

# Therapeutic targeting of the p300/CBP bromodomain for the treatment of castration resistant prostate cancer

CellCentric

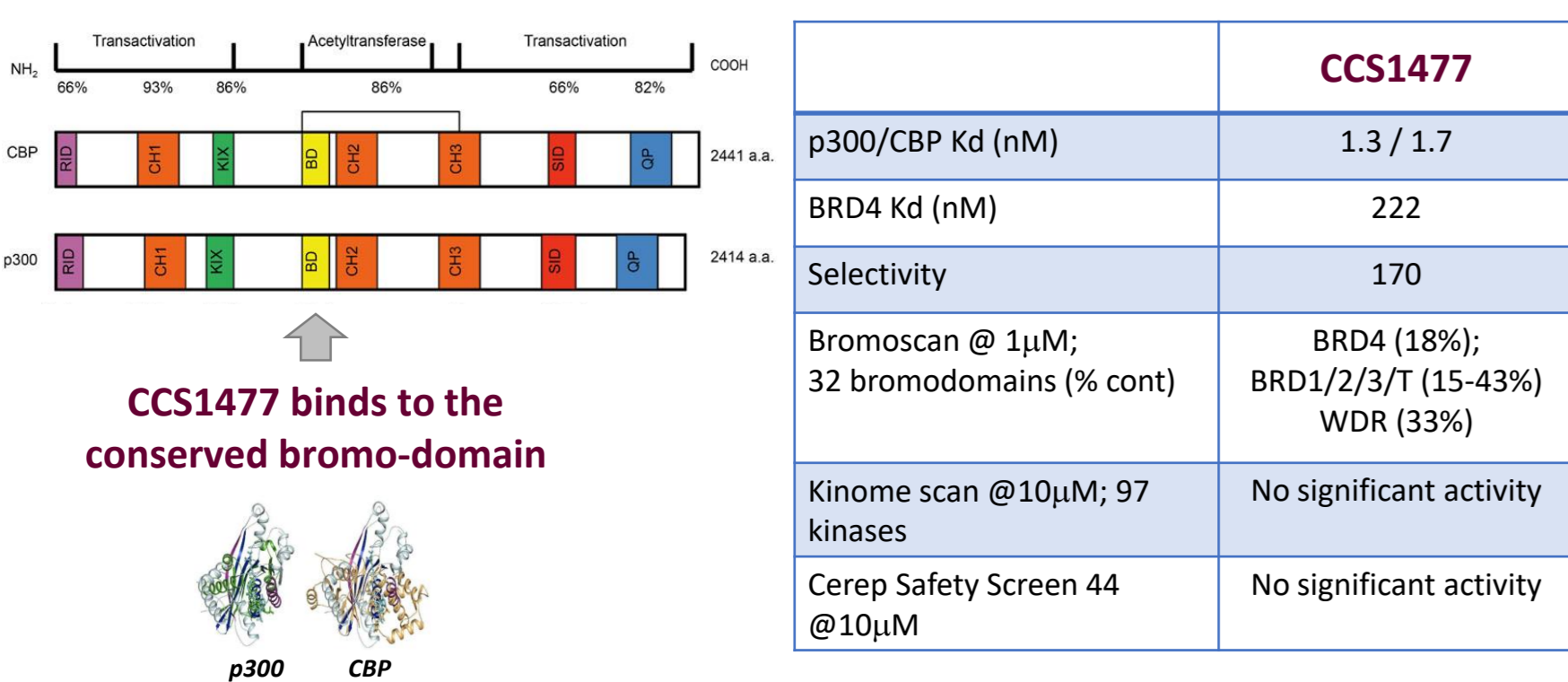
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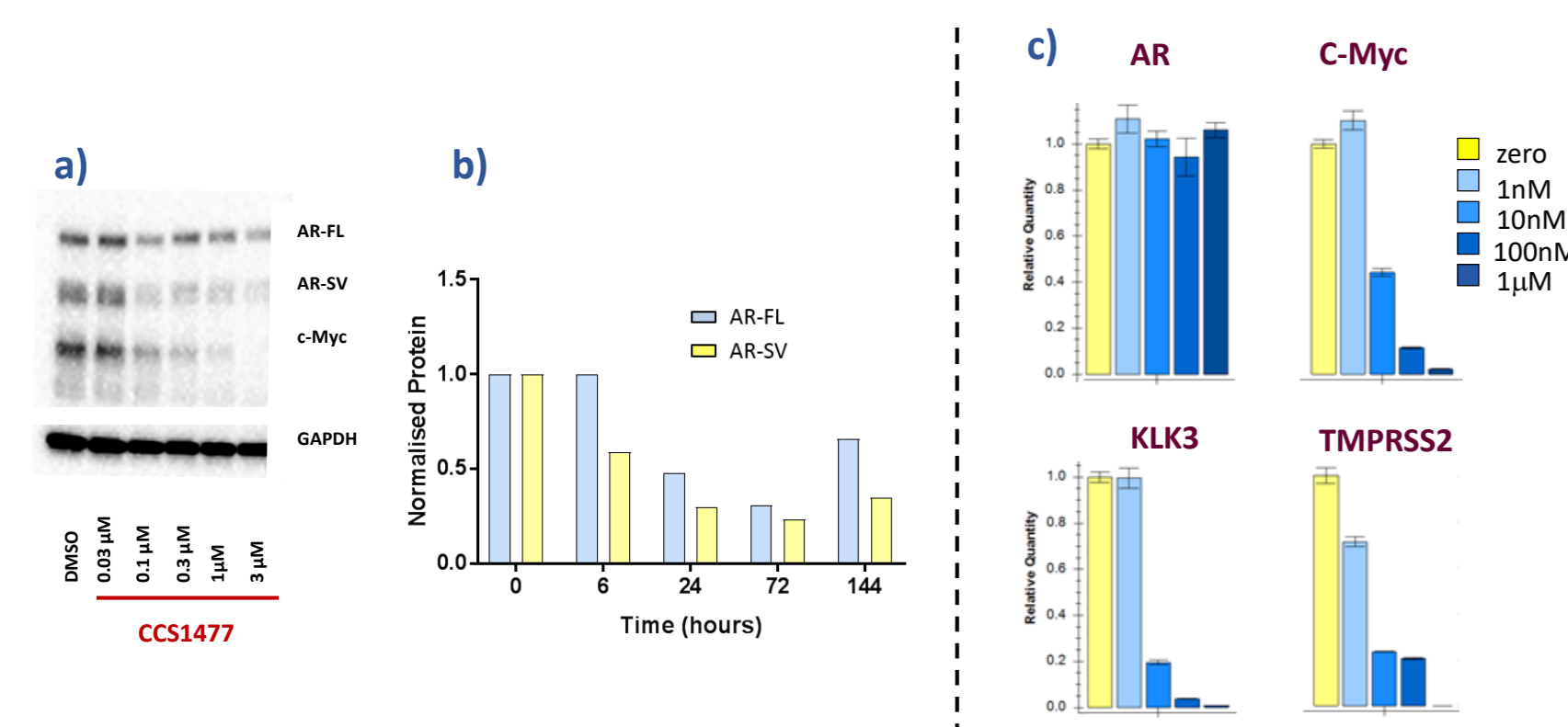
## Introduction

