Degradation of Epigenetic Machinery for the Treatment of Cancer

Helai Mohammad, Ph.D.
VP, Head of Biology
SK Life Science Labs

Keystone Symposia, Epigenetic Mechanisms and Cancer Treatment
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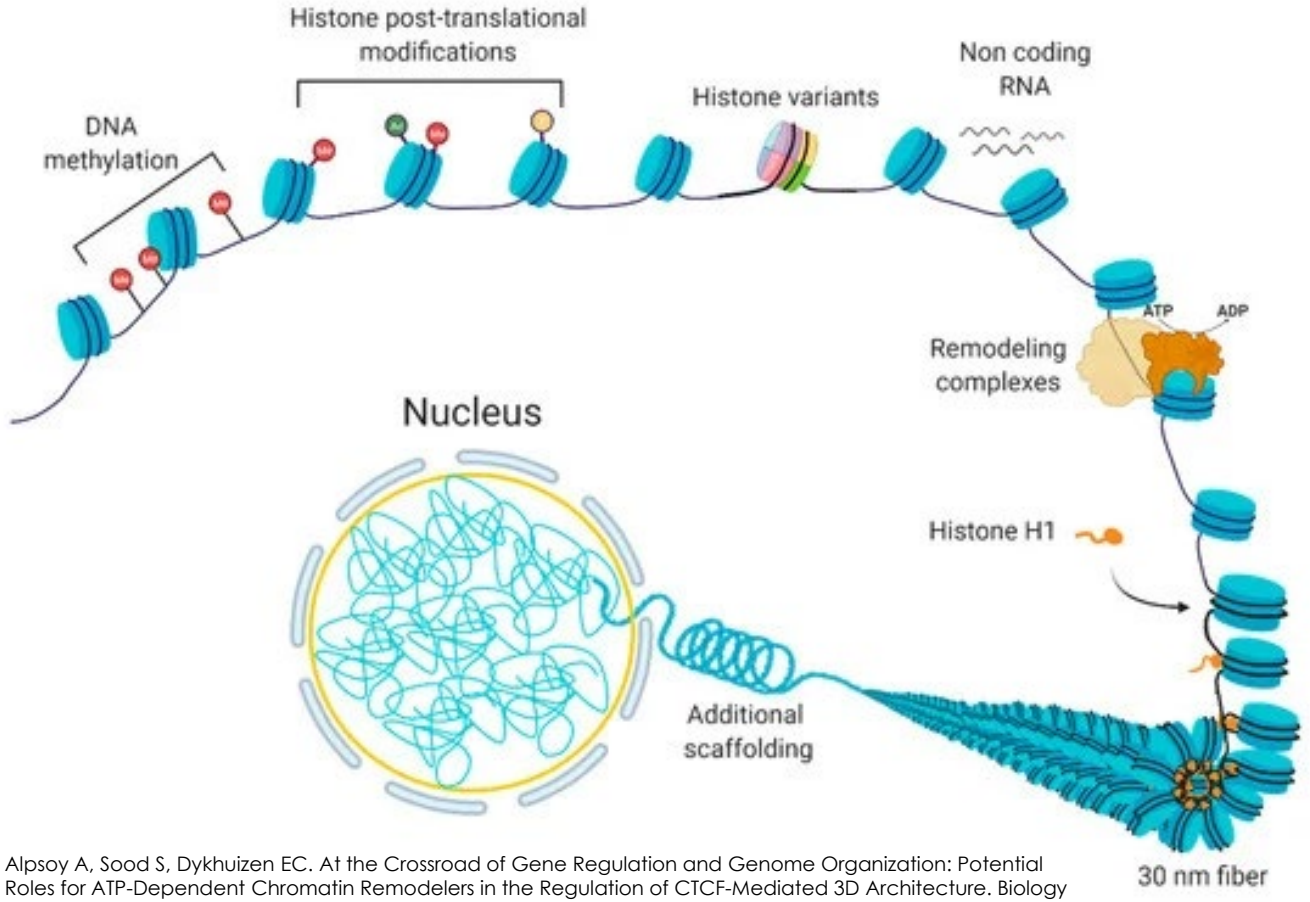
Packaging the genome and positioning genes for transcriptional outcomes requires tightly controlled interplay of epigenetics

Machinery can read, write, erase, and remodel



Alpsoy A, Sood S, Dykhuizen EC. At the Crossroad of Gene Regulation and Genome Organization: Potential Roles for ATP-Dependent Chromatin Remodelers in the Regulation of CTCF-Mediated 3D Architecture. Biology (Basel). 2021 Mar 27;10(4):272.

- **DNMTs** methylate CpG dinucleotides, frequently in the context of CpG rich regions
- **Histone modifying enzymes** catalyze the addition or removal of a variety of post-translational modifications (PTMs) including acetylation, phosphorylation, methylation, and many more
  - **Epigenetic readers**, including proteins that contain bromodomains or chromodomains, interpret the histone PTMs
- **Chromatin access** requires the activity of SWI/SNF ATP dependent remodeling complexes

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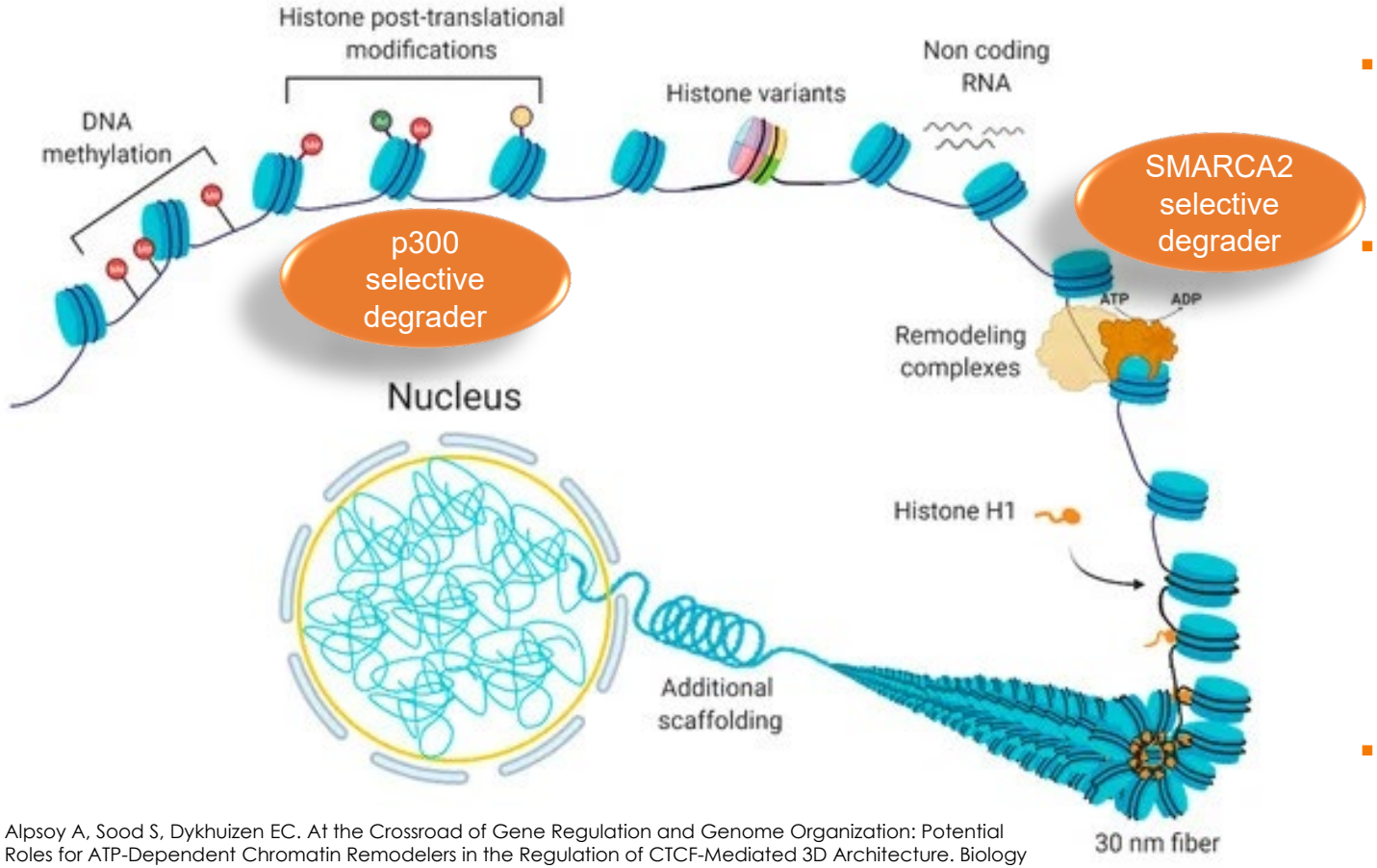

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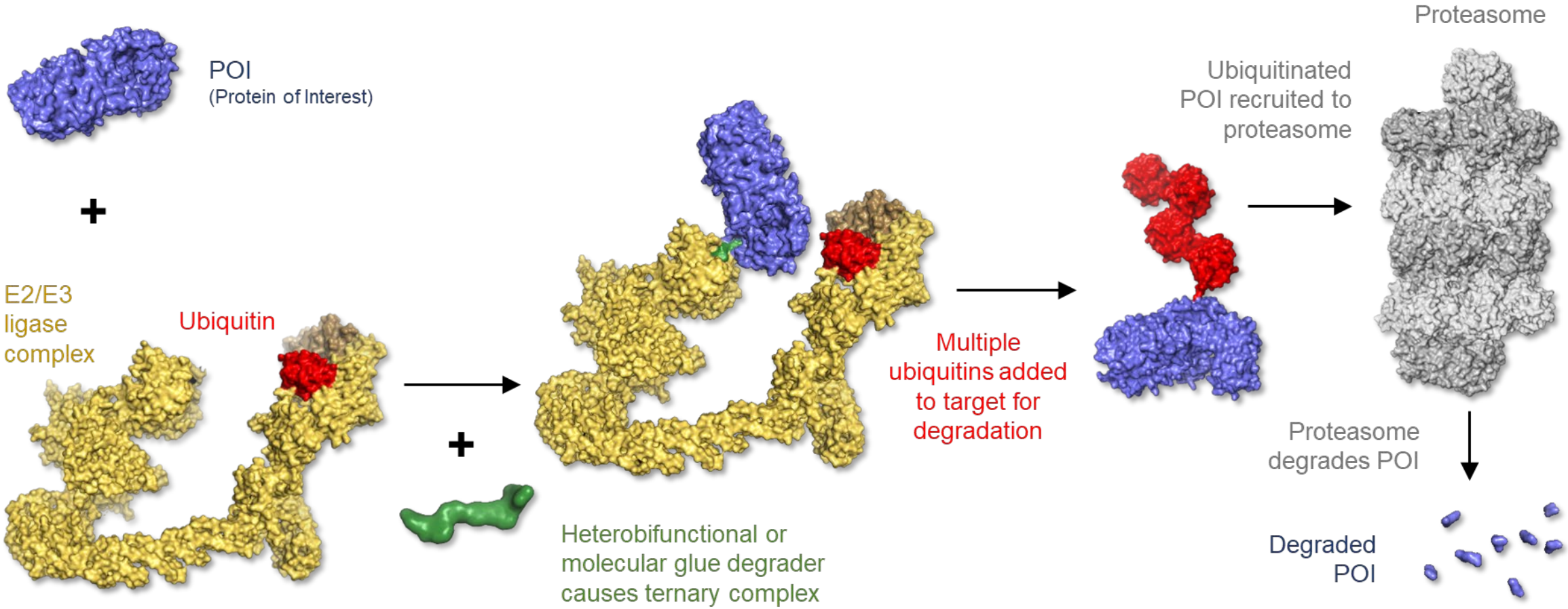
Targeted protein degradation

