Abstract # 1019

p300/CBP inhibitor CCS1477 targets 22Rv1 prostate tumour AR and c-Myc gene expression *in vivo*.

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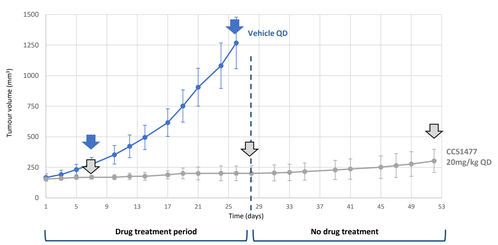
Introduction

- Histone acetyl transferases E1A binding protein (p300) and CREB binding protein (CBP) are co-activators of key transcription factors that contribute to prostate tumour progression including androgen receptor (AR), HIF1α, and c-Myc.
- A large proportion of AR regulated gene expression has been shown to be dependent on p300/CBP either through direct regulation of AR interaction with promoters of AR regulated genes or subsequent histone modification events. Both p300 and CBP are highly expressed in advanced prostate cancer and androgen deprivation leads to upregulation of both proteins.
- CCS1477 is a potent, selective inhibitor of the bromodomain in p300/CBP that has been shown to inhibit prostate tumour cell proliferation *in vitro* and tumour growth *in vivo*.

1. CCS1477 significantly inhibited 22Rv1 tumour growth with prolonged tumour growth inhibition after cessation of treatment



22Rv1 tumour cells were transplanted s/c and mice bearing established tumours ~150mm3 were treated with CCS1477 p.o. once daily 20mg per kg. Treatment was terminated after 28 days and tumour growth in animals treated with CCS1477 was monitored to day 52. Tumours were recovered from 3 animals in control or treated groups at the times indicated (⬇ controls, ⇩ treated)

Methods

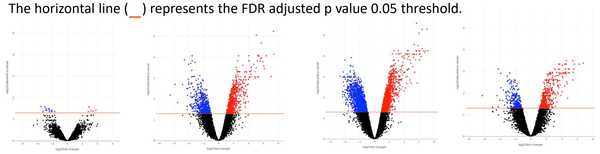
- Gene expression analysis was performed using Affymetrix Clariom D microarrays. Data QC and statistical analysis was performed to identify potential outliers using Hoeffding's D-statistic, Pearson correlation, Euclidean distance and Kolmogorov-Smirnov tests. Pairwise comparisons were performed to identify differentially expressed genes.

  CCS1477 20mg per kg day 7 vs. Vehicle control day 7
  CCS1477 20mg per kg day 28 vs. Vehicle control day 28
  CCS1477 20mg per kg day 52 vs. Vehicle control day 28
  CCS1477 20mg per kg day 52 vs. Vehicle control day 7

- Fold change cut-off values of 1.5 and 2 and a False Discovery Rate (FDR) p-value <0.05 were used to identify significant changes in gene expression.

2. CCS1477 significantly modified the expression of a subset of expressed genes

Volcano plots showing $\log_2$ fold-change plotted against -$\log_{10}$ transformed p-values are shown below. Each dot represents an individual gene plotted as up- (red) or down-regulated (blue) dots. The horizontal line (_) represents the FDR adjusted p value 0.05 threshold.



CCS1477 20mg per kg day 7 vs Vehicle day 7 | **CCS1477 20mg per kg day 28 vs Vehicle day 28** | **CCS1477 20mg per kg day 52 vs Vehicle day 28** | **CCS1477 20mg per kg day 52 vs Vehicle day 7**

3. CCS1477 p300/CBP inhibition both increased and reduced the expression of similar numbers of genes

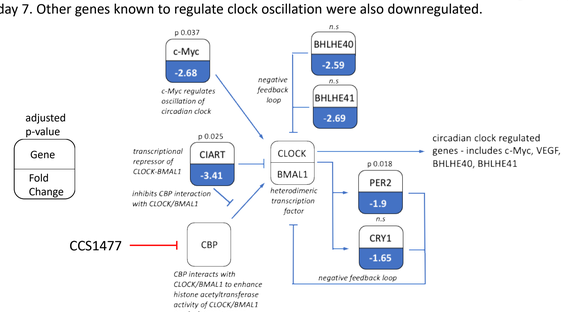
The number of up- and down-regulated genes in CCS1477 treated tumours with an adjusted p-value <0.05 for each comparison together with the number of genes with >2 fold change and adjusted p-value <0.05 is shown in the table below:

Comparison	Number significant p<0.05	> 2 fold change and p<0.05	
		Increased	Reduced
CCS1477 20mg per kg Day 7 vs Vehicle control Day 7	20	6	13
CCS1477 20mg per kg Day 28 vs Vehicle control Day 28	1355	350	283
CCS1477 20mg per kg Day 52 vs Vehicle control Day 28	3144	436	772
CCS1477 20mg per kg Day 52 vs Vehicle control Day 7	791	220	132

In addition to know targets of p300/CBP, a threshold of >2 fold change and adjusted p-value <0.05 at more than one timepoint was used to identify up- and down-regulated gene expression in response to CCS1477 treatment.