- Sustained androgen receptor pathway activation is the hallmark of castration resistant prostate cancer (CRPC).
- Therapeutic strategies for CRPC include targeted degradation of the androgen receptor (AR) and AR variants (ARV).
- E1A binding protein (p300) and CREB binding protein (CBP) are two closely related histone acetyl transferase proteins that are critical transcriptional regulators of the androgen receptor.
- We have developed CCS1477 which is a potent, selective and orally active small molecule inhibitor of the bromodomain of p300/CBP and have investigated its role in regulating androgen receptor expression and function.

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



## 2. CCS1477 degrades AR-FL, AR-SV and c-Myc proteins and reduces expression of AR-target genes



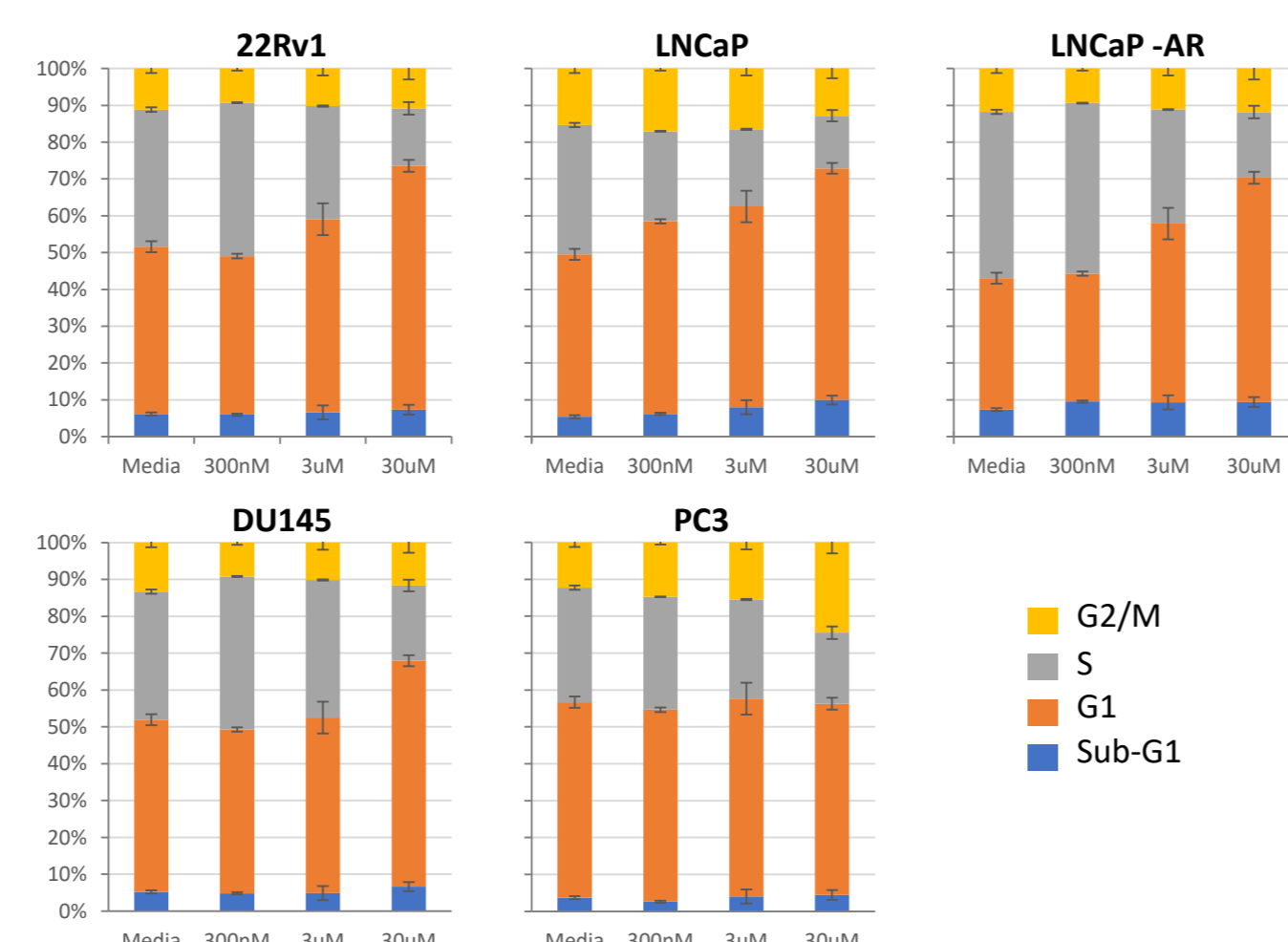
- a) Representative Western analysis of AR-FL, AR-SV (V7) and c-Myc protein in 22Rv1 cells after 24h treatment with CCS1477
- b) 22Rv1 cells were maintained in 10% charcoal stripped FCS and treated with 1µM (IC90) CCS1477 for the times indicated. Western analysis of AR protein expression quantified by densitometry.
- c) qPCR analysis of AR and down-stream genes after treatment with increasing doses of CCS1477 for 72h.