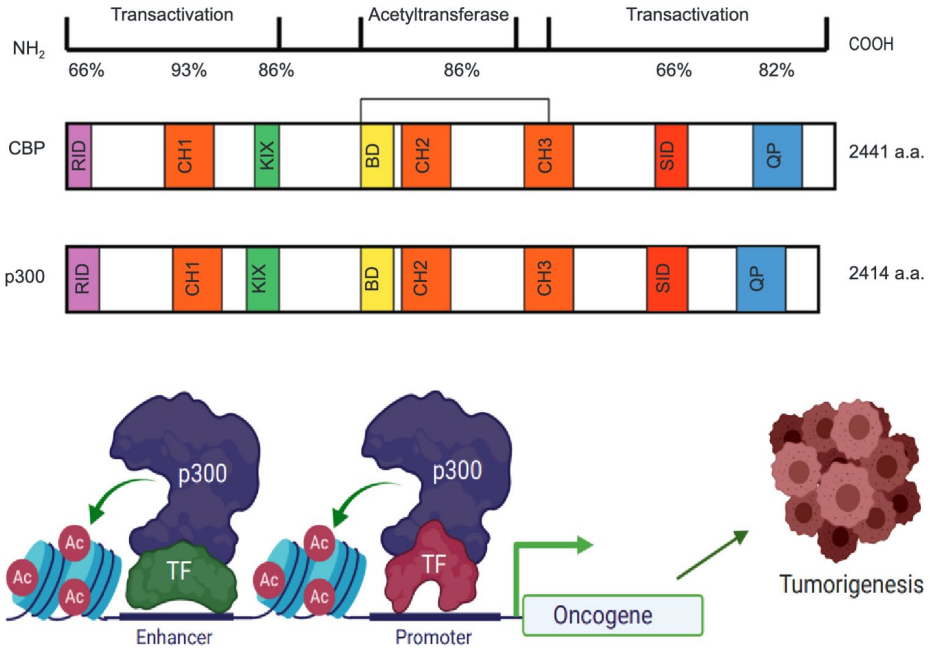
Ternary complex formation can enhance selectivity



Discovery and Characterization of Orally Bioavailable p300 Selective Degraders

p300 and CBP are Paralogous HAT Enzymes with High Sequence Similarity

Dual Targeting has Faced Challenges in Clinical Development due to Hematopoietic Toxicity

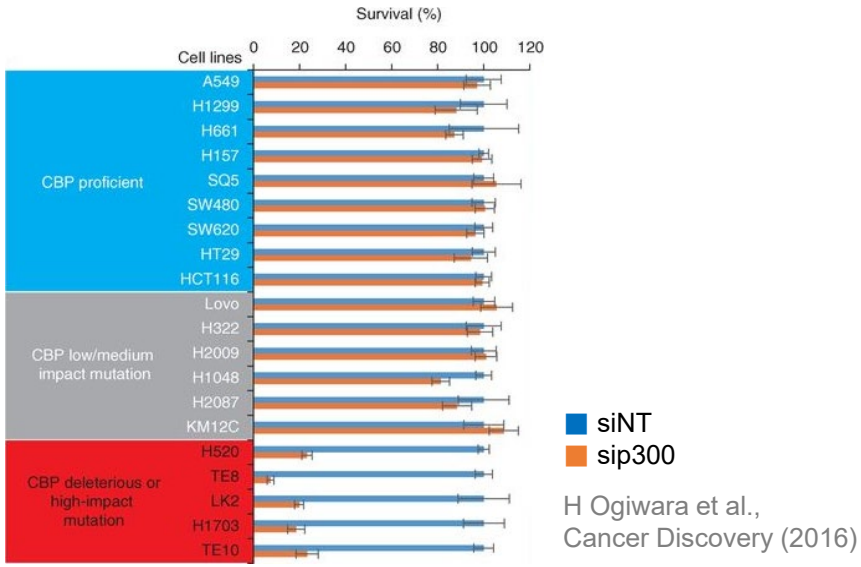


MV Karamouzis et al., Cell Research (2007),
AR Waddell et al., Cancers (2021)

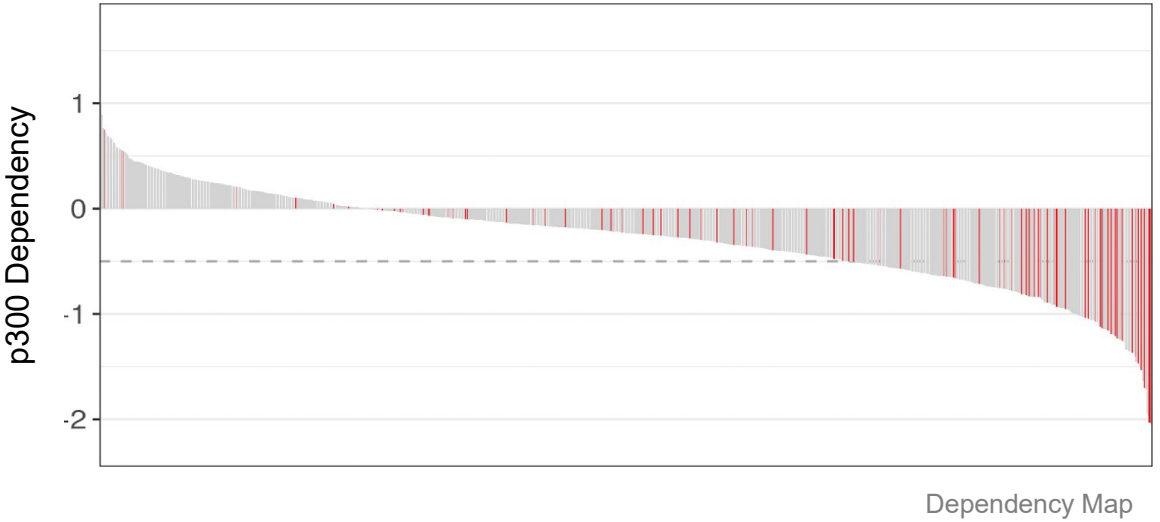
- DNA is packaged into nucleosomes that are comprised of the DNA itself wrapped around histones
- The ‘tails’ of histones can be modified by epigenetic machinery to permit or prevent access to transcription factors
- p300 and CBP are HAT (histone acetyl transferase) enzymes that mark histones with an acetyl group to activate expression of genes that are important in normal and cancer cell biology
- p300 and CBP share high sequence similarity across functional domains
- This has posed an unresolved challenge in discovery of selective functional domain targeted inhibitors; therefore, only dual inhibitors have entered the clinic despite narrow therapeutic margins

p300 Depletion Is Synthetic Lethal with CBP Mutation

Synthetic Lethality Offers Tumor-Specific Vulnerability and Improved Tolerability



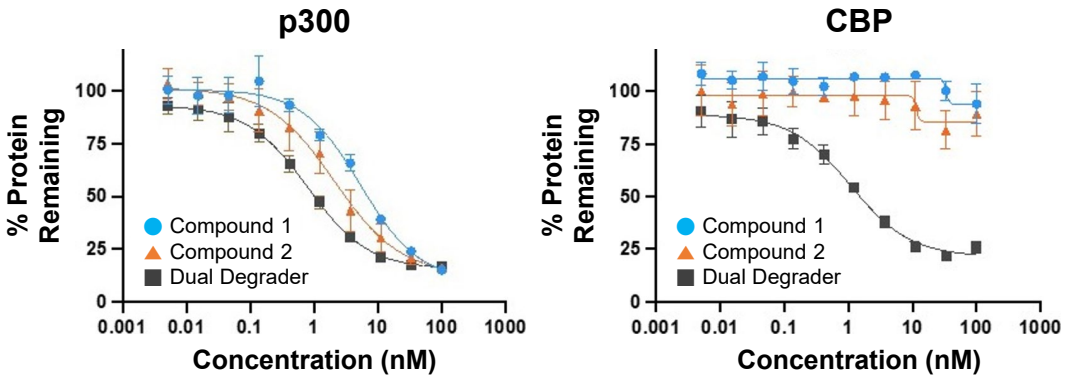
H Ogiwara et al., Cancer Discovery (2016)



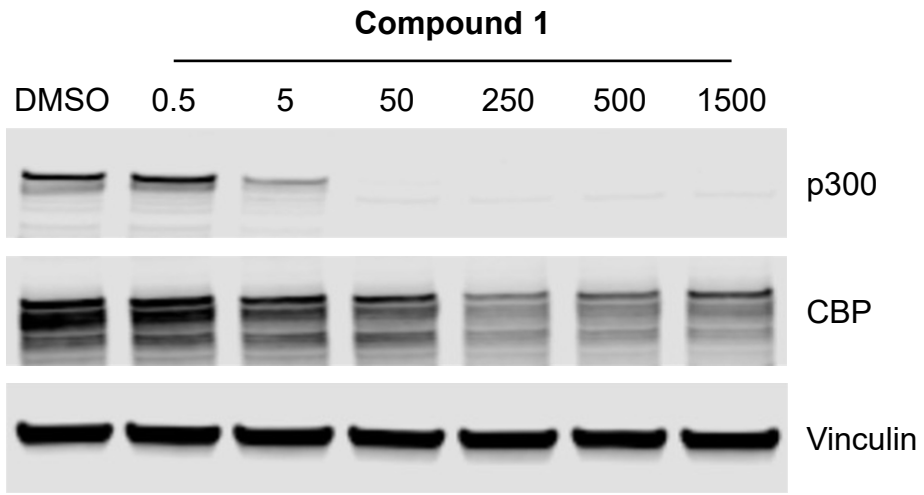
- CBP deleterious mutations confer sensitivity to p300 depletion
- p300 knock-down is synthetically lethal in presence of CBP mutations allowing for selective growth inhibition in this tumor-specific context
- CBP mutations are present in up to 15% of hematological malignancies and solid tumors
- Mutations in CBP present a predictive biomarker for a p300 selective degrader

p300 Degraders Show Potent and Selective Degradation

p300 Degraders Show Low nM Potency with Minimal Impact on CBP



	P300		CBP	
	D_{max} (%)	DC_{50} (nM)	D_{max} (%)	DC_{50} (nM)
Compound 1	85	6.5	13	> max
Compound 2	85	2.0	27	> max
Dual Degrader	84	1.0	79	1.5

