HDACs Related *In Vivo* Models

WuXi AppTec Research Service Division, Oncology & Immunology Unit

2021.07

Outline

- HDACs biology
- HDACs inhibitors as cancer therapeutics
- HDACs inhibitors in CDX models

HDACs family and their substrates

Classification of HDACs

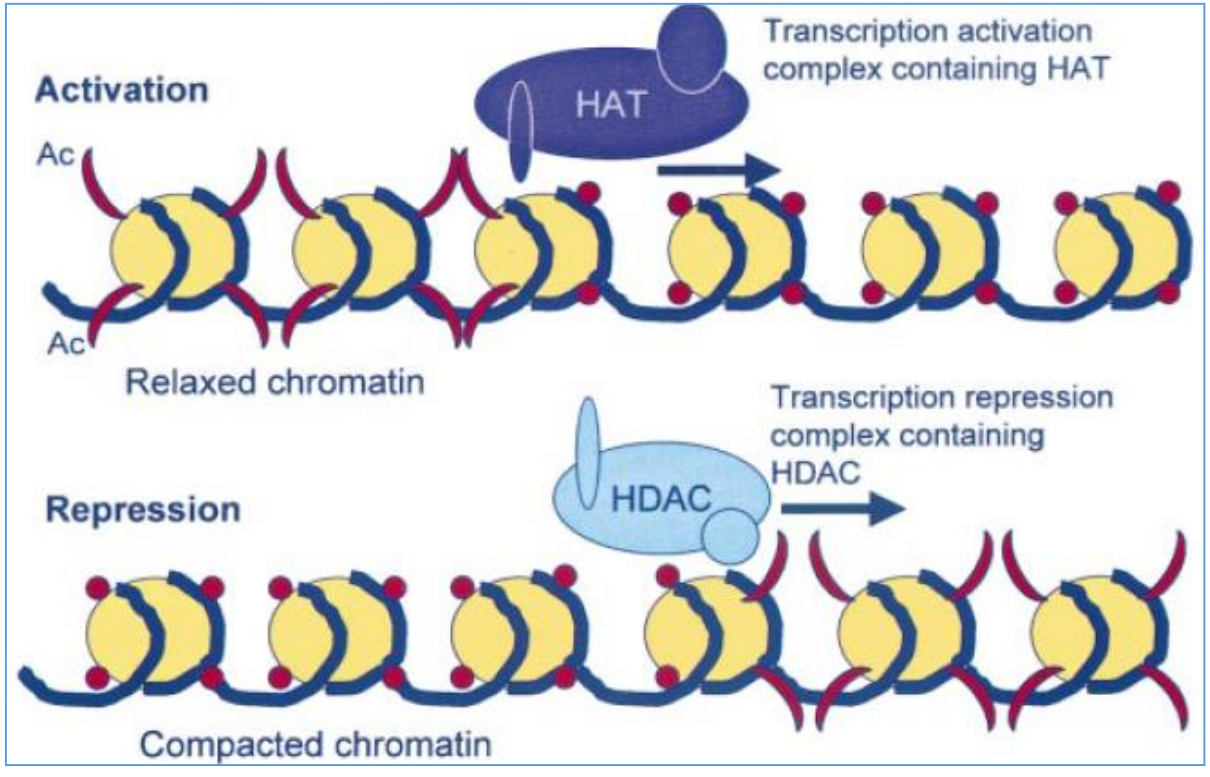
Classification	HDAC	Cytogenetic location	Subcellular localization	Non-histone substrates (partly shown)
I	HDAC1	1p35.1	nucleus	RB1, SHP, p53, MyoD, E2F1, STAT3, NF-κB, CtIP, AMPK
	HDAC2	6q21	nucleus	GCCR, BCL6, STAT3, YY1
	HDAC3	5q31.3	nucleus	SHP, YY1, GATA1, p65, STAT3, MEF2D
	HDAC8	Xq13.1	nucleus	SMC3, actin
IIa	HDAC4	2q37.3	nucleus/cytoplasm	GATA1, HP1
	HDAC5	17q21.31	nucleus/cytoplasm	SMAD7, HP1
	HDAC7	12q13.11	nucleus/cytoplasm	PLAG1, PLAG2
	HDAC9	7p21.1	nucleus/cytoplasm	---
IIb	HDAC6	Xp11.23	mostly cytoplasm	α-tubulin, HSP90, SHP, SMAD
	HDAC10	22q13.31-q13.33	nucleus/cytoplasm	---
III	SIRT1	10q21.3	nucleus/cytoplasm	p53, β-catenin, Ku70, E2F1, Rb, NF-κB, PGC1α, PPARγ, MyoD, PCAF, FOXO3, HIF1α
	SIRT2	19q13.2	cytoplasm	α-tubulin, FOXO1
	SIRT3	11p15.5	nucleus/mitochondria	IDH2, SDH, CypD, p53, FOXO3A, MRPL10, GDH, LCAD, Ku70, LKB1, NDUFA9
	SIRT4	12q24.31	mitochondria	IDE, ANT2/3, GDH
	SIRT5	6p23	mitochondria	CPS1, Cytochrome C
	SIRT6	19p13.3	nucleus	NF-κB, CtBP, DNA PK, PARP1, HIF1α
	SIRT7	17q25.3	nucleolus	p53
IV	HDAC11	3p25.2	nucleus/cytoplasm	---