## 3. CCS1477 preferentially inhibits AR driven prostate cancer cells

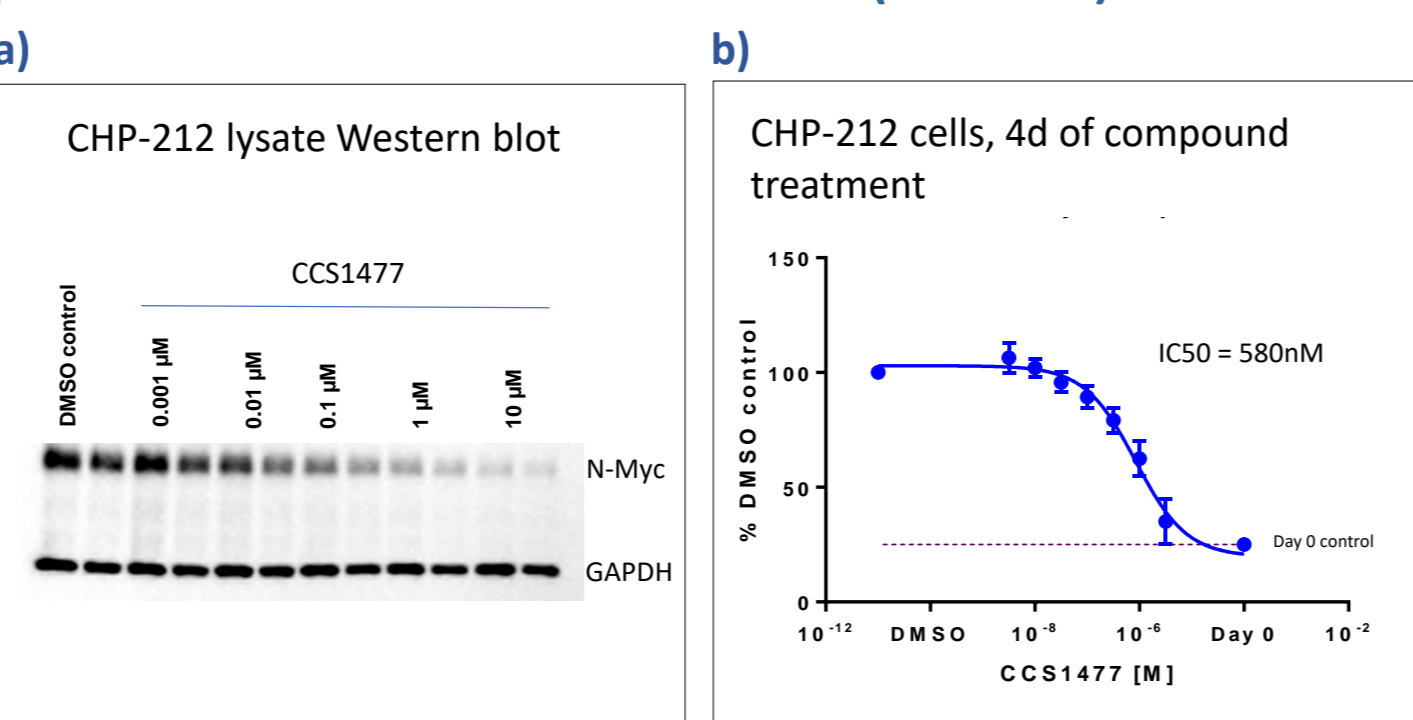
Cell Line	AR status	Model	CCS1477 Proliferation IC50 µM
LNCaP	AR-FL	Hormone responsive	0.230
LNCaP-AR	AR-FL over-expressed	CRPC	0.150
VCaP	AR-FL AR-SV	CRPC	0.049
22Rv1	AR-FL AR-SV	CRPC	0.096
DU145	AR negative	Hormone independent	1.280
PC3	AR negative	Hormone independent	1.490

Proliferation 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.