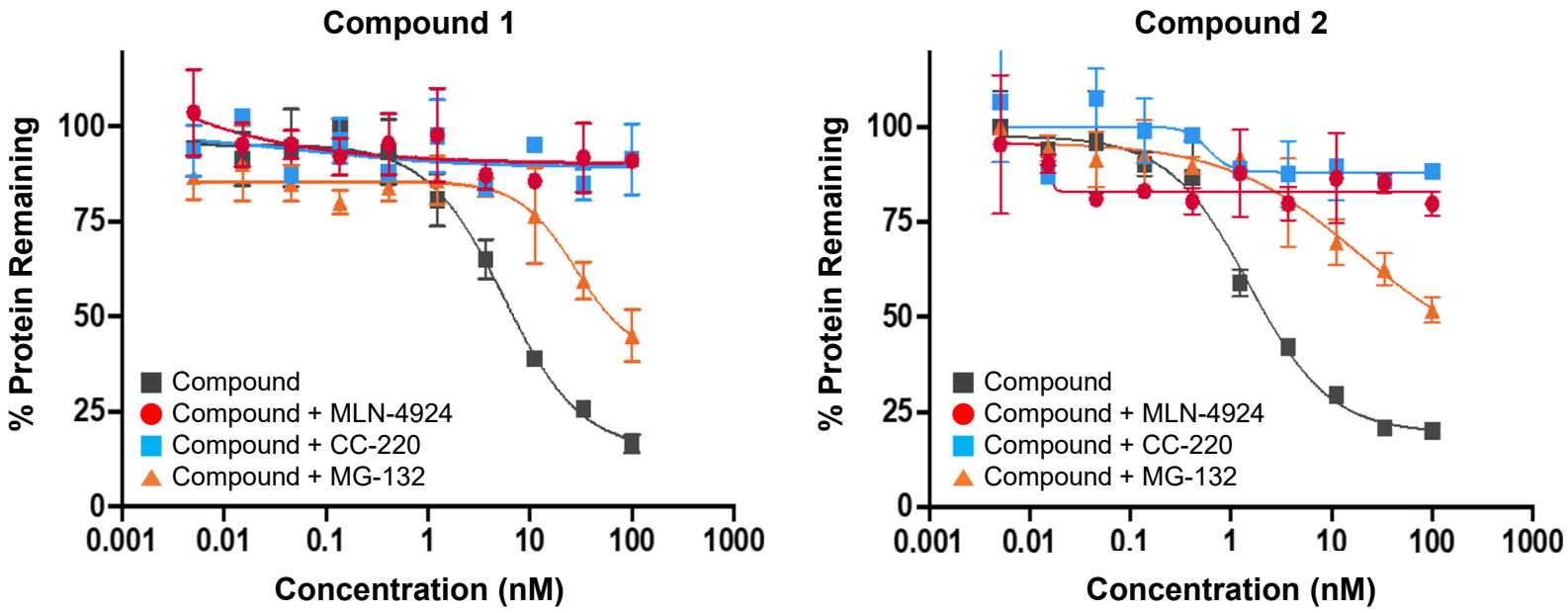


- Selectivity of degradation measured by HiBiT knock-in of either p300 (left) or CBP (right) in A549 cells

- Dose response by western blot confirms selectivity for p300 in H1299 cells expressing endogenous and untagged proteins

p300 Selective Degraders are UPS Dependent

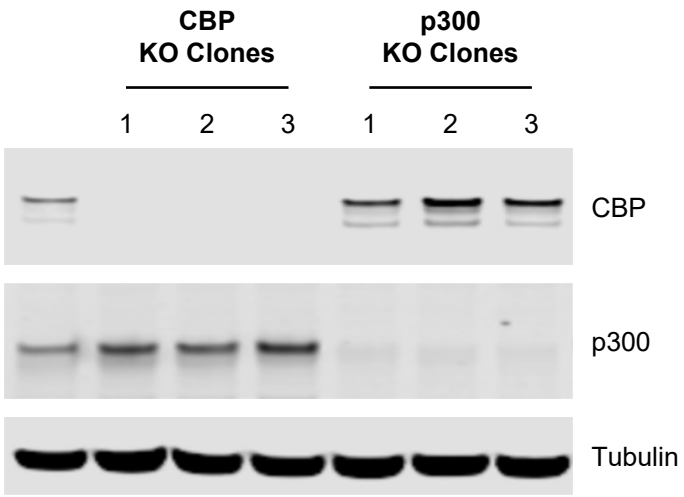
UPS (ubiquitin proteasome system) Inhibitors Block p300 Degradation

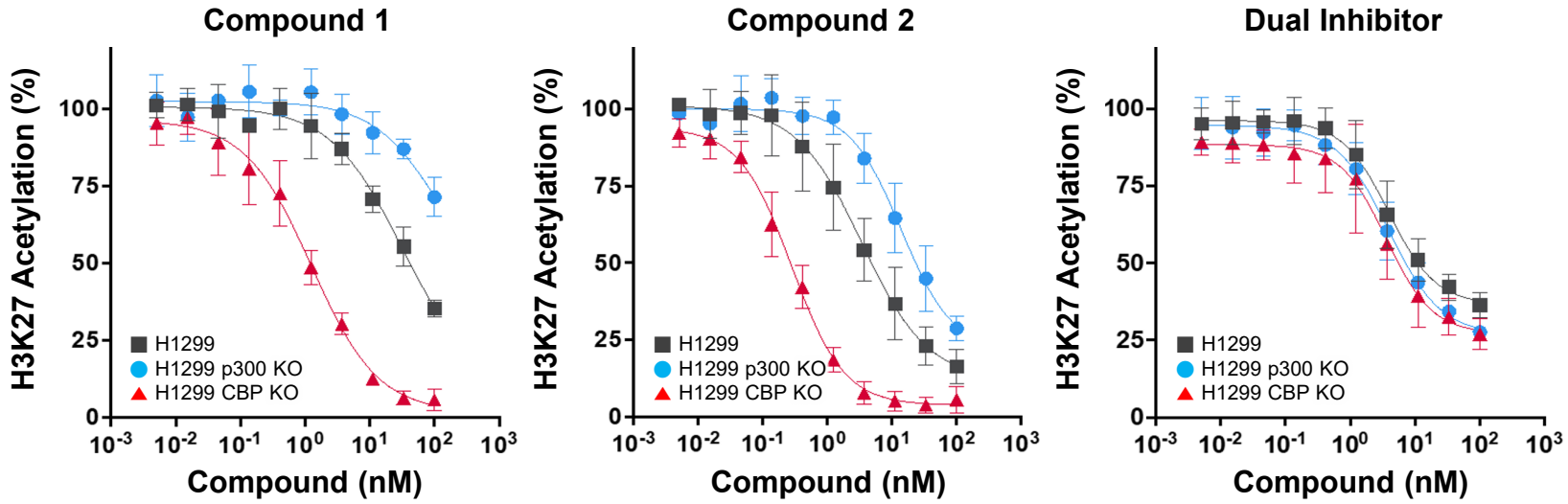


- Pretreatment with inhibitors of neddylation (MLN-4924, 1μM), cereblon (CC-220, 1μM) or proteasome (MG-132, 1μM) attenuates protein degradation

p300 Degradation Impacts Target Pharmacology

Complete Suppression of Histone Acetylation Can be Achieved in CBP KO Cells

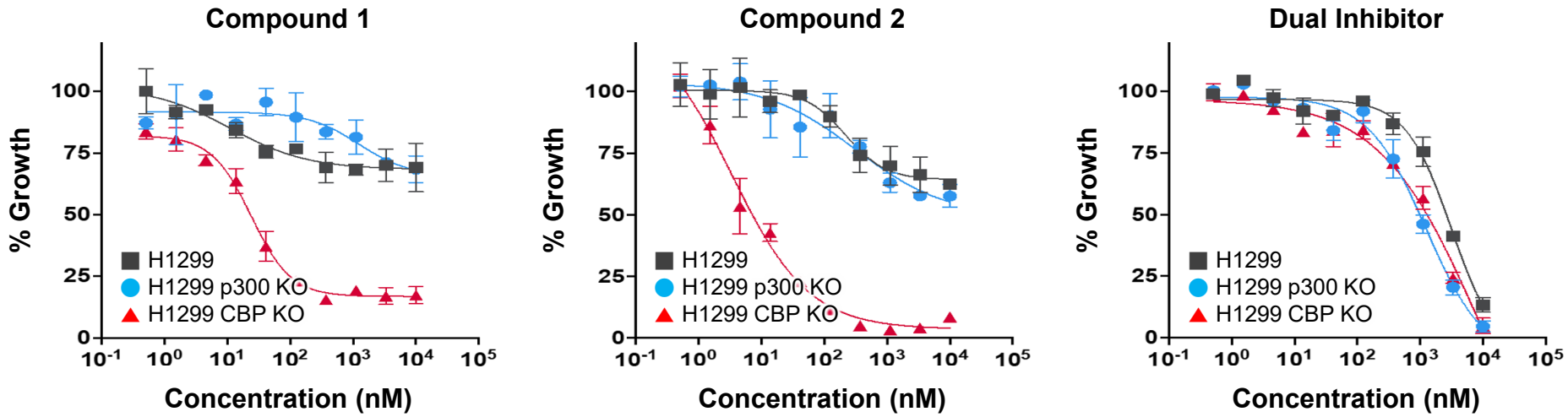




- CRISPR mediated genome editing was used to KO (knock-out) p300 or CBP in H1299 cells
- In-cell western blot was established to evaluate histone H3K27Ac (Histone H3, lysine 27 acetylation) in each cell line
- While a clinical stage dual p300/CBP inhibitor suppresses H3K27Ac in all contexts, p300 degraders lead to robust decrease in H3K27Ac with attenuated impact in wild-type or p300 knock-out cells, demonstrating selective pharmacology

p300 Degraders Result in Selective Growth Inhibition in Synthetic Lethal Context

Potent Growth Inhibition is Observed in CBP KO Cells

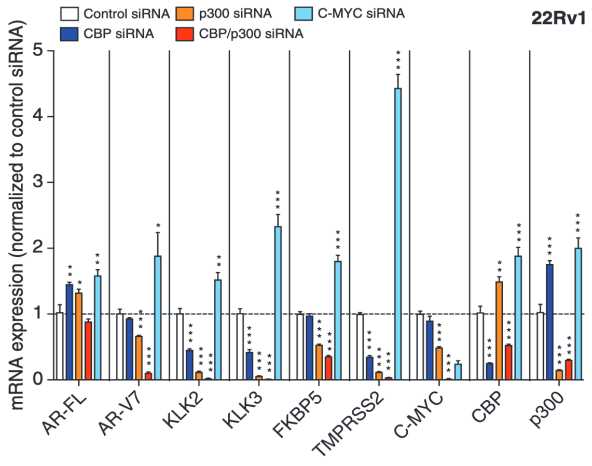


- Engineered cell lines were utilized to investigate effects on cell growth in 6-day proliferation assay
- While a clinical stage dual p300/CBP inhibitor inhibits growth in all contexts, p300 degraders lead to growth inhibition in CBP knock-out cell line minimal impact to growth of p300 knock-out or wild-type parental cell line