Int. J. Biol. Sci. 2014, 2014; 10(7): 757-770

BioMed Research International, 2016, http://dx.doi.org/10.1155/2016/8797206

HDACs biology: Deacetylation of histones

- Acetylation of ε-amino groups of lysine residues within histone tails neutralizes their positive charge, thereby relaxing chromatin structure and increase the accessibility of transcription factors to their target genes;
- Conversely, histone deacetylation favours transcriptional repression by allowing for chromatin compaction.
- Direct acetylation and deacetylation of **transcription factors** has been shown to have positive and negative consequences on gene transcription, respectively.



Nat Rev Genet. 2009 January ; 10(1): 32–42.
Biochem. J. (2003) *370, 737±749*

HDACs biology: Deacetylation of non-histone proteins

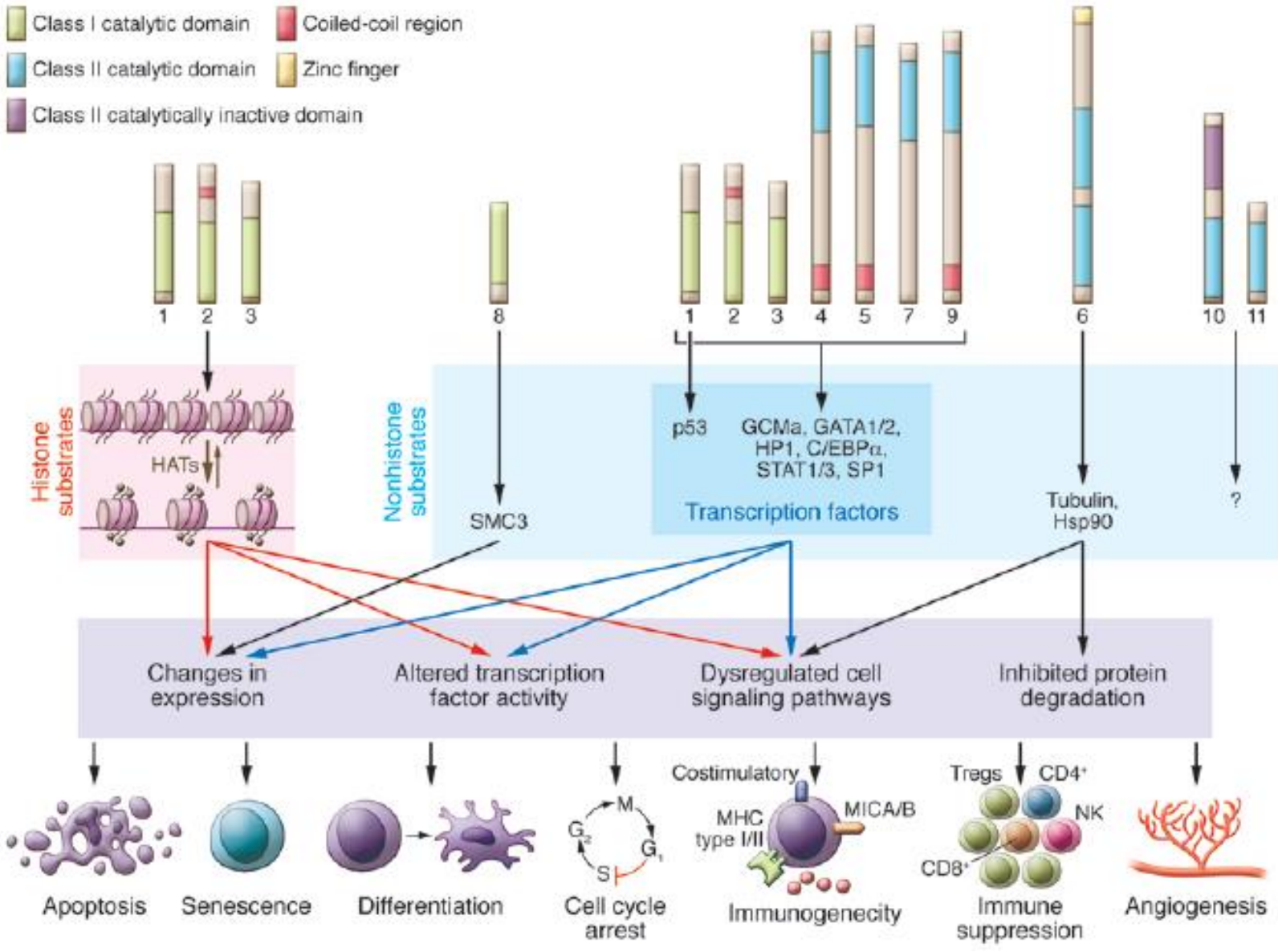
Selected non-histone proteins and functional consequences of their acetylation

