

# CCS1477, a potent and selective p300/CBP bromodomain inhibitor, is targeted and differentiated from BET inhibitors in prostate cancer cell lines *in vitro*

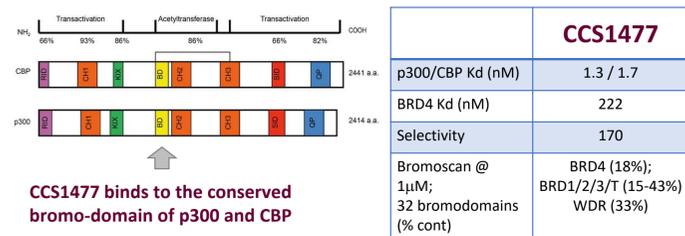
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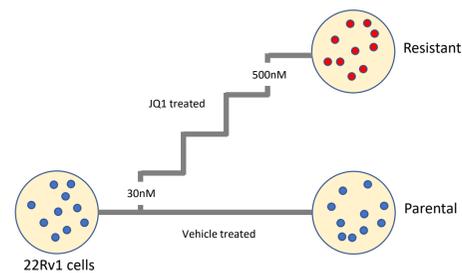
## Introduction

- CCS1477 is a potent and selective p300/CBP bromodomain inhibitor, currently in a Phase 1 clinical trial for patients with metastatic castrate resistant prostate cancer (mCRPC)
- CCS1477 inhibits the expression and function of the androgen receptor, including mutant, amplified and splice variant forms, as well as c-Myc
- Bromodomain and extra-terminal domain (BET) inhibitors are also undergoing clinical evaluation in prostate cancer
- We have established a BET inhibitor (BETi) resistant 22Rv1 prostate cell line and used this, alongside parental 22Rv1 cells, to characterize the differential effects of CCS1477 p300/CBP vs BET bromodomain inhibition

### 1. CCS1477 is a potent and selective inhibitor of p300/CBP bromodomains

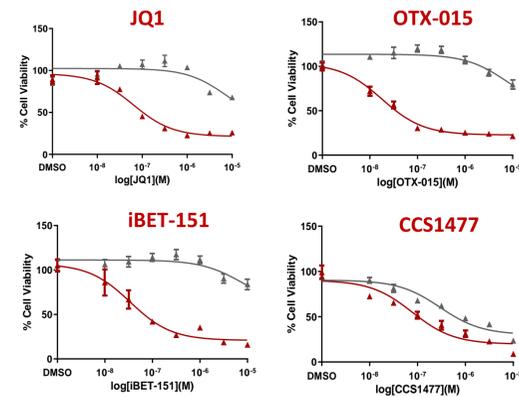


### 2. Development of JQ1 resistant 22Rv1 cells



Cell lines were generated by incubating 22Rv1 cells with increasing doses of JQ1 (Resistant) or with vehicle (Parental) over a 6 month period.

### 3. CCS1477 retains antiproliferative activity in JQ1 resistant 22Rv1 cells

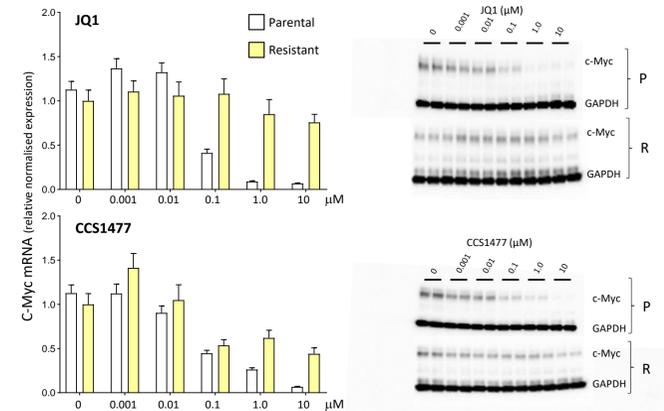


Proliferation was measured with a cell viability assay (CellTiter Glo) after compound treatment for 72h. Red line = Parental; Grey line = Resistant

	Parental 22Rv1 IC50 (µM)	Resistant 22Rv1 IC50 (µM)	Ratio
JQ1	0.06	7.3	122
iBET-151	0.03	7.7	257
OTX-015	0.02	7.7	385
<b>CCS1477</b>	<b>0.08</b>	<b>0.3</b>	<b>3.8</b>

