Abstract # 3991

Novel small molecule inhibitor of p300/CBP down-regulates androgen receptor (AR) and c-Myc for the treatment of prostate cancer and beyond

CellCentric

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Introduction

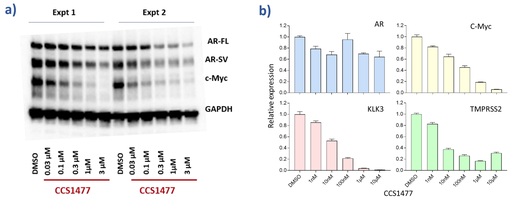
- Targeted degradation of androgen receptor (AR) and androgen receptor variants (AR-SV) remains an important therapeutic opportunity for patients with castration resistant prostate cancer.
  - E1A binding protein (p300) and CREB binding protein (CBP) are two closely related histone acetyl transferase proteins that act as translational co-activators of AR.
  - We have developed the clinical candidate, CCS1477, which is a potent, selective and orally active small molecule inhibitor of the bromodomain of p300/CBP and we report here its impact on AR, AR-SV and c-Myc expression and function.
- We have also extended the evaluation of CCS1477 into other disease settings, including haematological cancers, and those tumours with loss of function mutations in p300/CBP providing susceptibility to synthetic lethality (e.g. bladder cancer).

1. CCS1477: Inhibits prostate cancer cell 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.

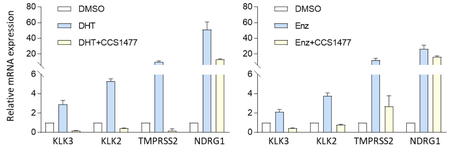
2. CCS1477 degrades AR-FL, AR-SV & c-Myc protein in 22Rv1 cells: Including downstream genes



Representative Western analysis of AR-FL, AR-SV (V7) and c-Myc protein in 22Rv1 cells after 24h treatment with CCS1477

qPCR of analysis of AR, c-Myc and AR-target genes after 24h incubation in 22Rv1 cells

3. CCS1477 inhibits DHT and enzalutamide agonist activity at AR F876L



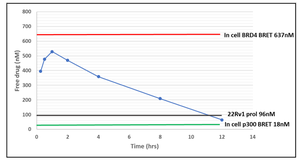
LNCaP-AR$_{F876L}$ cells stably-express AR$_{F876L}$ and demonstrate enhanced AR target gene expression in response to both DHT and enzalutamide. Cells were grown in steroid-depleted media for 48 hours prior to stimulation with 1nM DHT or 1µM enzalutamide in the presence and absence of 0.5µM CCS1477 for 24hrs.

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

a) Mouse pharmacokinetics

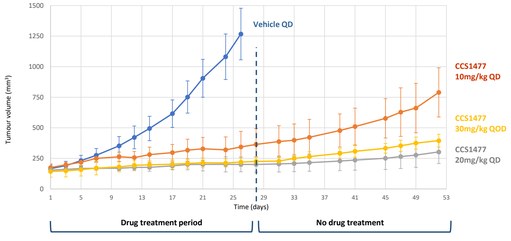
0.5mg/kg iv; 3mg/kg po	
T1/2 (hr)	0.96
Cl obs (ml/min/kg)	14
Vss (L/kg)	1.1
F (%)	73

b) Free drug exposure @ 30mg/kg po



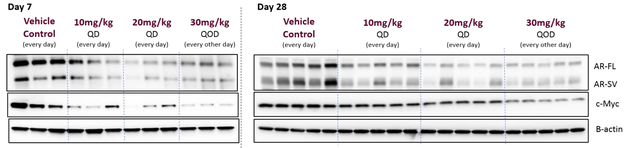
a) Pharmacokinetic parameters after iv (0.5mg/kg in 5% DMSO/HPBCD) and oral (3mg/kg in 5% DMSO/MC) dosing; b) Plasma levels of CCS1477 (free drug) following an oral dose of 30mg/kg in mouse (**blue**); IC50 value for CCS1477 for inhibition of proliferation of 22Rv1 cells (black); IC50 value for in cell binding of CCS1477 to p300 using BRET assay (**green**); IC50 value for in cell binding of CCS1477 to BRD4 using BRET assay (**red**)

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



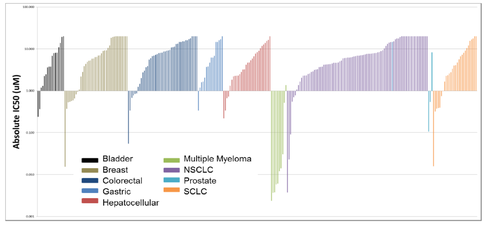
22Rv1 tumour bearing athymic nude mice were treated with CCS1477 by oral gavage, once daily (10/20mg/kg) or once every other day (30mg/kg). Vehicle (5% DMSO:95% methylcellulose [0.5%w/v]) was dosed once daily.