4. CSS1477 potentially disrupts circadian regulation

c-Myc and CBP are key regulators of the circadian clock that regulates the expression of ~10% of all expressed genes. CIART, a repressor of CLOCK/BMAL1 was the most significantly downregulated gene at day 7. Other genes known to regulate clock oscillation were also downregulated.



5. CCS1477 inhibited androgen regulated gene expression

TMPRSS2, TMEFF2 and TM4SF1 were all significantly downregulated at day 28. Modest reduction in AR expression was maintained after treatment cessation. Expression of KLK3 (PSA) was not significantly changed (data not shown). ETS2 has been reported to be regulated by both AR and AR variants.

Fold change in expression compared to control is indicated for each comparison after 7 and 28 days treatment. Fold change at day 52, 24 days after treatment cessation is presented compared to day 28 (52^{28}) or day 7 (52^{7}) vehicle controls. Significant fold change with adjusted p-value <0.05 is indicated (*).

6. CCS1477 inhibited c-Myc, VEGF and HIF regulated gene expression

c-Myc expression was downregulated at day 7 and day 28 and was increased with tumour regrowth at day 52. NRDG1, a target of both c-Myc and AR was downregulated at day 28. VEGFA was downregulated at all timepoints. The histone lysine demethylase KDM3A and two of its target genes PLOD2 and P4HA1 implicated in HIF1α hypoxia response were significantly downregulated at day 28 and day 52.

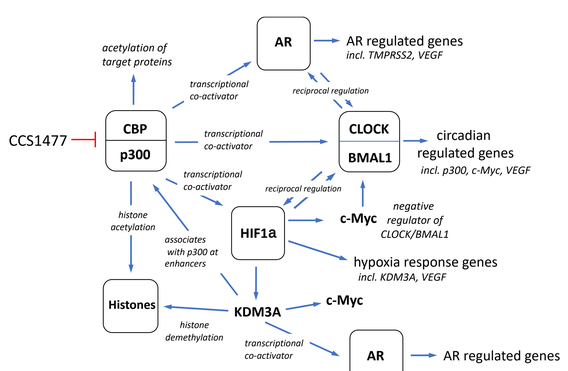
7. CCS1477 significantly upregulated histone expression

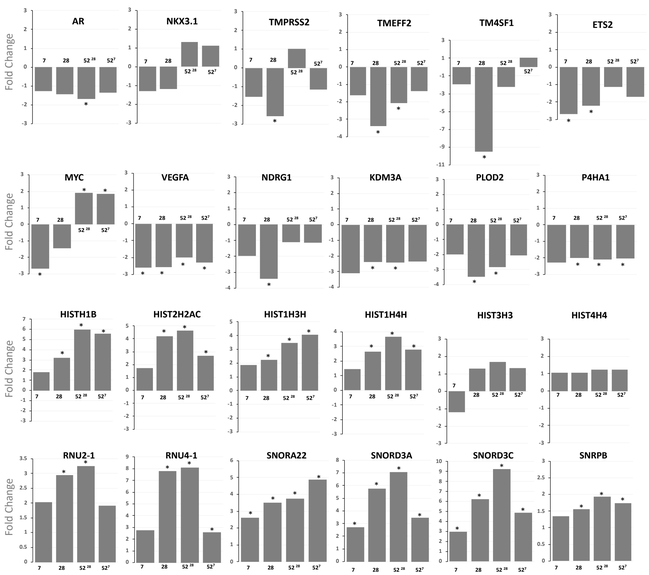
Histone RNAs comprising the core nucleosome (H2, H3, H4) and linker histone (H1) were among the most highly upregulated targets (examples shown here). Accumulation of histones may be a contributor to growth suppression through genomic instability and cell cycle disruption.

8. CCS1477 upregulated non-coding RNAs

Non-protein coding RNAs representing core components of the spliceosome were significantly increased at all timepoints suggesting a widespread disruption of RNA translation. c-Myc has been implicated as a master regulator of ribosome biogenesis and spliceosome function.

Potential impact of CCS1477 on key pathways in prostate cancer





Conclusions

1. Inhibition of p300/CBP activity in 22Rv1 prostate tumour xenografts resulted in widespread alteration of gene expression.
2. Histone and non-protein coding RNAs were significantly upregulated at all timepoints suggesting disruption of transcription and translation in addition to the impact of p300/CBP inhibition on protein and histone acetylation
3. Downregulation of VEGFA expression together with downregulation of hypoxia related genes KDM3A, PLOD2 and P4HA1 suggest that angiogenesis is inhibited in response to CCS1477 treatment.
4. Response to p300/CBP inhibition and the prolonged duration of tumour growth inhibition following treatment withdrawal likely results from a complex interacting network of key pathways regulated by AR, c-Myc, and chromatin disruption.