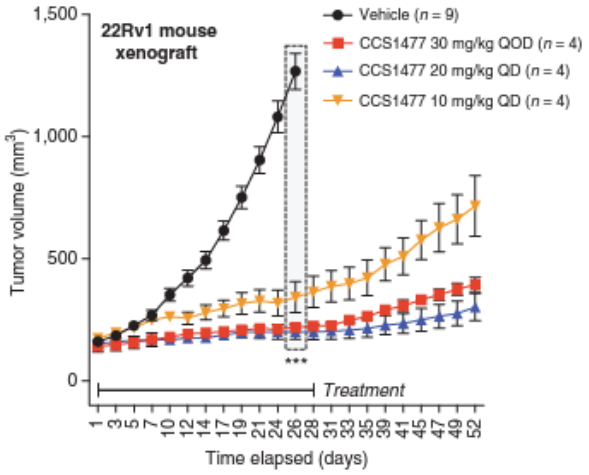
mCRPC Translational Biology

AR Positive Prostate Cancer may Show Exquisite Sensitivity to p300 Degraders

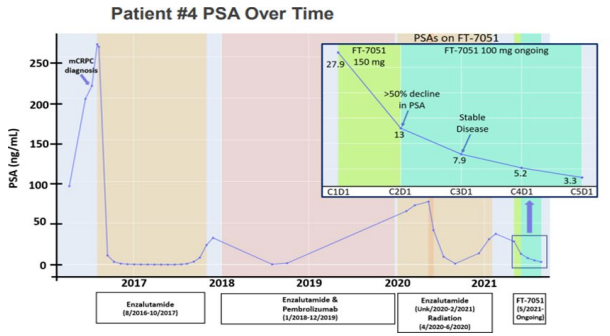
Prostate Cancer Cells Depend on p300 for Growth



J Welti, et al.
Cancer Discovery (2021)



Patient #4 achieved a >80% PSA response by Week 16 with stable disease by radiography



Baseline Characteristics

Demographics: 66-year-old white male

Node-only measurable disease

Chemotherapy naïve with prior enzalutamide, pembrolizumab and radiation

Biomarker Data

Positive for AR F877L matation

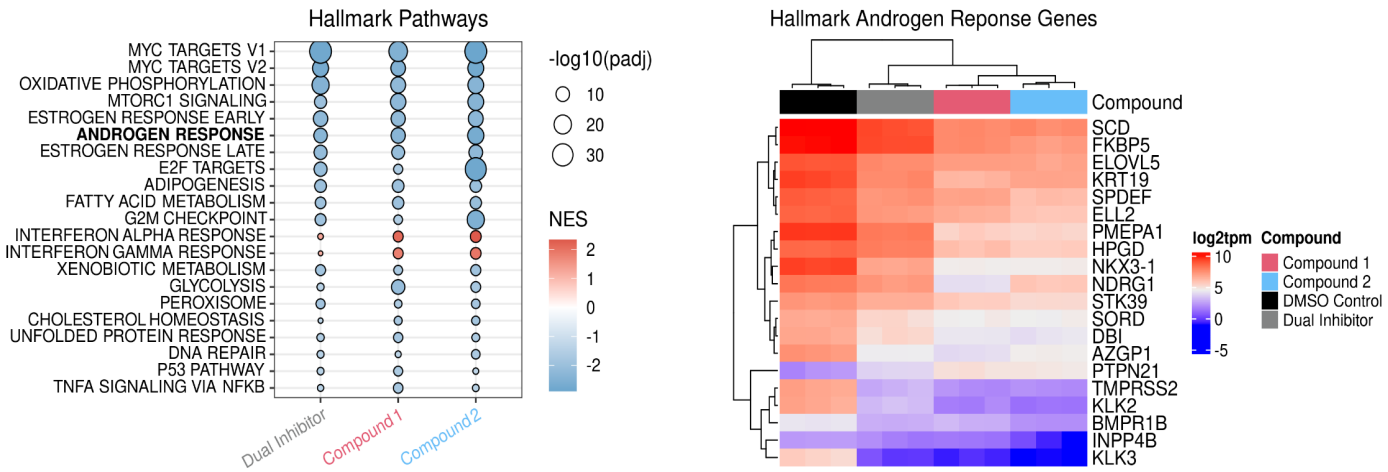
No CTCs at baseline for AR-v7 testing

Forma Therapeutics
AACR-NCI-EORTC Presentation (2021)

- Depletion of p300, but not CBP, results in growth inhibition of AR (androgen receptor) dependent prostate cancer cells through modulation of AR target genes
- Dual inhibitor shows efficacy in vivo in mCRPC (metastatic castration-resistant prostate cancer) cell line
- Dual CBP/p300 inhibitors have shown some promising clinical activity, however, therapeutic margin may be limited

p300 Degraders Result in Suppression of AR-Mediated Gene Signatures

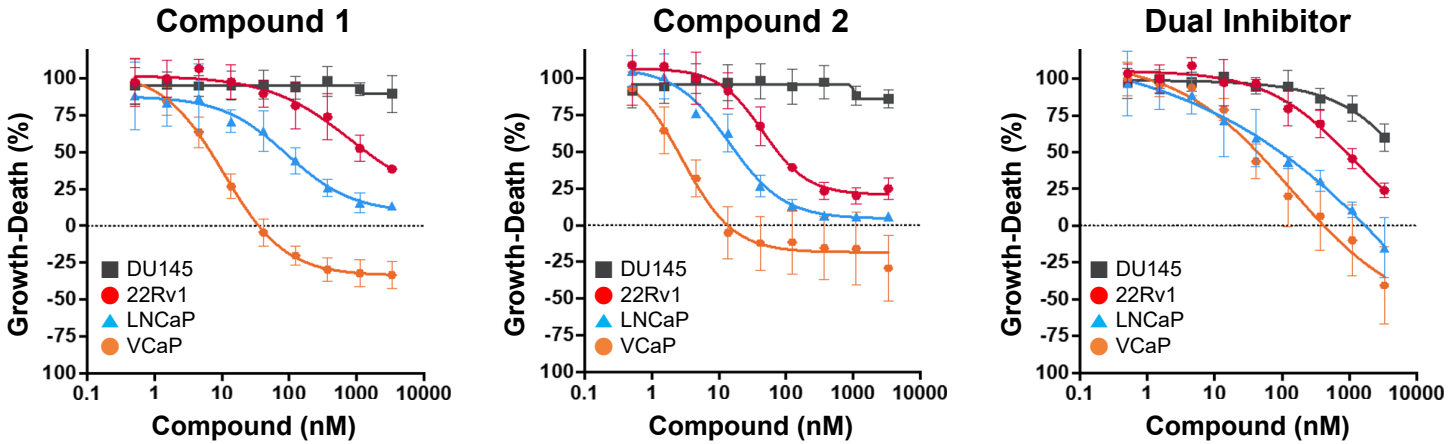
Selective Degrader Impact on Gene Expression is Equivalent to a Dual Inhibitor

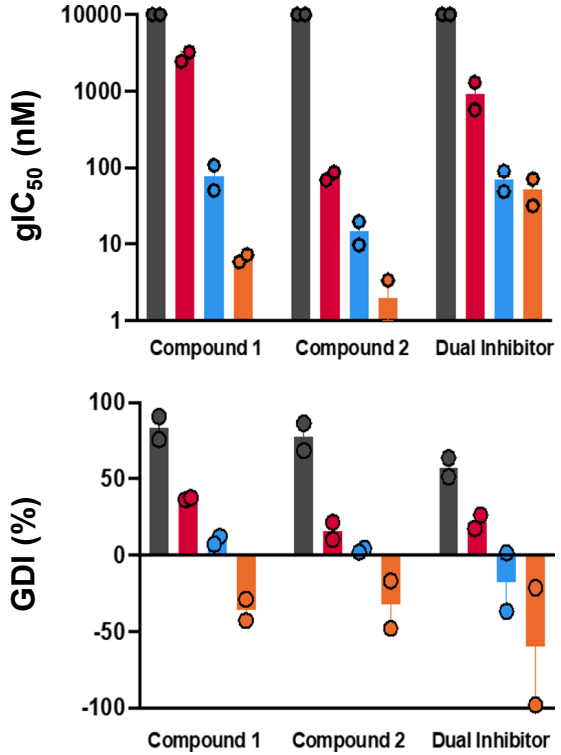


- mCRPC cell line was treated for 48 h with p300 degraders or clinical stage dual inhibitor
- GSEA dot plot and heat map of heat map of differentially expressed genes within the androgen response signature confirms that p300 selective degraders can suppress AR target gene signatures
- AR positive prostate cancer cell growth is clinically correlated to unabated AR dependent gene expression, therefore, perturbation of this pathway can lead to meaningful clinical response

p300 Degraders Exhibit Selective Growth Inhibition of AR Positive Prostate Cancer Cells

CBP Perturbation is not Required for Cell Growth Inhibition



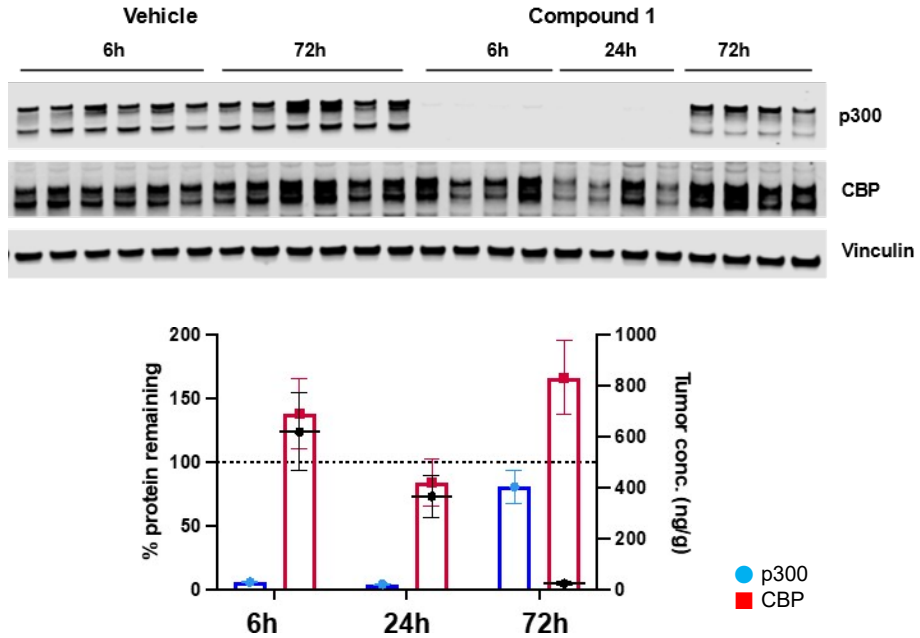


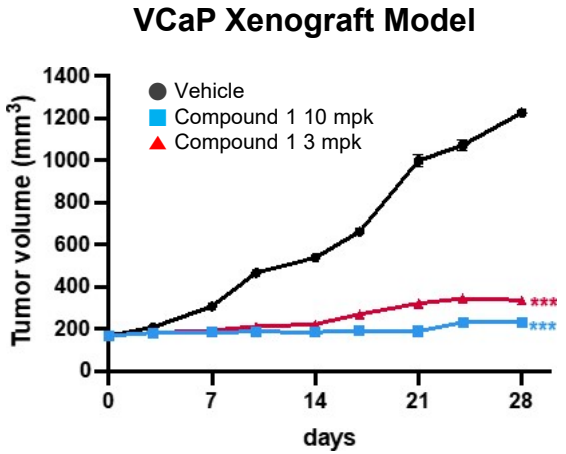
- Treatment of AR positive prostate cancer cells with a p300 degrader results in cell growth inhibition superior to what is observed with a clinical stage dual p300/CBP inhibitor
- AR null DU145 cells show no response to p300 degraders indicating growth inhibition is selective for AR positive cells