## 4. CCS1477 inhibits G1/S transition in AR driven cells

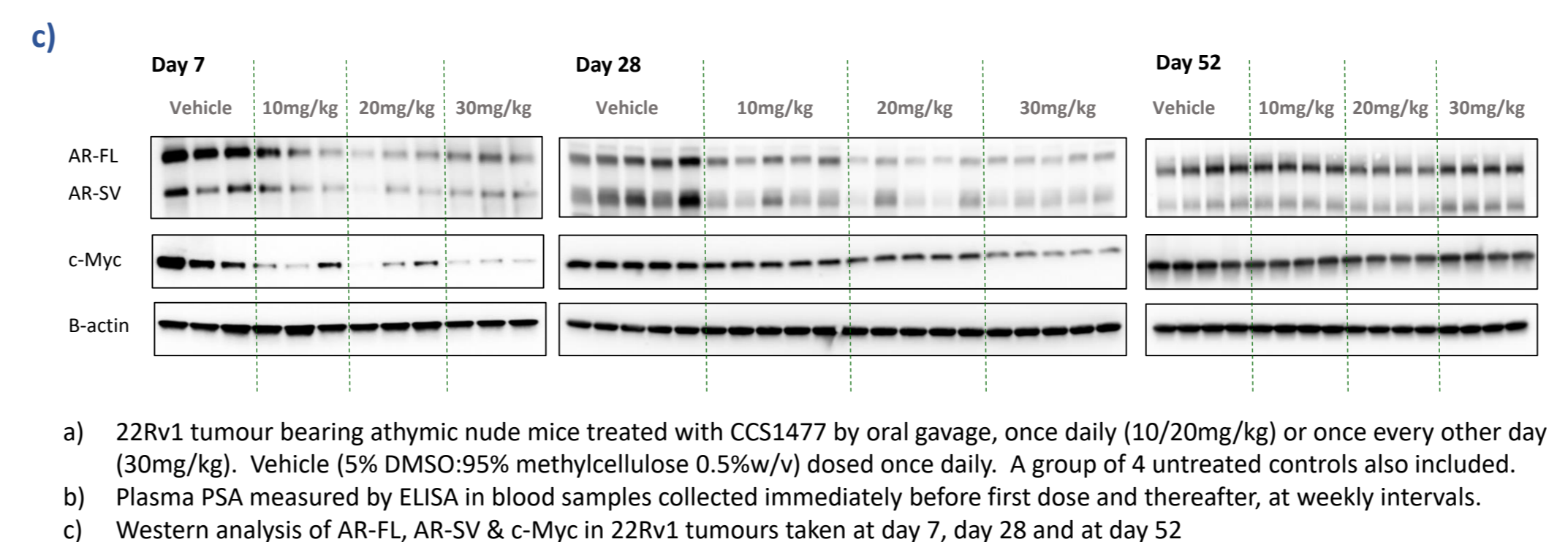
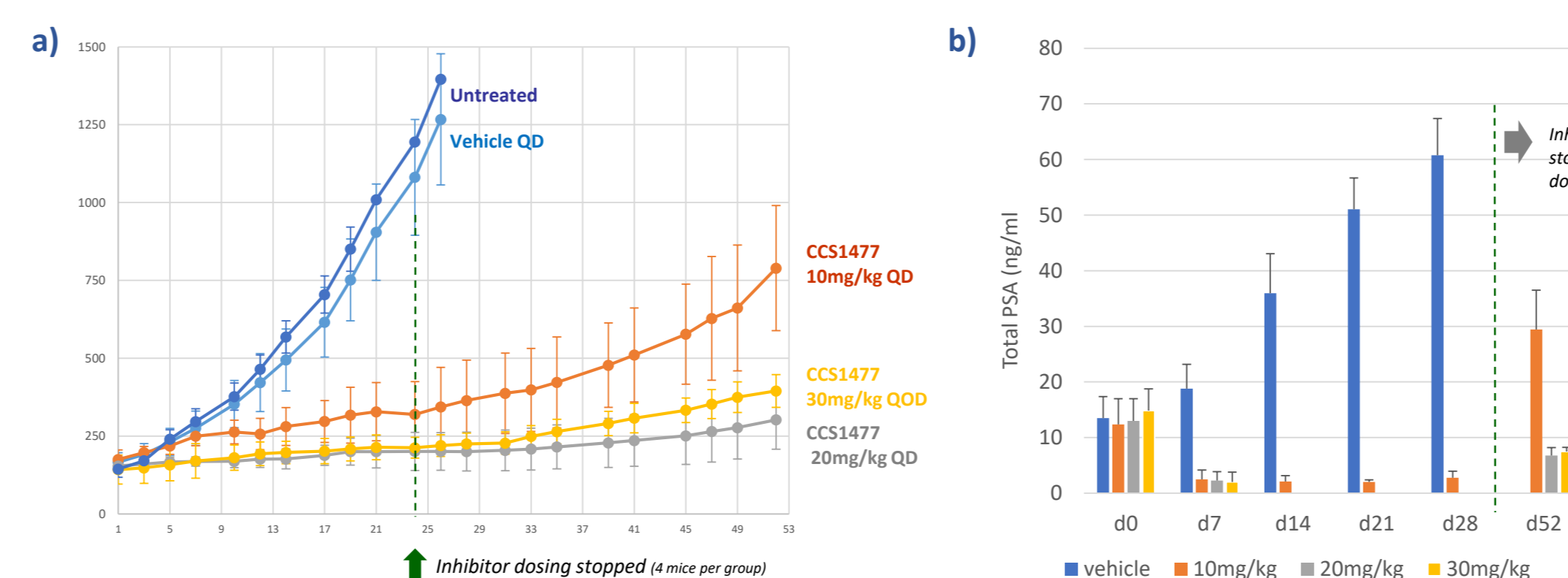


