL AR-SV	CRPC	0.096
DU145	AR negative	Hormone independent	1.280
PC3	AR negative	Hormone independent	1.490

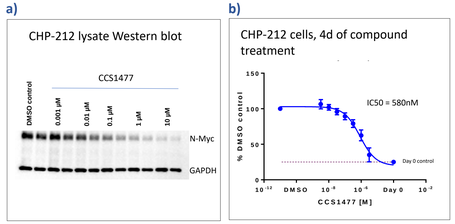
Proliferation measured with a cell viability assay (CyQuant Direct Cell Proliferation or CellTiter Glo) in prostate cancer cells (maintained in 10% FCS) after compound treatment for 72h.

4. CCS1477 inhibits G1/S transition in AR driven cells



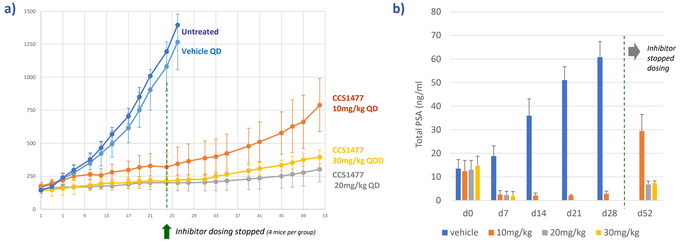
Cell cycle progression was analysed by propidium iodide flow cytometry.

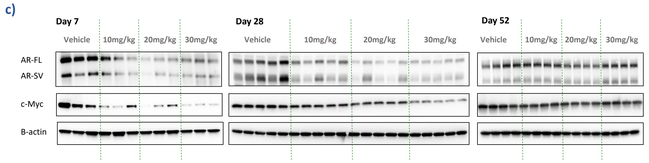
5. CCS1477 inhibits n-Myc protein expression and *in vitro* proliferation in neuroblastoma cells (CHP-212)



a) Representative Western analysis of n-Myc protein expression in CHP-212 cells treated with CCS1477 for 72h.
b) CHP-212 were treated with CCS1477 for 4 days and cell proliferation measured by CellTitre Glo.

6. *In vivo* efficacy in 22Rv1 xenograft: Including continued tumour growth block following drug withdrawal

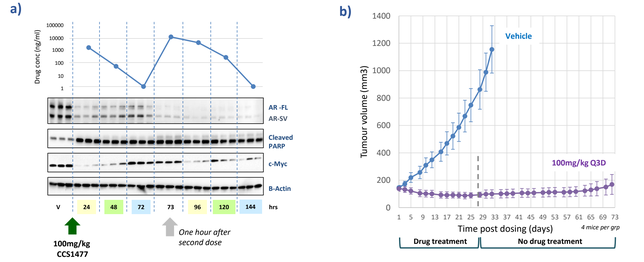




a) 22Rv1 tumour bearing athymic nude mice treated with CCS1477 by oral gavage, once daily (10/20mg/kg) or once every other day (30mg/kg). Vehicle (5% DMSO:95% methylcellulose 0.5%w/v) dosed once daily. A group of 4 untreated controls also included.
b) Plasma PSA measured by ELISA in blood samples collected immediately before first dose and thereafter, at weekly intervals.
c) Western analysis of AR-FL, AR-SV & c-Myc in 22Rv1 tumours taken at day 7, day 28 and at day 52

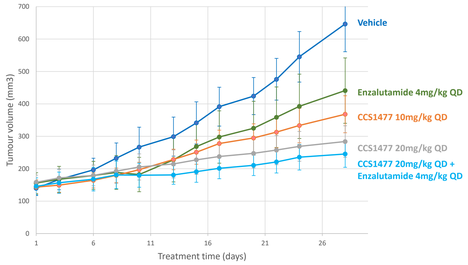
Note: Plasma and tumour levels of CCS1477 are undetectable at day 52.

7. Tumour regression with intermittent high dose of CCS1477: Associated with significant and sustained reduction of AR/cMyc and increase in cleaved PARP



a) Protein biomarkers were measured by Western in tumour lysates collected from 22Rv1 tumours, at 24, 48 and 72h after two oral doses of CCS1477 (100mg/kg) given 0 and 72h. Drug concentration was measured in plasma by LC-MS/MS.
b) In a separate study, 22Rv1 tumour bearing athymic nude mice were treated for 28d with CCS1477 by oral gavage, once every three days (100mg/kg) Vehicle (5% DMSO:95% methylcellulose [0.5%w/v]) was dosed every three days. At day 28, group of 4 animals from the CCS1477 treated group were left untreated, and tumour growth was measured for a further 44 days. Plasma and tumour levels of CCS1477 are undetectable at day 73.

8. *In vivo* efficacy in Bicalutamide resistant LNCaP xenograft: Active as monotherapy and in combination with Enzalutamide

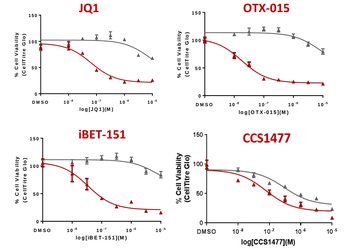


Breakthrough growth of Enzalutamide treatment around day 12-14.

CCS1477 alone, and in combination, has a more sustained effect.

Bicalutamide resistant LNCaP tumour bearing athymic nude mice were treated with Enzalutamide by oral gavage, once daily (4mg/kg), or CCS1477 by oral gavage, once daily (10/20mg/kg) or with Enzalutamide (4mg/kg) and CCS1477 (20mg/kg) combination once daily. Vehicle (5% DMSO:95% methylcellulose [0.5%w/v]) was dosed once daily.

9. CCS1477 retains activity in a BETi resistant 22Rv1 cell-line



	22Rv1_V1 IC50 (µM)	22Rv1_J1 IC50 (µM)	Ratio
JQ1	0.06	7.3	122
JQ2	0.01	3.4	340
iBET-151	0.03	7.7	257
OTX-015	0.02	7.7	385
CCS1477	0.08	0.3	3.8
GNE-272	6	>10	NA
CPI-637	3.5	38	10.8

BETi differentiation

- CCS1477 synergy with JQ1 in 22RV1 cell proliferation assay
- CCS1477 degrades AR protein (FL & SV) but JQ1 has no effect at a comparative proliferation IC50
- Cell line panel testing clearly demonstrates that BETi are more promiscuous compared to CCS1477

A JQ1 resistant cell line was generated by incubating 22Rv1 cells with increasing doses of either JQ1 (22Rv1-J1) or with vehicle (22Rv1-V1) over a 9 month period. Proliferation was measured with a cell viability assay (CellTiter Glo) after compound treatment for 72h.

Conclusions

- CCS1477 is a potent, selective and orally bioavailable inhibitor of p300/CBP bromodomains.
- CCS1477 causes profound tumour growth inhibition in xenograft models of prostate cancer at tolerated doses. Changes in biomarkers that are consistent with an androgen receptor-mediated mechanism of action.
- Extended duration of tumour growth inhibition in the absence of drug.
- Bromodomain inhibition of p300/CBP represents a differentiated approach to targeting androgen receptor pathway activation in castration resistant prostate cancer.
- CCS1477 is currently in pre-clinical evaluation in preparation for initiation of a Phase 1/1b clinical programme in 2018.