Abstract# 11590

Characterisation of CCS1477: A novel small molecule inhibitor of p300/CBP for the treatment of castration resistant prostate cancer

CellCentric

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Introduction

- Targeted degradation of androgen receptor (AR) and androgen receptor variants (AR-SV) remains an important therapeutic opportunity for patients with castration resistant prostate cancer.
- E1A binding protein (p300) and CREB binding protein (CBP) are two closely related histone acetyl transferase proteins that act as translational co-activators of 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

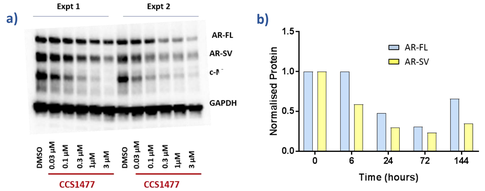
	CCS1477 Clinical candidate
p300/CBP Kd (nM)	1.3/1.7
BRD4 Kd (nM)	222
Selectivity	170
Bromoscan @ 1μM; 32 bromodomains (% control)	BRD4 (18%); BRD1/2/3/T (15-43%) WDR (33%)
Kinome scan @10μM; 97 kinases	No significant activity
Cerep Safety Screen 44 @10μM	No significant activity

2. Inhibition of *in vitro* proliferation

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

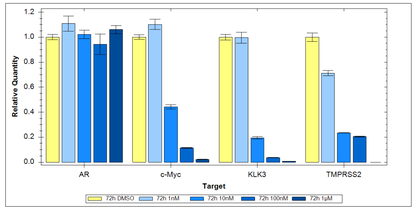
Proliferation was measured with a cell viability assay (CyQuant Direct Cell Proliferation or CellTiter Glo) in prostate cancer cells (maintained in 10% FCS) after compound treatment for 72h.

3. 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.

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



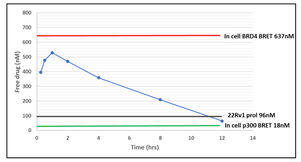
qPCR analysis of AR & down-stream genes after treatment with increasing doses of CCS1477 for 72h.

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

a) Mouse pharmacokinetics

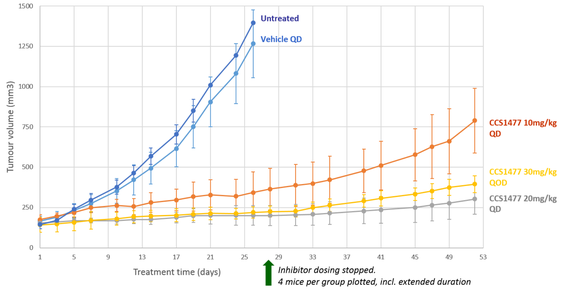
0.5mg/kg iv; 3mg/kg po	
T1/2 (hr)	0.96
Cl obs (ml/min/kg)	14
Vss (L/kg)	1.1
F (%)	73

b) Free drug exposure @ 30mg/kg po



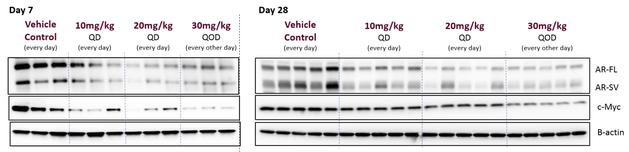
a) Pharmacokinetic parameters dosing iv (0.5mg.kg in 5% DMSO/HPBCD) and oral (3mg/kg in 5% DMSO/MC)
b) Plot of plasma levels of CCS1477 (free drug) following dose of 30mg/kg po in mouse (blue); IC50 value for CCS1477 for inhibition of proliferation of 22Rv1 cells (black); IC50 value for in cell binding 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



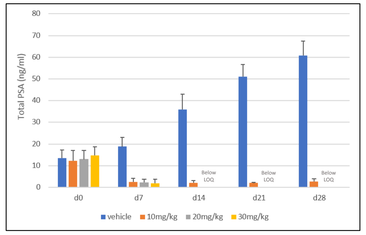
22Rv1 tumour bearing athymic nude mice were treated with CCS1477 by oral gavage, once daily (10/20mg/kg) or once every other day (30mg/kg). Vehicle (5% DMSO:95% methylcellulose [0.5%w/v]) was dosed once daily. A group of 4 untreated controls were also included.

7. Protein biomarkers are reduced in 22Rv1 tumour bearing animals treated with CCS1477 for 7 and 28 days



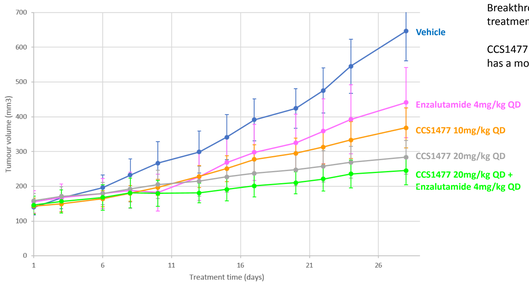
Western analysis of AR-FL, AR-SV, c-Myc in 22Rv1 tumours taken from a satellite group at day 7 of the study shown in Fig. 6 and at the end of the same study at day 28.

8. Plasma PSA is reduced in 22Rv1 tumour bearing animals treated with CCS1477



Plasma PSA was measured by ELISA in blood samples collected immediately before first dosing and thereafter, at weekly intervals from the mice treated with CCS1477 in the 22Rv1 xenograft study shown in Fig. 6

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

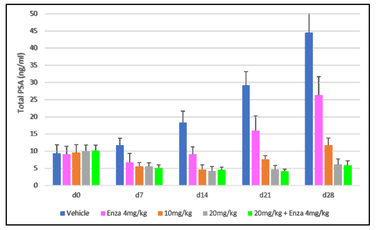


Breakthrough growth of Enzalutamide treatment ~day 12-14.

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.

10. Plasma PSA is reduced in bicalutamide resistant LNCaP tumour bearing animals treated with CCS1477



Plasma PSA was measured by ELISA in blood samples collected immediately before first dosing and thereafter, at weekly intervals from the mice treated with CCS1477 in the bicalutamide resistant LNCaP xenograft study shown in Fig. 9

Conclusions

- **Small molecule inhibition of the bromodomain of p300/CBP, 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. The tumour growth inhibition caused by CCS1477 is sustained 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.**
- **CCS1477 is a potential first-in-class p300/CBP inhibitor for the treatment of CRPC, and potentially in the future, of tumours harbouring p300 and CBP mutations.**