## 5. CCS1477 inhibits n-Myc protein expression and *in vitro* proliferation in neuroblastoma cells (CHP-212)

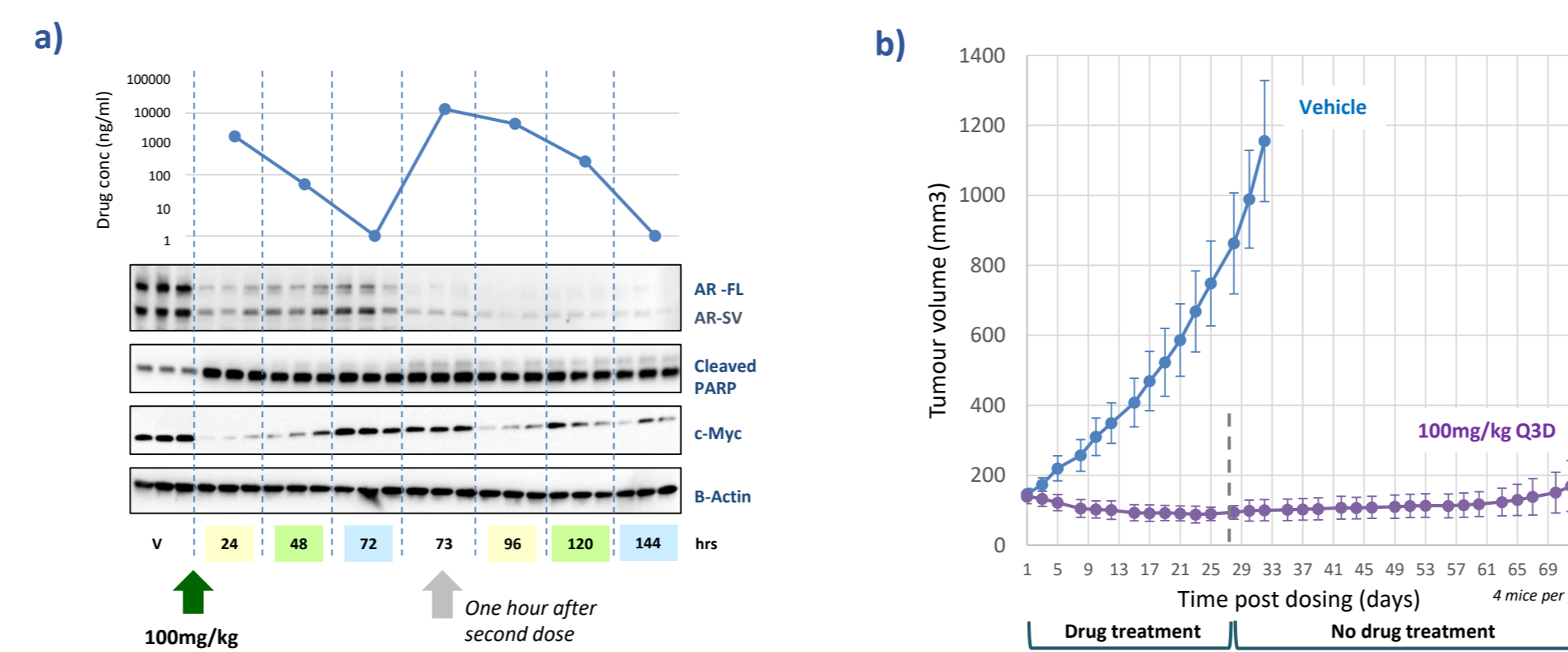


- a) Representative Western analysis of n-Myc protein expression in CHP-212 cells treated with CCS1477 for 72h.
- b) CHP-212 were treated with CCS1477 for 4 days and cell proliferation measured by CellTiter Glo.