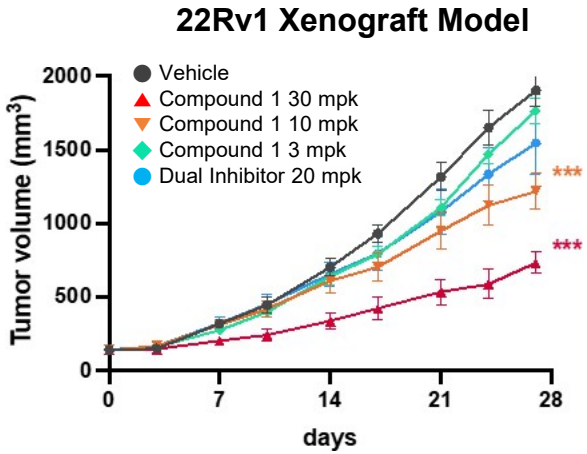
p300 Degrader is Efficacious in AR Positive Prostate Cancer Models

Orally Administered p300 Degrader Induces Tumor Regression

Compound 1, 10 mpk, *in vivo*





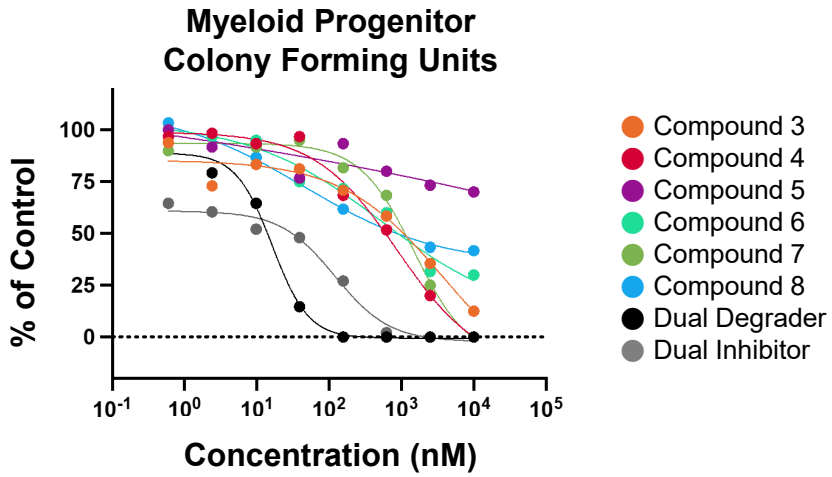


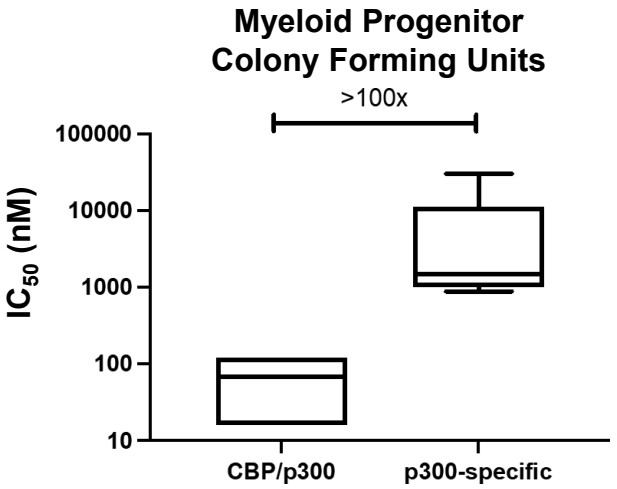
- Oral administration (once daily) of Compound 1 to mice with H1703 xenografts demonstrates >90% reduction of p300 within tumor cells

- Oral administration (once daily) of Compound 1 to mice with prostate cancer xenografts demonstrates substantial tumor growth inhibition at pharmacologically relevant doses

p300 Degraders Show Limited Activity in Hematopoietic Progenitor Ex Vivo Toxicity Study

p300 Degraders Show Less Potency than Dual Inhibitor or Dual Degrader

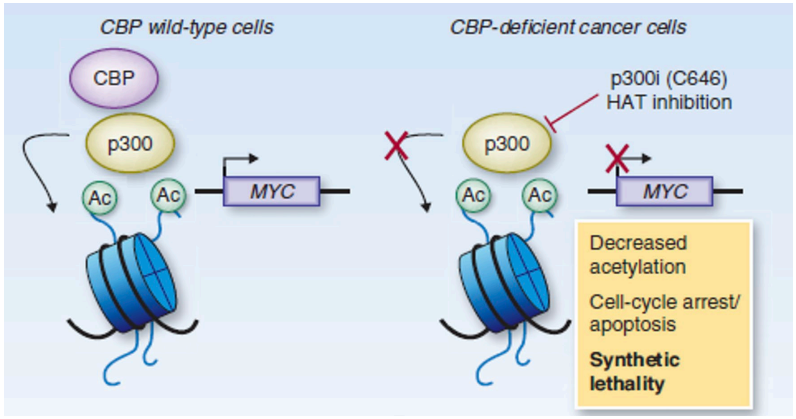




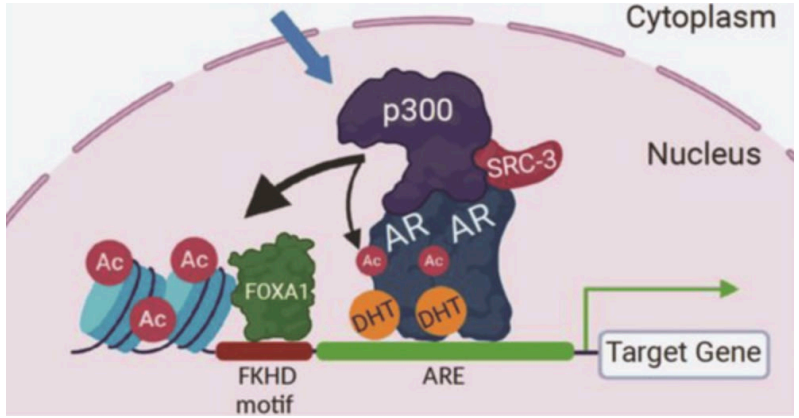
- Bone marrow derived hematopoietic stem cells were differentiated ex vivo for toxicity assessment
- Dual degrader and dual inhibitor inhibit the growth of myeloid progenitor cells
- p300 selective degraders show markedly less potency in bone marrow toxicity assay suggesting a better therapeutic index

Summary

Discovery of p300 Selective Heterobifunctional Degraders for CBP Mutant Cancer and mCRPC



C Kadoch et al., Cancer Discovery (2016), AR Waddell et al., Cancers (2021)



Abbreviations : CBP, CREB-binding protein; mCRPC, metastatic castration-resistant prostate cancer

p300/CBP Regulate Histone Acetylation & Gene Expression

Proprietary, Potent & Selective p300 Heterobifunctional Degrader

Selective Growth Inhibition of CBP mutant Cancer Cells

Orally Bioavailable & Low Clearance

Tumor Regression in Prostate Cancer CDX Model

Over 100X Improved Margin in HemeTox Assay (vs Clinical Compound)

Acknowledgements

p300 Project Team

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Winston Wu
Zhihua Sui

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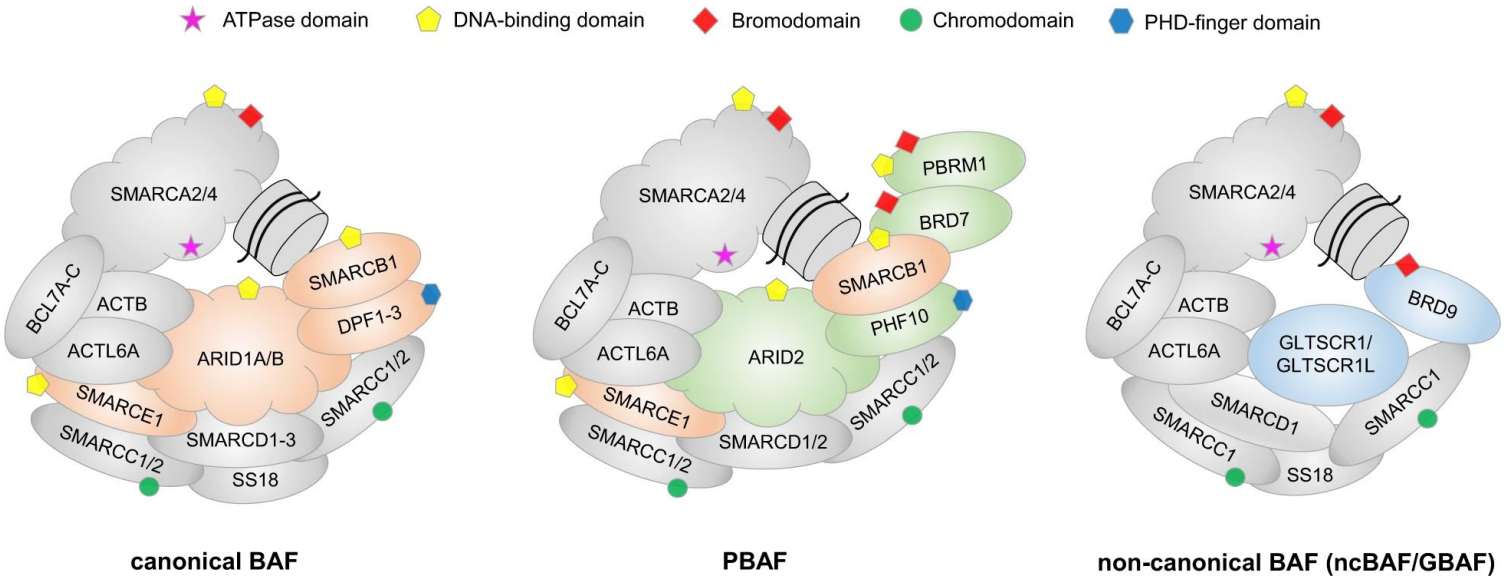
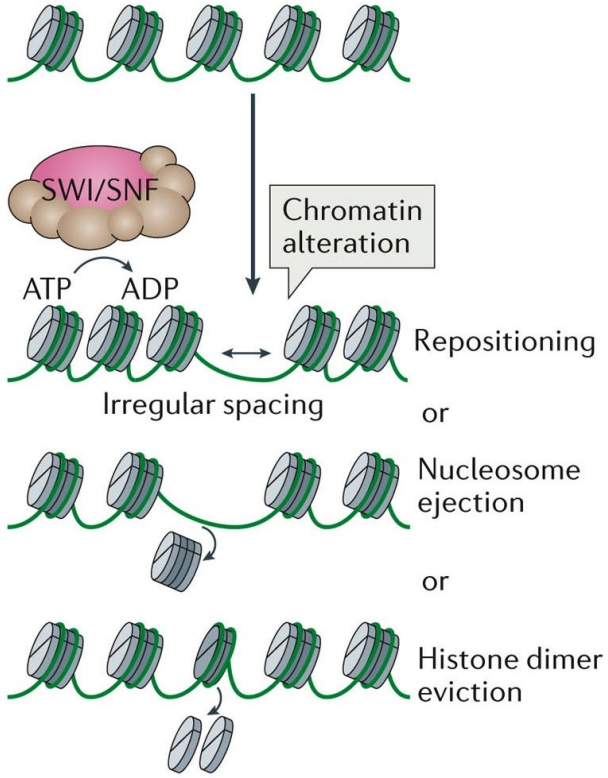
Christine Stuhlmiller

