A novel small molecule inhibitor of p300/CBP for the treatment of castration resistant prostate cancer – preclinical evaluation

CellCentric

Nigel Brooks, Neil Pegg, Jenny Worthington[#], Barbara Young[+], Amy Prosser[+], Jordan Lane[+], David Taddei[+], Matthew Schiewer*, Nicolas Gordon*, Karen Knudsen*

CellCentric Ltd, Cambridge UK; [#]Axis Bioservices, Coleraine, UK; [+]Sygnature Discovery, Nottingham UK; *The Sidney Kimmel Cancer Centre at Thomas Jefferson University, Philadelphia, USA

Introduction

- Targeted degradation of androgen receptor (AR) and androgen receptor variants (ARV) 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
- p300/CBP, as well as AR and ARV, are elevated in prostate cancer tumours following androgen deprivation therapy and through progression to castrate resistant prostate cancer (CRPC)
- We have developed potent, selective and orally active small molecule inhibitors of the bromodomain of p300/CBP and report here for the first time, their impact on AR and ARV expression and function.

1. CCS series selectivity

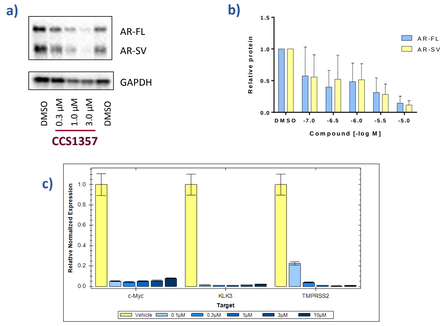
	CCS1357	CCS1477
	In vitro probe compound	Clinical candidate
p300/CBP Kd (nM)	3.6/5.0	1.3/1.7
BRD4 Kd (nM)	245	222
Selectivity	68	170
Bromoscan @ 1µM; 32 bromodomains (% control)	BRD4 (15%); BRD1/2/3/T (15-48%) WDR (23%); SMARCA4 (7%)	BRD4 (18%); BRD1/2/3/T (15-43%) WDR (33%)
Kinome scan @10µM; 97 kinases	No significant activity	No significant activity
Cerep Safety Screen 44 @10µM	No significant activity	No significant activity

2. Inhibition of *in vitro* proliferation by CCS1357

Cell Line	AR status	Model	CCS1357 Proliferation IC50 µM	CCS1477 Proliferation IC50 µM
LNCaP	AR-FL	Hormone responsive	1.3	
LNCaP-AR	AR-FL over-expressed	CRPC	0.13	
VCaP	AR-FL AR-SV	CRPC	0.4	0.049
22Rv1	AR-FL AR-SV	CRPC	0.3	0.096
C42	AR-FL	CRPC	0.24	
DU145	AR negative	Hormone independent	2.7	
PC3	AR negative	Hormone independent	2.0	

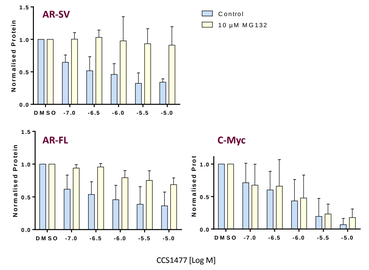
Proliferation was measured with a cell viability assay (CellTitre Glo) in 22Rv1 cells (maintained in 10% FCS) after compound treatment for 72h.

3. CCS1357 degrades AR-FL & AR-SV protein & reduces expression of AR-target genes in 22Rv1 cells



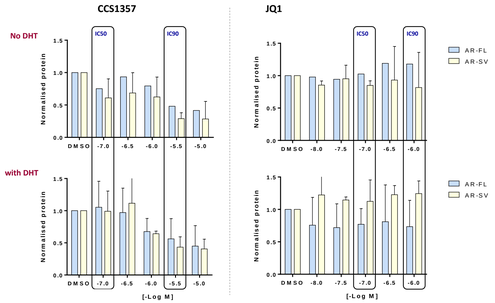
a) Representative Western analysis of AR and ARSV (V7) in 22Rv1 cells after 72h of cpd treatment. b) Average (+/-SD) protein expression quantified by densitometry from 3 independent cell culture experiments. c) qPCR analysis of down-stream genes after treatment with increasing doses of cpd for 24h.

4. CCS1357 degradation of AR, but not c-Myc protein, is reversed by simultaneous inhibition of MG132, a proteasome inhibitor



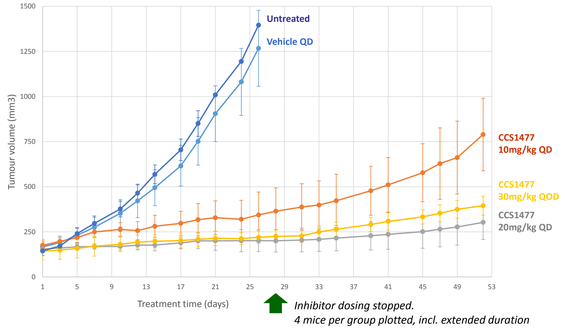
22Rv1 cells were maintained in 10% charcoal stripped FCS and treated for 24h with CCS1357 given alone or in combination with MG132 (10µM)

5. CCS1357 degrades AR & ARV protein; JQ1 (BRD4 inhibitor) has no effect



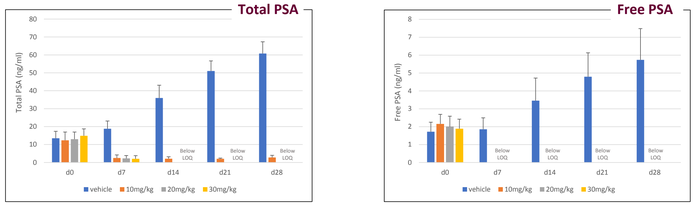
22Rv1 cells were maintained in 10% charcoal stripped FCS in the absence or presence of 10nM DHT and treated for 72h with increasing doses of CCS1357 or JQI. The 22Rv1 cell proliferation IC50s and IC90s for each compound are highlighted by the boxes.

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



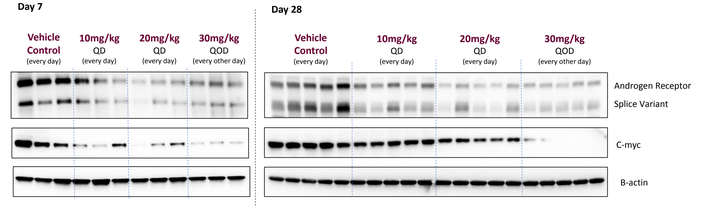
22Rv1 tumour bearing athymic nude mice were randomisation to control and treatment arms when tumours had reached a volume of approximately 160mm^3. Animal body weight and individual tumour volumes were measured 3x week. Compound was dosed once daily (10/20mg/kg) or once every other day (30mg/kg) by oral gavage. Vehicle (5% DMSO:95% methylcellulose (0.5%w/v) was dose once daily. A group of 4 untreated controls were also included.

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



Plasma PSA (free and total) 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 Section 6.

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



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

Conclusions

- Small molecule inhibition of the bromo-domain 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. Inhibition is seen of tumour AR, AR-SV and c-Myc, as well as plasma PSA.
- The tumour growth inhibition caused by CCS1477 is sustained following drug withdrawal.
- Inhibiting the bromo-domain of p300/CBP represents a promising new approach to targeting AR expression and function.
- CC1477 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.