Targeting the bromodomain of p300/CBP for the treatment of castrate resistant prostate cancer

CellCentric

Nigel Brooks, Neil Pegg and Will West. CellCentric Ltd, Chesterford Research Park, Cambridge CB10 1XL, UK

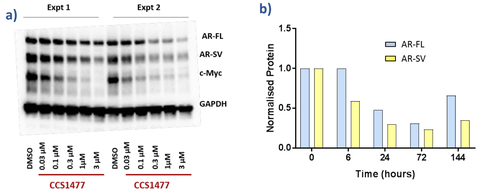
Introduction

- Therapeutic strategies for castrate resistant prostate cancer (CRPC) include targeting degradation of androgen receptor (AR) and androgen receptor variants (AR-SV).
- E1A binding protein (p300) and CREB binding protein (CBP) are two closely related histone acetyl transferase proteins that transcriptionally co-activate AR.
- We have developed potent, selective and orally active small molecule inhibitors of the bromodomain of p300/CBP and report here, their impact on AR, AR-SV and c-Myc expression and function.
- We have also examined their effects in xenograft models of castration resistant prostate cancer as monotherapy and in combination with standard of care agents.

1. CCS1477 potency and selectivity

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

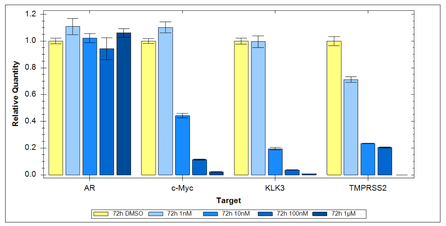
2. CCS1477 degrades AR-FL, AR-SV & c-Myc protein: Including time course of AR-FL, AR-SV reduction



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 1uM (IC90) CCS1477 for the times indicated. Western analysis of AR protein expression quantified by densitometry.

3. CCS1477 reduces expression of AR-target genes in 22Rv1 cells



qPCR analysis of AR and down-stream genes after treatment with increasing doses of CCS1477 for 72h.

4. Inhibition of *in vitro* proliferation

Cell Line	AR status	Model	CCS1477 Proliferation IC50 uM
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

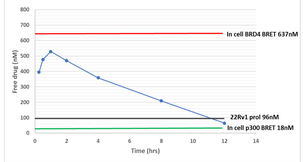
Proliferation was measured with a cell viability assay (CyQuant Direct Cell Proliferation or CellTiter Glo) in prostate cancer cells (maintained in 10% FCS) after compound treatment for 72h.

5. Good oral exposure in mouse: blood levels exceed 22Rv1 proliferation IC50 for several hours

a) Mouse pharmacokinetics

0.5mg/kg iv; 3mg/kg po	
T1/2 (hr)	0.96
Cl obs (ml/min/kg)	14
Vss (L/kg)	1.1
F (%)	73

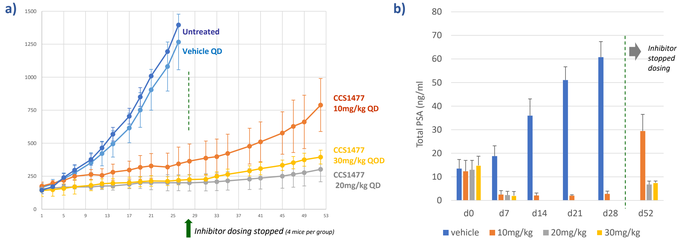
b) Free drug exposure @ 30mg/kg po

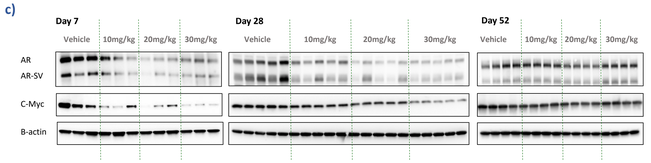


a) Pharmacokinetic parameters dosing iv (0.5mg.kg in 5% DMSO/HPBCD) and oral (3mg/kg in 5% DMSO/MC)

b) Plot of plasma levels of CCS1477 (free drug) following dose of 30mg/kg po in mouse (blue); IC50 value for CCS1477 for inhibition of proliferation of 22Rv1 cells (black); IC50 value for in cell binding of CCS1477 to p300 using BRET assay (green); IC50 value for in cell binding of CCS1477 to p300 using BRET assay (red)

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



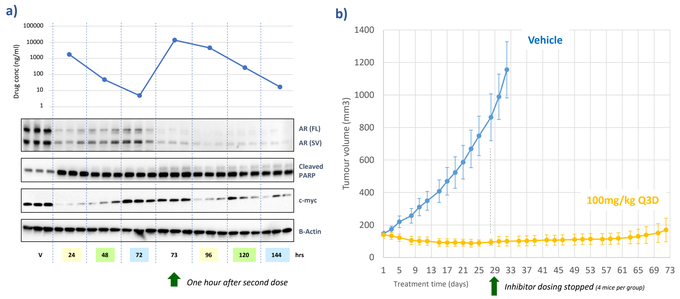


a) 22Rv1 tumour bearing athymic nude mice treated with CCS1477 by oral gavage, once daily (10/20mg/kg) or once every other day (30mg/kg). Vehicle (5% DMSO:95% methylcellulose 0.5%w/v) dosed once daily. A group of 4 untreated controls also included.

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.

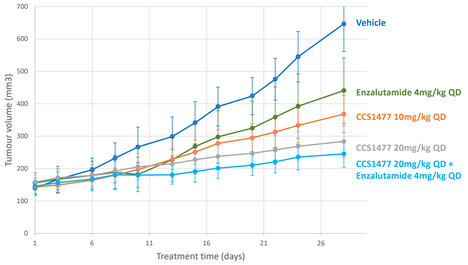
7. Tumour regression with intermittent high dose of CCS1477; associated with significant and sustained reduction of AR/cMyc and increase in cleaved PARP



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

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

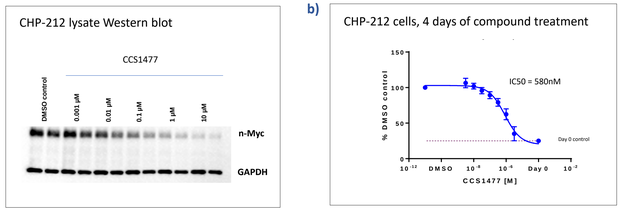


Breakthrough growth of Enzalutamide treatment ~day 12-14.

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 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 72 hours.

b) CHP-212 were treated with CCS1477 for 4 days and cell proliferation measured by CellTitre Glo.

Conclusions

- Small molecule inhibition of the p300/CBP bromodomain leads to down-regulation of AR, AR-SV and c-Myc, as well as inhibiting key downstream genes, including PSA and TMPRSS2.
- CCS1477, a clinical candidate, causes complete tumour growth inhibition in a 22Rv1 xenograft model at doses which are well tolerated. Tumour regression is observed with high dose intermittent doses of CCS1477 and is associated with increased cleaved PARP. The tumour growth inhibition caused by CCS1477 is sustained (>6wks) following drug withdrawal.
- CCS1477 is also efficacious in a bicalutamide resistant LNCaP xenograft model as monotherapy or in combination with Enzalutamide, demonstrating a more sustained effect.
- CCS41477 inhibits n-Myc, an important driver of neuroendocrine prostate cancer.
- CCS1477 is a potential first-in-class p300/CBP inhibitor for the treatment of CRPC