Biological implication	Proteins affected by acetylation	
Protein stability	Acetylation increases stability p53, p73, Smad7, c-Myc, Runx3, AR, H2A.z, E2F1, NF-E4, ER81, SREBP1a, HNF6, BACE1	Acetylation decreases stability GATA1, HIF-1α, pRb, SV40 T-Ag
DNA binding	Increased DNA binding p53, SRY, STAT3, GATA transcription factors, E2F1, p50 (NFκB), Erα, p65 (NFκB), c-Myb, MyoD, HNF-4, AML1, BETA2, NF-E2, KLF13, TAL1/SCL, TAF(I)68, AP endonuclease	Decreased DNA binding YY1, HMG-A1, HMG-N2, p65 (NFκB), DEK, KLF13, Fen-1
Gene expression	Transcriptional activation p53, HMG-A1, STAT3, AR, ERα (basal), GATA transcription factors, EKLF, MyoD, E2F1, p65(NFκB), GR, p73, PGC1α, MEF2D, GCMa, PLAG1, PLAGL2, Bcl-6, β-Catenin, KLF5, Sp1, BETA2, Cart1, RIP140, TAF(I)68	Transcriptional inactivation Erα (ligand-bound), HIF-1α, STAT1, FOXO1, FOXO4, RIP140
Protein interactions	Enhanced STAT3, AR, EKLF, Importin A, STAT1, TFIIB, α-Tubulin, actin, cortactin	Decreased p65(RelA), Ku70, Hsp90
Localisation	Ac → nucleus PCAF, SRY, CtBP2, POP-1, HNF-4, PCNA Sub-nuclear WRN, PCNA	Ac → cytosol c-Abl, p300, PAP
mRNA stability	Increased p21, Brm	Decreased Tyrosinhydrolase (Th), eNOS
Enzymatic activity	Enhanced p300, ATM	Decreased PTEN, HDAC1, Mdm2, ACS, Neil2, Polβ
Mitochondrial proteins	ACS (Ac-CoA-Synthetase), Sod1/2, Profilin I, Thioredoxin; multiple components of metabolic and oxidative phosphorylation machinery	
Viral proteins	E1A, S-HDAg, L-HDAg, HIV Tat, SV40 T-Ag	

The international journal of biochemistry & cell biology 2009 Jan; 41(1):185-98.