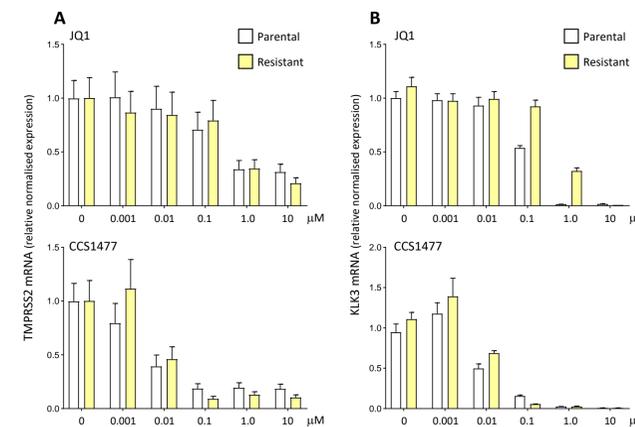
Comparison of proliferation IC50 between parental vs resistant cell lines after 72h treatment with various BET inhibitors (JQ1, iBET, OTX) or CCS1477.

### 4. Inhibitory effect of JQ1 on c-Myc gene and protein expression is abrogated in resistant cells



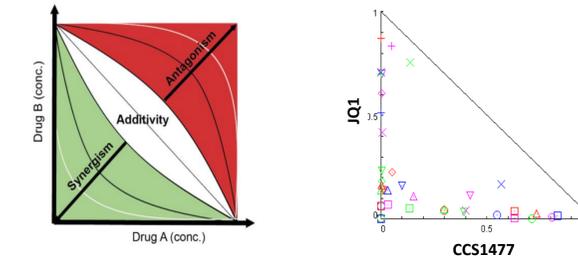
1. c-Myc mRNA expression following 24h treatment with JQ1 or CCS1477 in parental and resistant cell lines. Bars represent quadruplicate measurements with error bars representing SEM. c-Myc protein measured by Western analysis (Abcam, ab32072).
2. No difference in expression of AR-full length or ARV7 splice variant mRNA/protein between JQ1 and CCS1477 and no difference between parental and resistant cells.

### 5. Potent inhibitory effect of CCS1477 on TMPRSS2 & KLK3 gene expression is greater vs. JQ1



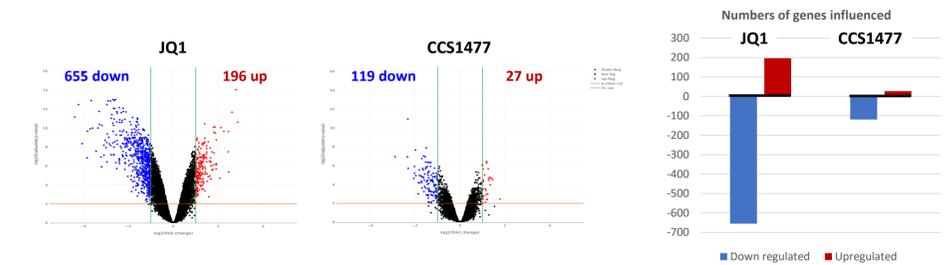
- A) TMPRSS2 mRNA expression following 24h treatment with JQ1 or CCS1477 in parental and resistant cell lines. Bars represent quadruplicate measurements with error bars representing SEM.
- B) KLK3 mRNA expression following 24h treatment with JQ1 or CCS1477 in parental and resistant cell lines.

### 6. Synergy between CCS1477 and JQ1 in parental 22Rv1 cells indicates differentiated mode of action



Cell proliferation was measured with a cell viability assay (CellTiter Glo) after treatment of parental 22Rv1 cells with various doses of CCS1477 in combination with JQ1. Combination effects were assessed using an Isobologram analysis.

### 7. Significantly fewer genes are altered after CCS1477 vs. JQ1 in parental 22Rv1 cells



Volcano plot/histogram showing up and down regulated genes after treatment of 22Rv1 cells treated with either JQ1 or CCS1477. Parental 22Rv1 cells were treated for 24h with 500nM of CCS1477 or JQ1. Extracted RNA was profiled on Affymetrix gene expression microarrays (Clariom D)

## Conclusions

These studies provide three lines of evidence for a differentiated mode of action of CCS1477 vs BETi

1. CCS1477 continues to inhibit proliferation and relevant response biomarkers in a cell line that is resistant to BETi
2. There is a synergistic, rather than additive effect of combining CCS1477 with JQ1 on cell proliferation
3. Significantly fewer genes and a distinct pattern of gene change after CCS1477 vs JQ1

Collectively, these data point to a differentiated and more selective profile of p300/CBP inhibition with CCS1477