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



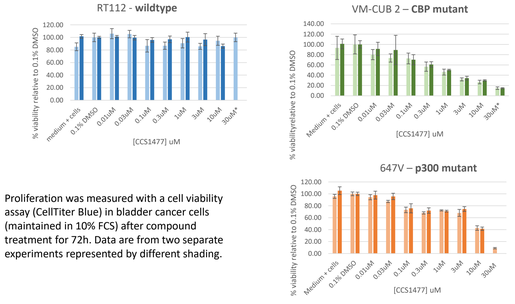
Western analysis of AR-FL, AR-SV, c-Myc in 22Rv1 tumours taken from a satellite group at day 7 of the study shown in Fig. 5 and at the end of the same study at day 28.

7. Breadth of efficacy screening with CCS1477 demonstrates subsets of cell lines which are sensitive: including prostate cancer and beyond



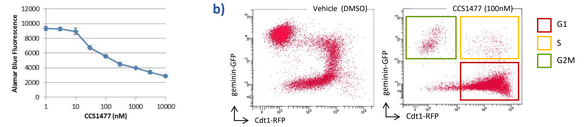
Proliferation was measured with a cell viability assay (CellTiter Glo) in panel of 277 cancer cell lines after compound treatment for 7 days.

8. Differential sensitivity to CCS1477 in bladder cancer cell lines with p300 or CBP mutations compared to wild type



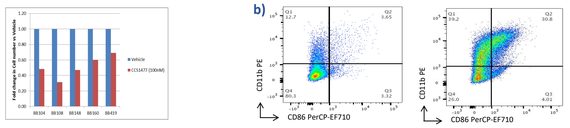
Proliferation was measured with a cell viability assay (CellTiter Blue) in bladder cancer cells (maintained in 10% FCS) after compound treatment for 72h. Data are from two separate experiments represented by different shading.

9. CCS1477 inhibits the proliferation of AML cells mediated by G1 cell cycle arrest



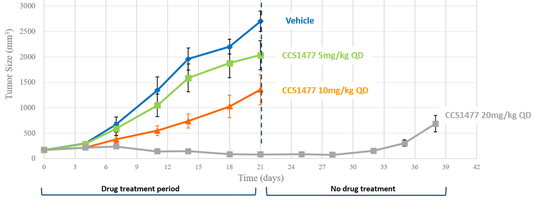
(a) Inhibition of proliferation of THP-1 cells after 48hrs incubation; (b) Fucci flow analysis of THP-1 cells following incubation with DMSO vehicle or CCS1477 (100nM) for 48 hrs .

10. CCS1477 inhibits the proliferation of patient derived primary AML cells and promotes myeloid differentiation



(a) Inhibition of proliferation of patient derived primary AML cells after 7 days treatment with 100nM CCS1477; (b) Flow cytometry analysis of differentiation markers (CD86 and CD11b) in patient derived cells (patient BB108) following treatment with DMSO vehicle or CCS1477 (100nM) for 7 days.

11. *In vivo* efficacy in AML MOLM16 xenograft: Including continued tumour growth inhibition following drug withdrawal



MOLM-16 tumour bearing NOD / SCID mice were treated with CCS1477 by oral gavage, once daily (5/10/20mg/kg). Vehicle (5% DMSO:95% methylcellulose [0.5%w/v]) was dosed once daily.

Conclusions

- CCS1477, a p300/CBP inhibitor and clinical candidate, demonstrates complete tumour growth inhibition in selected xenograft models at doses which are well tolerated.
- The tumour growth inhibition caused by CCS1477 is sustained following drug withdrawal.
- These data support the clinical testing of CCS1477 in patients in three settings (i) CRPC through down-regulating AR, AR-SV and c-Myc; (ii) haematological cancers, in particular AML, through effects on cell cycle arrest and myeloid differentiation, and (iii) in patients with loss of function mutations in p300 or CBP by driving synthetic lethality.