HDACs biology

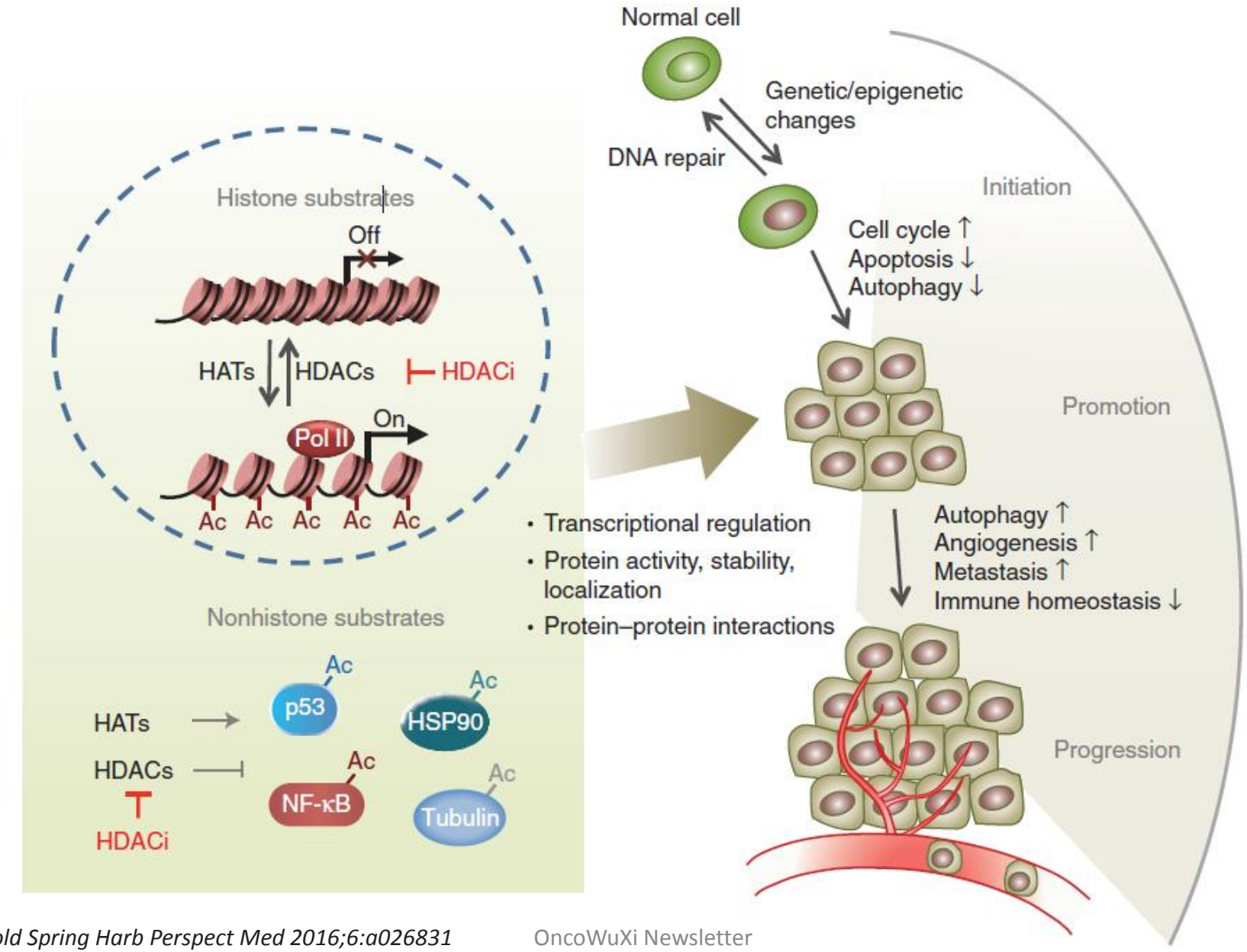
The biological processes HDACs involve



J Clin Invest. 2014;124(1):30–39.

Role of HDACs in cancer

Diverse functions of HDACs and HDACi regulating different stages of cancer



Cold Spring Harb Perspect Med 2016;6:a026831

Role of HDACs in cancer

HDACs are aberrantly regulated in cancer.

Aberrant regulation of HDACs in cancer

HDAC	Normal and oncogenic protein associations	Expression in cancer	Genetic evidence
Class I (homologous to RDP3 yeast protein, nuclear location, ubiquitous tissue expression)			
HDAC1	HDAC2, CoREST, NuRD, Sin3, AML1-ETO, PML, PLZF, BCL6, p53, AR, ER, Rb/E2F1	Elevated in gastric[A], breast[B], colorectal, HL, lung[A], liver[A]	KD induced growth arrest, decreased viability, and increased apoptosis in colon, breast, and osteosarcoma cancer cells and increased survival of mice with overt PML-RARα–mediated APL; HDAC1 KO/HDAC2 KD induced growth arrest in fibroblasts; KO induced genomic instability and arrest and reduced survival of transformed cells in vivo
HDAC2	HDAC1, CoREST, NuRD, Sin3, AML1-ETO, PML, PLZF, Bcl6	Elevated in gastric[A], prostate[A], colorectal[A], HL, CTCL	KD induced growth arrest, decreased viability, and increased apoptosis in colon and breast cancer cells and induced apoptosis and decreased lung cancer in vivo; HDAC1 KO/HDAC2 KD induced growth arrest in fibroblasts; KO induced genomic instability and arrest and reduced survival of transformed cells in vivo; KD induced apoptosis and decreased lung cancer in vivo
HDAC3	HDAC4, HDAC5, HDAC7, NCoR/SMRT, AML1-ETO, PML, PLZF, PML-RARα, PLZF-RARα, Bcl6, STAT1, STAT3, GATA1, GATA2, NF-κB	Elevated in gastric[A], breast[AB], ALL, colorectal, HL; decreased in liver	KD in colon cancer cells decreased survival, increased apoptosis, and relieved transcriptional repression mediated by PML-RARα in APL cells
HDAC8		Elevated in neuroblastoma	KD reduced proliferation of lung, colon, and cervical cancer cells

J Clin Invest. 2014;124(1):30-9. doi: 10.1172/JCI69738

Role of HDACs in cancer (continued)

HDACs are aberrantly regulated in cancer.