Discovery and Characterization of Orally Bioavailable SMARCA2 Selective Degraders

SWI/SNF ATP-dependent chromatin remodeling is critical for nucleosome positioning

SMARCA2/4 are essential components of the complex



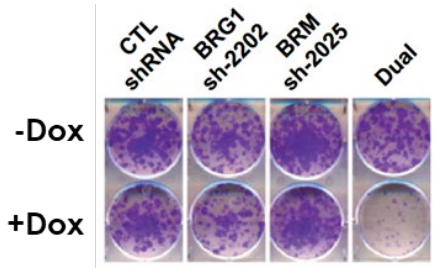


- ATPase function within the SWI/SNF complex is only provided by the mutually exclusive SMARCA2/4 paralogous subunits
- ATPase role of SMARCA is indispensable for the function of the SWI/SNF complex

Non-essential role of SMARCA2 and SMARCA4 BRD provides opportunity for driving selectivity through degradation

BRD domain targeting does not impact cell growth

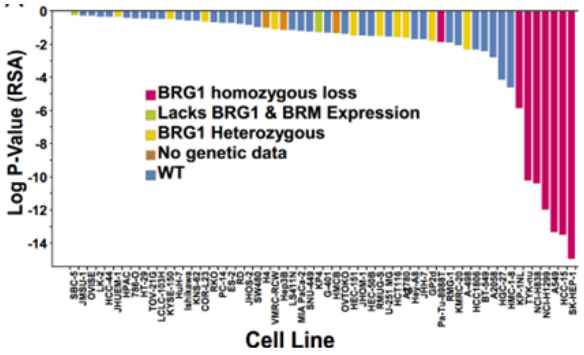
Simultaneous depletion of SMARCA2/4 is not tolerated



BRG1 = SMARCA4
BRM = SMARCA2

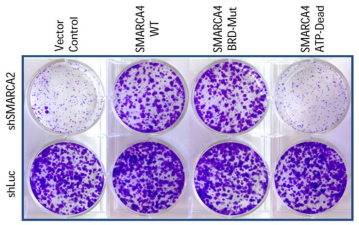
BEAS2B Cells
(SM4/BRG1 Wild-type)

SMARCA2 depletion is synthetic lethal in SMARCA4 deficient cells

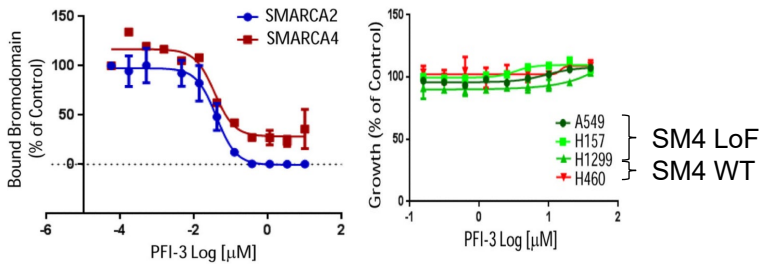


Hoffman Gregory R et al *PNAS* vol. 1118 (2014)

ATPase domain is vital for cell viability



The bromodomain is not essential for cellular growth inhibition

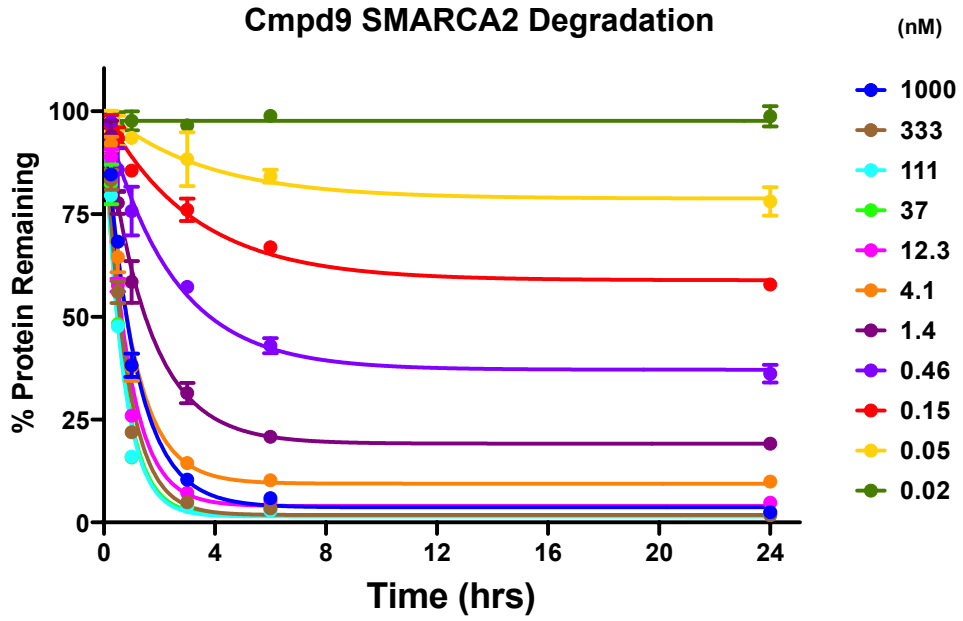


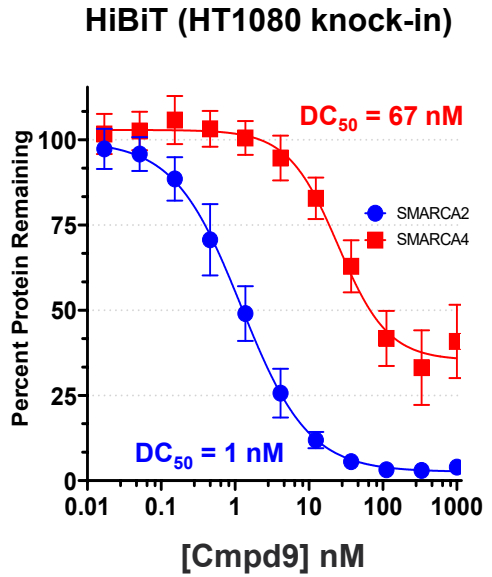
Vangamudi Bhavatarini et al. *Cancer research* vol. 7518 (2015): 3865-3878

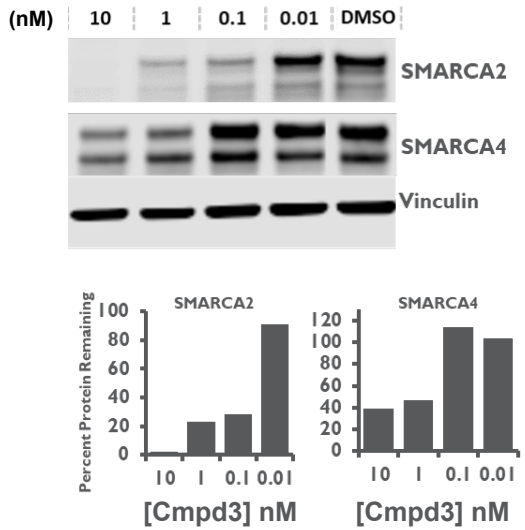
- The essential role of SMARCA2/4 provides a clear mechanistic basis for the synthetic lethal relationship between the paralogs
- ATPase domain is druggable however inhibitors have faced selectivity challenges
- SMARCA2 bifunctional degraders can leverage BRD binding to retain cellular selectivity and minimize systemic toxicity

Discovery of potent, selective, and rapid degraders of SMARCA2

Multiple orthogonal assays are utilized to characterize degraders





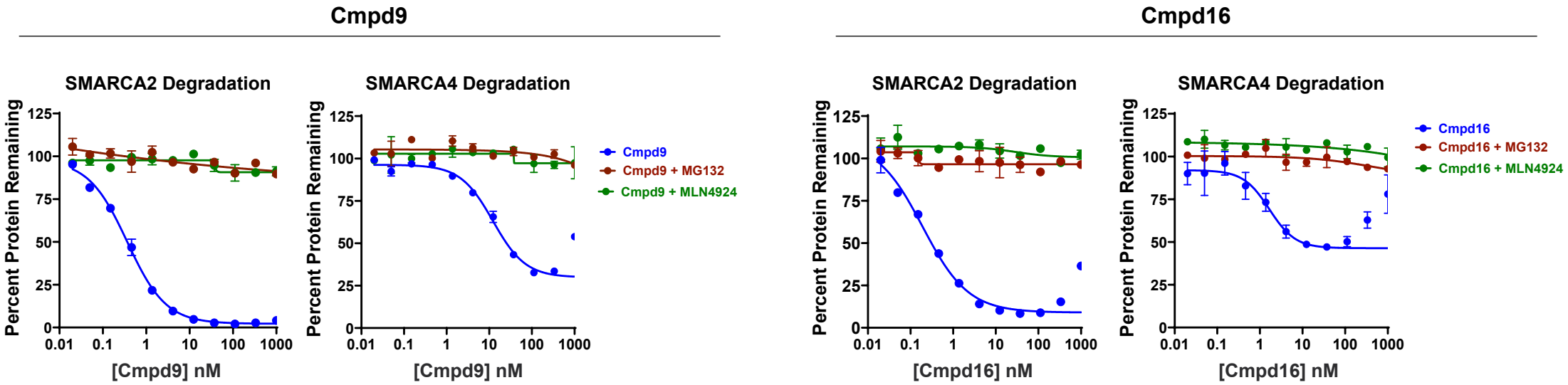


- SKLSL heterobifunctional degraders exhibit rapid kinetics
- Maximal degradation is achieved by 6 hours in HiBiT assay (HT1080 cells)

- HiBiT knock-in cell line utilized to determine degradation potency and selectivity
- Parental cell line exhibits similar response as HiBit degrader profiling cell line to confirm

Structurally diverse molecules demonstrate proteasome dependent degradation of SMARCA2 and 4

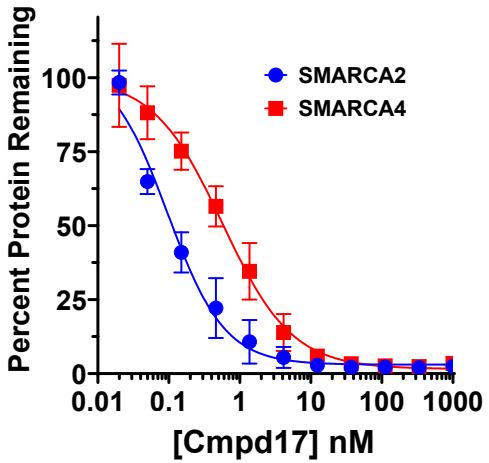
UPS mechanism is confirmed with various inhibitors of the complex