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

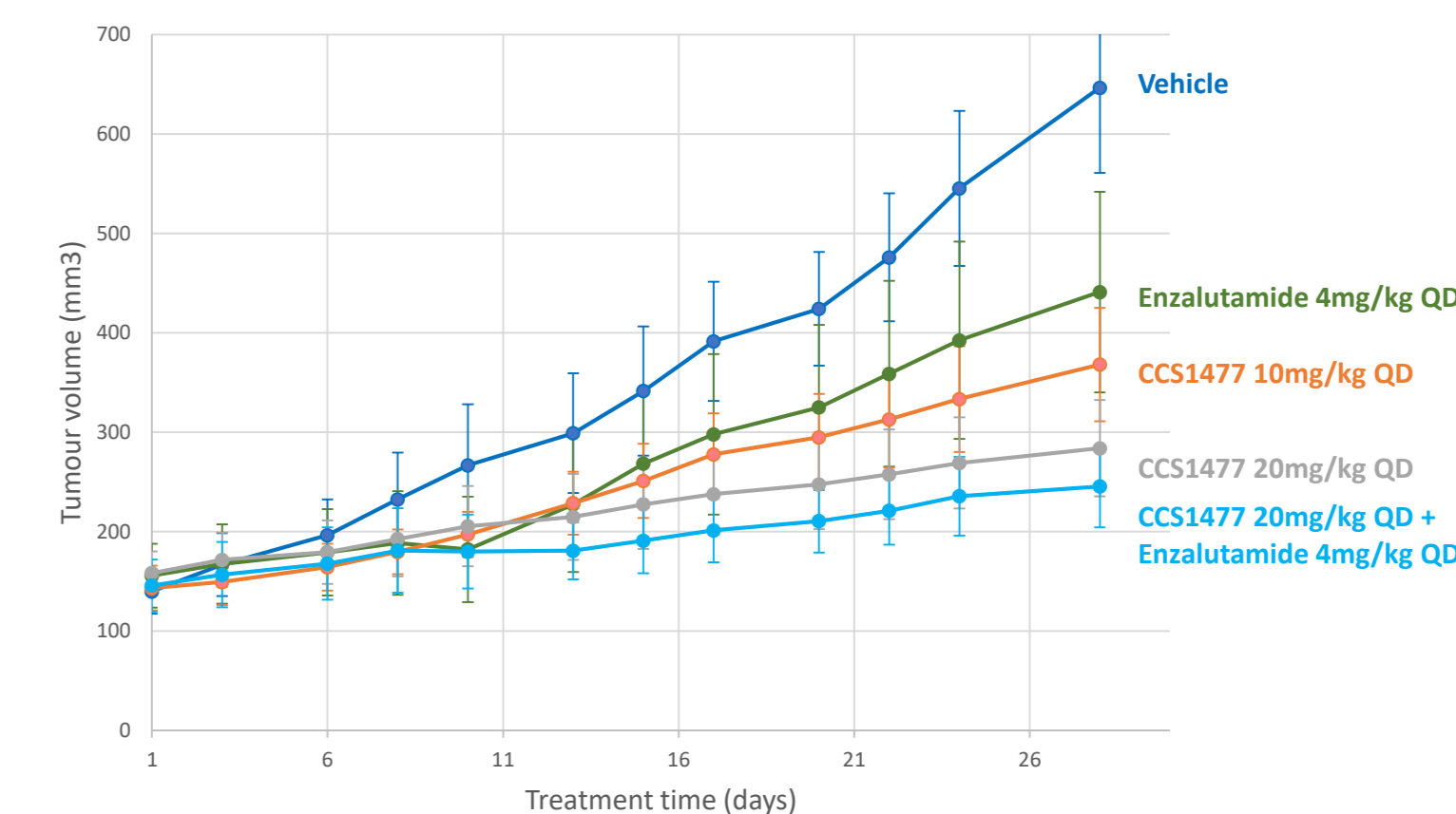


## 7. Tumour regression with intermittent high dose of CCS1477: Associated with significant and sustained reduction of AR/cMyc and increase in cleaved PARP



- a) Protein biomarkers were measured by Western in tumour lysates collected from 22Rv1 tumours, at 24, 48 and 72h after two oral doses of CCS1477 (100mg/kg) given 0 and 72h. Drug concentration was measured in plasma by LC-MS/MS.
- b) In a separate study, 22Rv1 tumour bearing athymic nude mice were treated for 28d with CCS1477 by oral gavage, once every three days (100mg/kg) Vehicle (5% DMSO:95% methylcellulose [0.5%w/v]) was dosed every three days. At day 28, group of 4 animals from the CCS1477 treated group were left untreated, and tumour growth was measured for a further 44 days. Plasma and tumour levels of CCS1477 are undetectable at day 73.

