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

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1. **CCS1477 potency and selectivity**

<table>
<thead>
<tr>
<th>Compound</th>
<th>p300/CBP Kd (nM)</th>
<th>Bromoscan @ 1uM</th>
<th>Selectivity (p300/CBP to BRD4)</th>
<th>Kinome scan @10uM</th>
<th>Cell viability</th>
<th>AR-FL</th>
<th>AR-SV</th>
<th>c-Myc</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCS1477</td>
<td>0.130</td>
<td>0.150</td>
<td>1.7</td>
<td>97</td>
<td>50</td>
<td>0.150</td>
<td>0.150</td>
<td>0.150</td>
</tr>
</tbody>
</table>

2. **CCS1477 degrades AR-FL, AR-SV & c-Myc protein:**

- **In-vitro proliferation**
  - **Cell Line**
    - LNCaP-AR: AR-FL over-expressed CRPC
    - DU145: AR negative Hormone independent
    - VCaP: AR-FL Hormone responsive
  - **CCS1477**
    - LNCaP-AR: 0.150 uM
    - DU145: 1.280 uM
    - VCaP: 0.230 uM

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

- Pharmacokinetic parameters dosing iv (0.5mg.kg in 5% DMSO/HPBCD) and oral (3mg/kg in 5% DMSO/MC)
  - **Vss (L/kg)**
    - 1.1
  - **Cl obs (ml/min/kg)**
    - 14

4. **Inhibition of ar stimulation**

- **Cell Line**
  - LNCaP-AR: AR-FL over-expressed CRPC
  - DU145: AR negative Hormone independent
  - VCaP: AR-FL Hormone responsive
- **CCS1477**
  - LNCaP-AR: 0.150 uM
  - DU145: 1.280 uM
  - VCaP: 0.230 uM

5. **CCS1477 reduces AR-target genes in 22Rv1 cells**

- CHP-212 were treated with CCS1477 for 72 hours.  Proportional analysis of n-Myc protein expression in CHP-212 cells treated for 72 hours with CCS1477

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

- Bicalutamide resistant LNCaP tumour bearing athymic nude mice were treated with Enzalutamide by oral gavage, once daily (4mg/kg), or CCS1477 for end point androgen withdrawal. Vehicle (5% DMSO:95% methylcellulose [0.5%w/v]) was dosed every three days. At day 28, group of 4 animals was dosed with Enzalutamide once daily (4mg/kg), or CCS1477 10mg/kg once every three days or 20mg/kg once every three day.  Tumour volume (mm3) was measured weekly until day 52.

7. **Tumour regression with intermittent high dose of CCS1477:**

- a) b) c) d) Representative Western analysis of n-Myc protein expression in CHP-212 cells treated with CCS1477 for 72 hours.  Plasma PSA measured by ELISA in blood samples collected immediately before first dose and thereafter, at weekly intervals.

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

- 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 (4-weeks) 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 efficacy. CCS1477 inhibits n-Myc, an important driver of neuroendocrine prostate cancer.

9. **CCS1477 inhibits n-Myc protein expression and in vivo 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-actin was used as a control.

10. **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 (4-weeks) 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 efficacy. CCS1477 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.