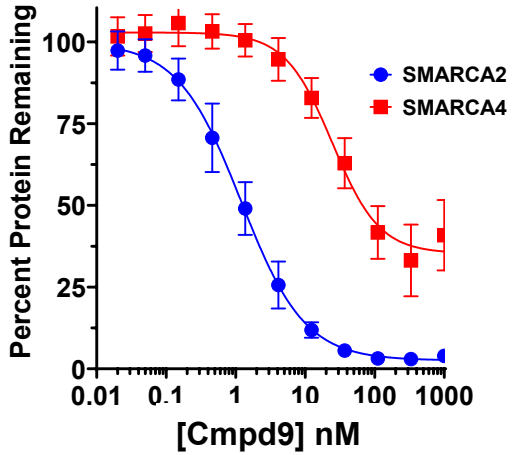


- Cmpd9 and Cmpd16 are two chemically diverse degraders
- Pre-incubation with neddylation inhibitor MLN4924 or proteasome inhibitor MG132 prevents degradation of SMARCA2 and 4
- Cmpd9 and Cmpd16 degradation of SMARCA2 and 4 exhibit cullin ring E3 ubiquitin ligase and proteasome dependence

SMARCA2 selective or dual degrader molecules allow for investigation of biology and in vitro to in vivo correlation

Cmpd17 represents an example of a dual mechanism while Cmpd9 is selective



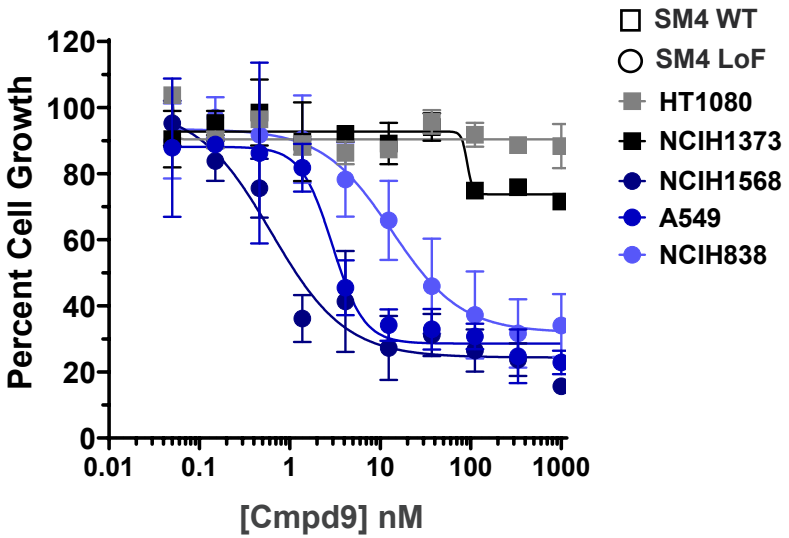


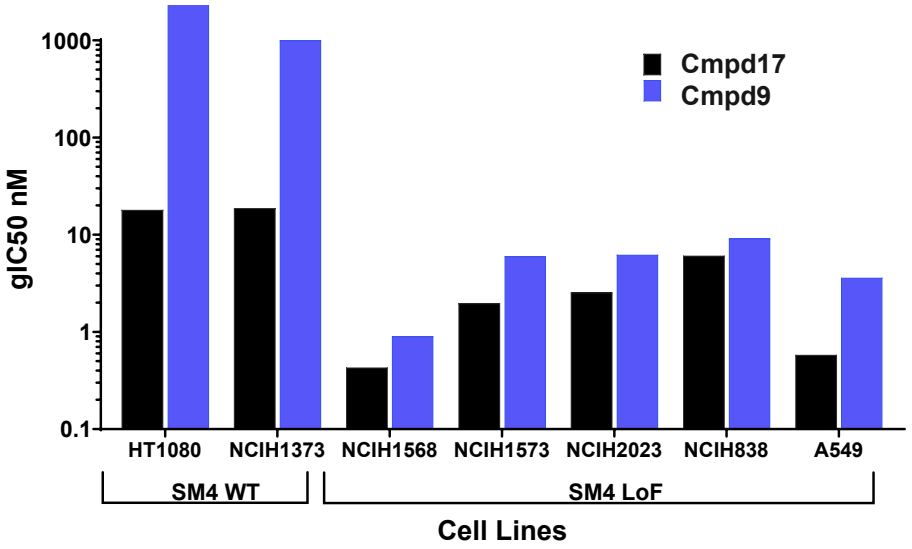
	SMARCA2		SMARCA4	
	D_{max} (%)	DC_{50} (nM)	D_{max} (%)	DC_{50} (nM)
Cmpd17	97	0.1	98	0.6
Cmpd9	97	1	65	67

- HT1080 HiBit assay utilized to evaluate degradation
- Cmpd17 and 9 exhibit differences in degradation potency and selectivity
- Cmpd9 is a potent and selective SMARCA2 degrader
- Cmpd17 and Cmpd9 exhibit equivalent plasma clearance and tumor exposure

SMARCA2 degradation selectivity leads to selective in vitro anti-proliferative activity

SMARCA4 LoF cells are sensitive to selective degraders while wild-type cells are unaffected

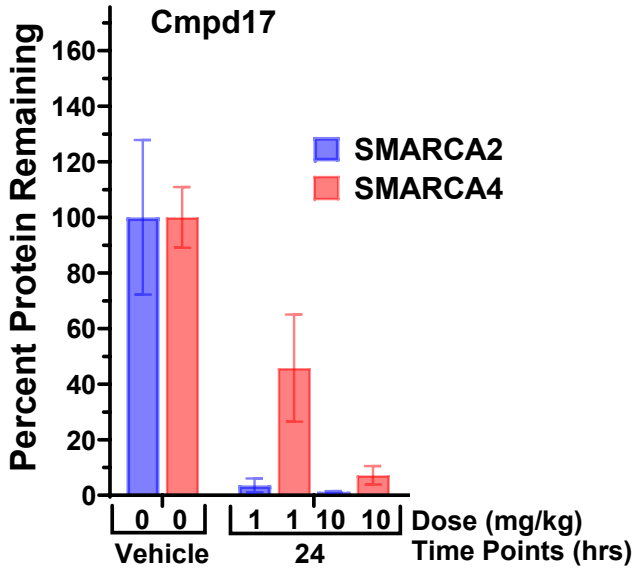


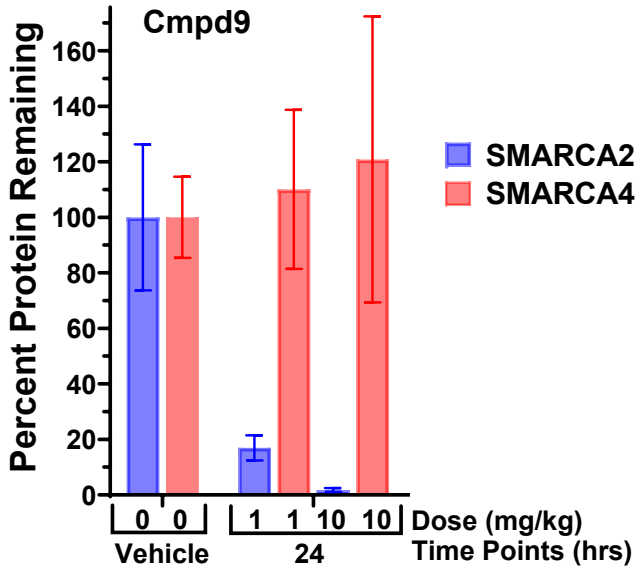


- 6-day proliferation assay used to investigate biological impact of SMARCA degradation
- Cmpd9 is a selective SMARCA2 degrader while Cmpd17 degrades SMARCA4 with similar potency
- Selective SMARCA2 degraders exhibit selective anti-proliferative activity on SMARCA4 LoF cells

In vitro SMARCA2 potency and selectivity translates to in vivo protein degradation

Degradation in wild-type xenograft tumor model confirms in vitro results

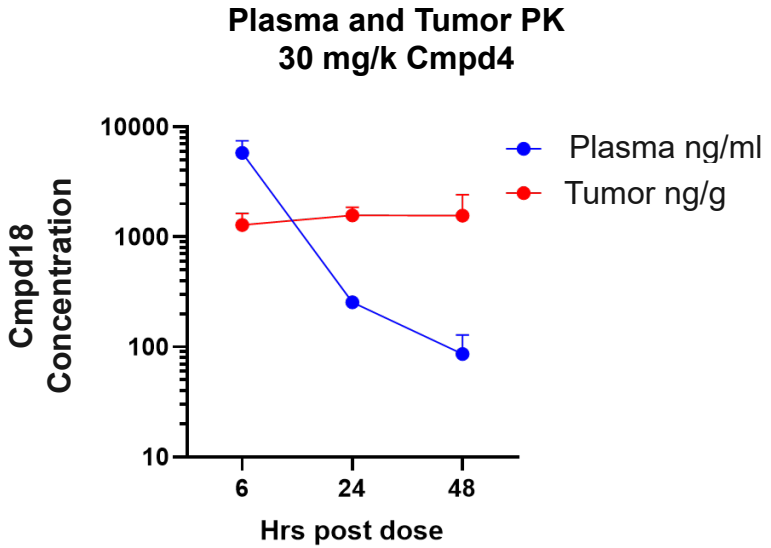


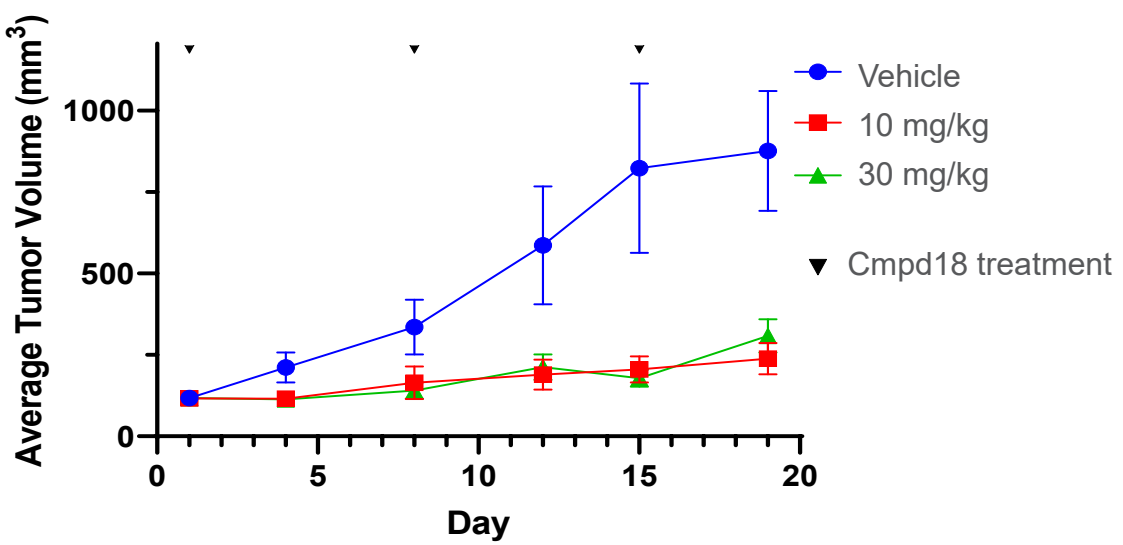


- HT1080 xenograft model established for in vivo assessment of degradation
- SMARCA2 and SMARCA4 degradation in tumors was assessed after single administration of Cmpd17 or Cmpd9
- Cmpd17 shows greater reduction of SMARCA2 at 1mpk but shows similar degradation of both SMARCA2/4 at 10mpk
- Cmpd9 maintains greater selectivity than Cmpd17 even at the higher dose level thereby correlating with in vitro results