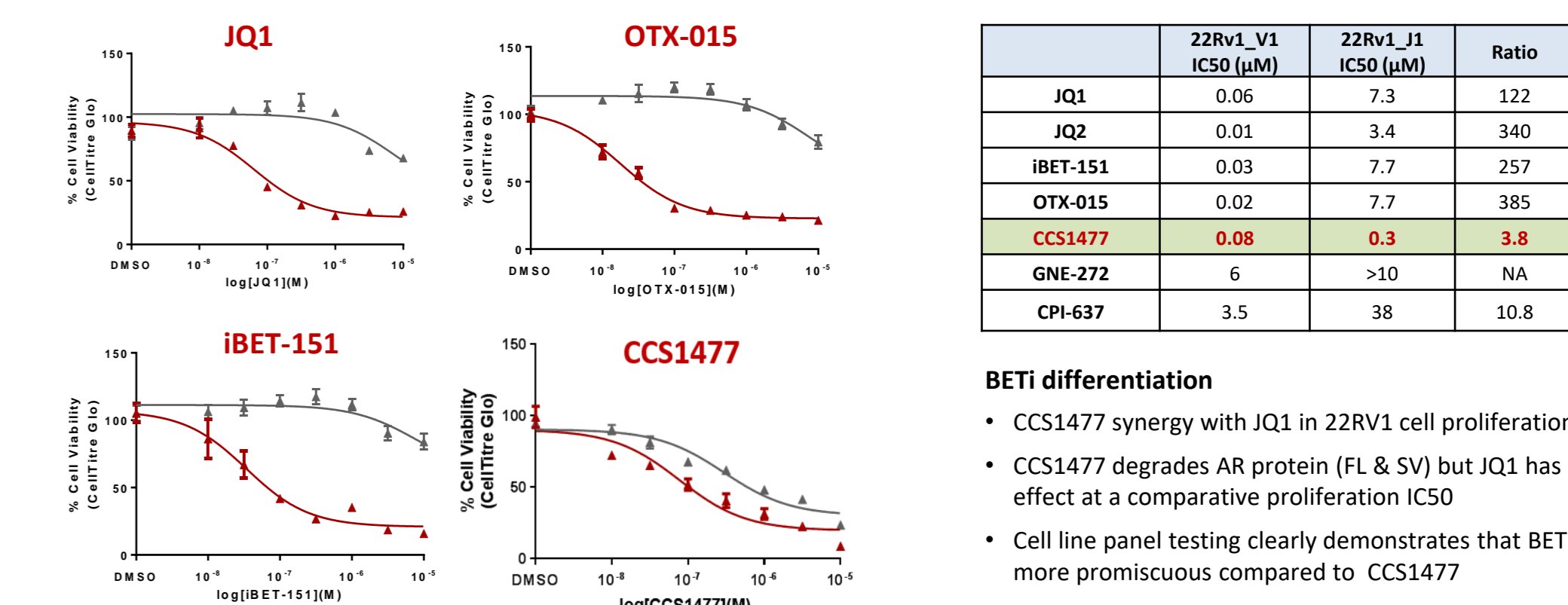
## 8. *In vivo* efficacy in Bicalutamide resistant LNCaP xenograft: Active as monotherapy and in combination with Enzalutamide



Bicalutamide resistant LNCaP tumour bearing athymic nude mice were treated with Enzalutamide by oral gavage, once daily (4mg/kg), or CCS1477 by oral gavage, once daily (10/20mg/kg) or with Enzalutamide (4mg/kg) and CCS1477 (20mg/kg) combination once daily. Vehicle (5% DMSO:95% methylcellulose [0.5%w/v]) was dosed once daily.

Breakthrough growth of Enzalutamide treatment around day 12-14. CCS1477 alone, and in combination, has a more sustained effect.

## 9. CCS1477 retains activity in a BETi resistant 22Rv1 cell-line



A JQ1 resistant cell line was generated by incubating 22Rv1 cells with increasing doses of either JQ1 (22Rv1-J1) or with vehicle (22Rv1-V1) over a 9 month period. Proliferation was measured with a cell viability assay (CellTiter Glo) after compound treatment for 72h.

## Conclusions

- CCS1477 is a potent, selective and orally bioavailable inhibitor of p300/CBP bromodomains.
- CCS1477 causes profound tumour growth inhibition in xenograft models of prostate cancer at tolerated doses. Changes in biomarkers that are consistent with an androgen receptor-mediated mechanism of action.
- Extended duration of tumour growth inhibition in the absence of drug.
- Bromodomain inhibition of p300/CBP represents a differentiated approach to targeting androgen receptor pathway activation in castration resistant prostate cancer.
- CCS1477 is currently in pre-clinical evaluation in preparation for initiation of a Phase 1/1b clinical programme in 2018.