Treatment with Cmpd18 leads to anti-tumor efficacy in SMARCA4 LoF NSCLC tumor model

Sustained tumor exposure leads to efficacy with once weekly administration

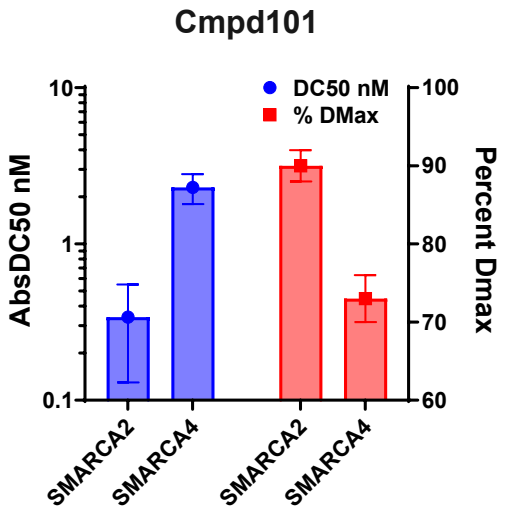


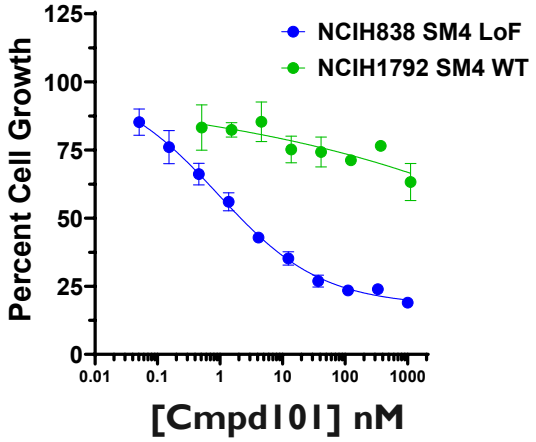


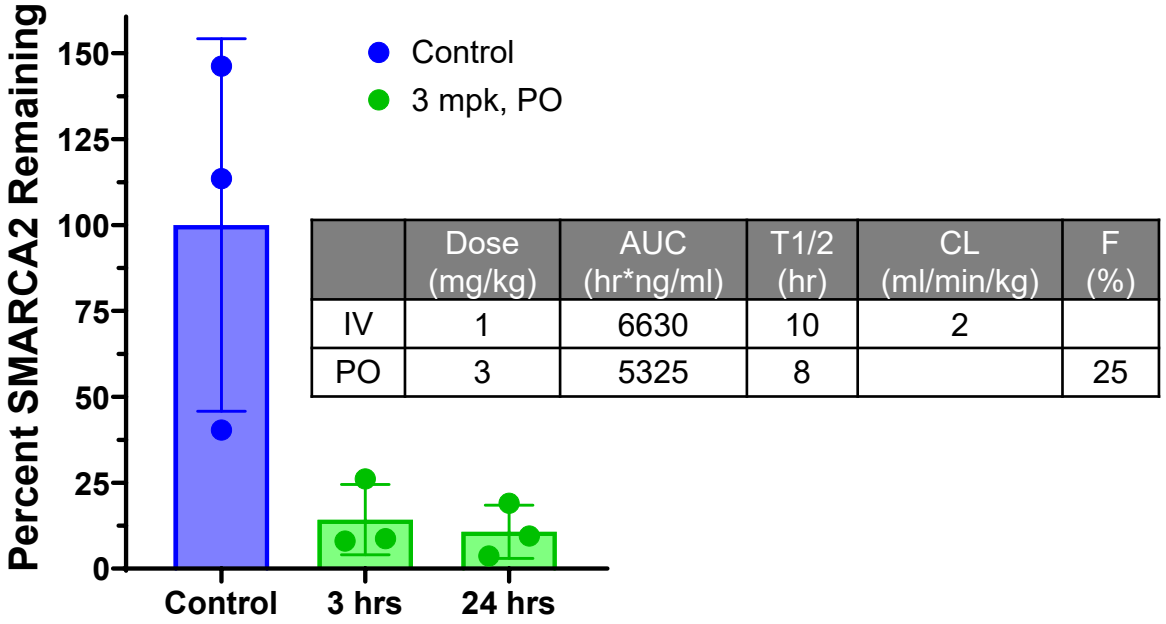
- Cmpd18 selectively degrades SMARCA2 over SMARCA4 in vitro and in vivo
- NCI-H838 SMARCA4 LoF xenograft model was established for in vivo evaluation of SMARCA2 degrader efficacy
- Cmpd18 demonstrates sustained tumor exposure in NCIH838 xenograft model
- Cmpd18 treatment leads to potent anti-tumor efficacy effect in SMARCA4 LoF tumor model

Cmpd101 demonstrates potent and selective SMARCA2 degradation in vitro and in vivo

Cmpd101 is orally bioavailable







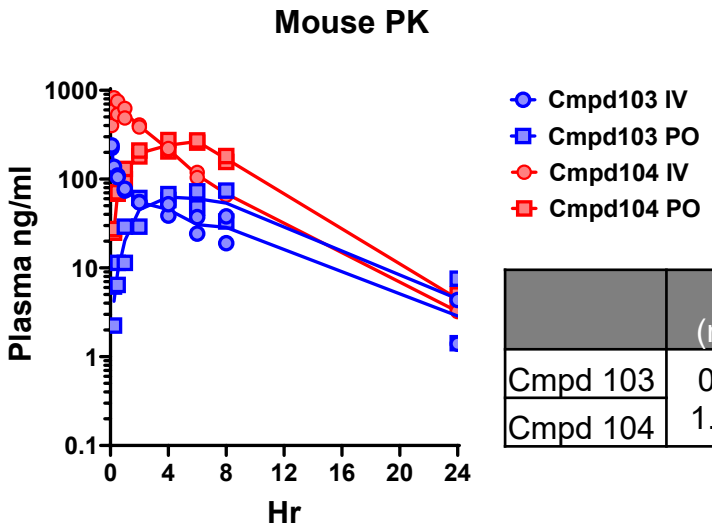
	Dose (mg/kg)	AUC (hr*ng/ml)	T1/2 (hr)	CL (ml/min/kg)	F (%)
IV	1	6630	10	2	
PO	3	5325	8		25

- Selective degradation evident in Hela HiBit assay
- Potent and selective antiproliferative activity on SMARCA4 LoF cells

- High circulating exposure and low clearance after Cmpd101 IV (1 mg/kg) and PO (3 mg/kg) administration
- Robust SMARCA2 degradation after single 3 mg/kg oral administration by 3 hours that persists through 24 hours

Two structurally unique series of SMARCA2 degraders demonstrate oral bioavailability

Cmpd103 and Cmpd104 represent exemplars from each series

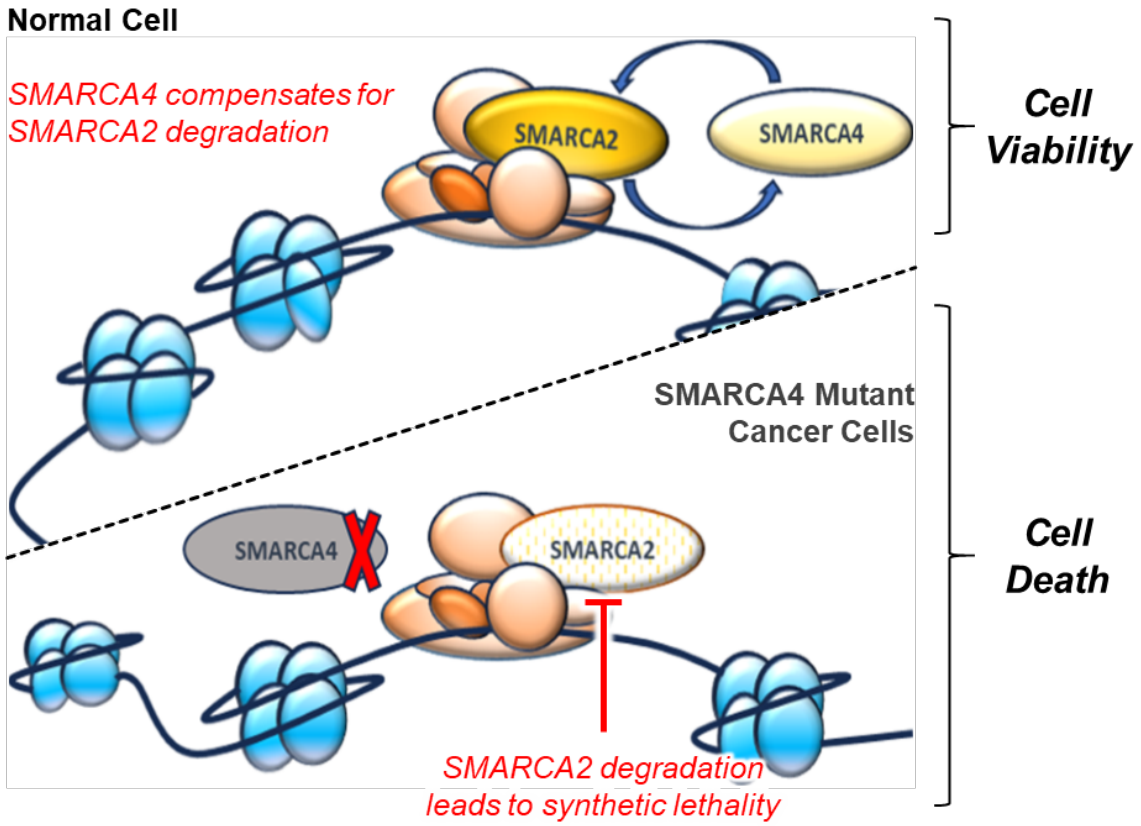


	Dose (mg/kg)	Vdss (L/kg)	T1/2 (hr)	CL (ml/min/kg)	F (%)
Cmpd 103	0.5 (IV) 1.0 (PO)	5.3	5.0	14	58
Cmpd 104		3.4	6.6	6.7	18

- SKLSL series of degraders exhibit oral bioavailability in mice ranging from 7-58 %F
- Rat oral bioavailability ranges from 9-31 %F
- Orally bioavailable degraders exhibit selective degradation of SMARCA2 and growth inhibition of SMARCA4 LoF cells

Summary

Orally Available SMARCA2 Selective Heterobifunctional Degrader for SMARCA4 Mutant Cancer



SMARCA2/4 Regulate Chromatin Accessibility & Gene Expression

Rapid, Potent & Selective SMARCA2 Heterobifunctional Degrader

Selective Growth Inhibition of SMARCA4 Mutant Tumors Representing up to 65,000 cases per year (US)

Orally Bioavailable & Low Clearance

Over 90% SMARCA2 Selective Degradation in Mice (P.O.)

Three Structurally Diverse Series of Heterobifunctional Degraders

Acknowledgements

SMARCA Project Team

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- Corey Strickland
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- Lingying Leng
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