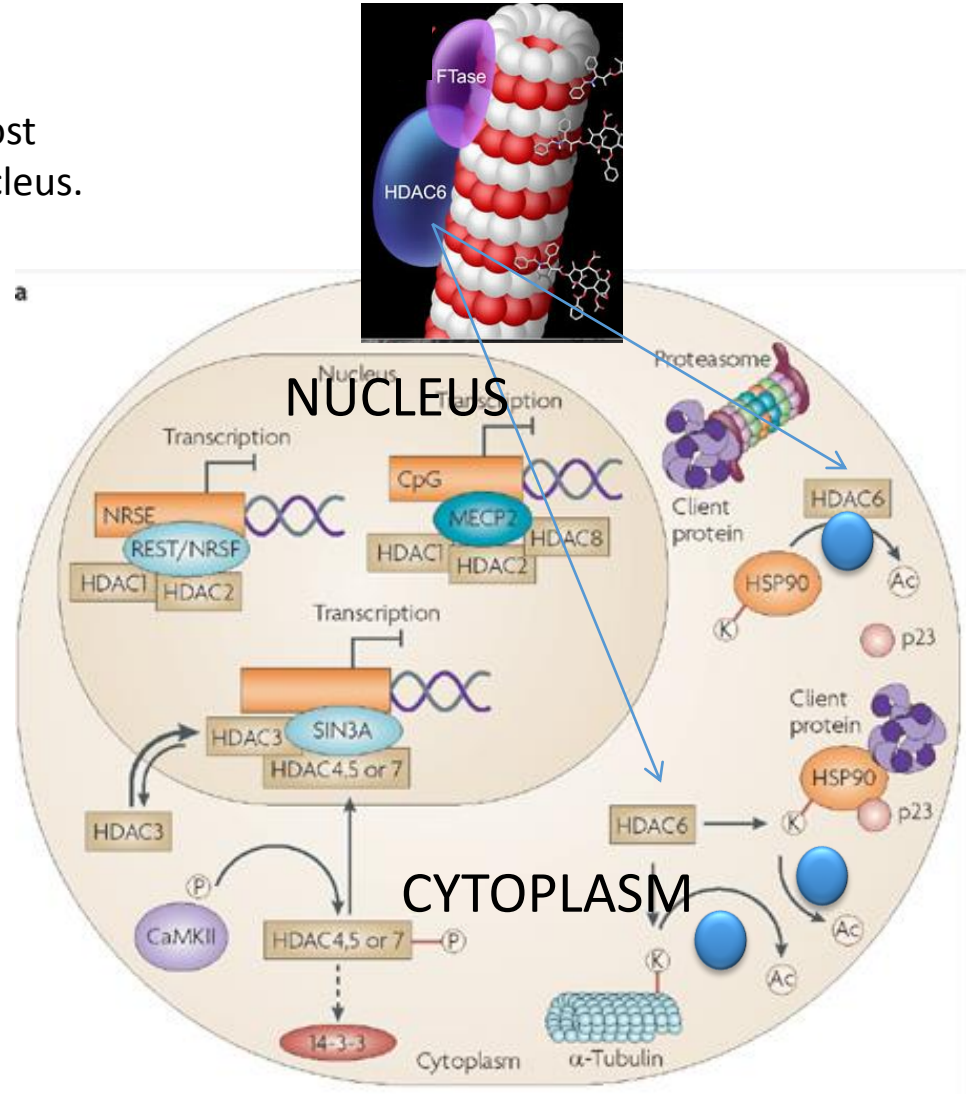
Class IIa (homologous to Hda1 yeast protein, shuttle between nucleus and cytoplasm, tissue-restricted expression)			
HDAC4	HDAC3-NCoR, GATA1		KD in chondrosarcoma cells increased VEGF expression and reduced growth and induced apoptosis of colon and glioblastoma tumors in vivo
HDAC5	HDAC3-NCoR, GATA1, GATA2	Elevated in medulloblastoma; decreased in lung	KD decreased medullablastoma cell growth and viability
HDAC7	HDAC3-NCoR, ERα	Elevated in ALL; decreased in lung	KD induced growth arrest in colon and breast cancer cells
HDAC9		Elevated in ALL, medullablastoma	KD of HDAC9/10 inhibited homologous recombination and increased sensitivity to DNA damage and decreased medullablastoma cell growth and viability
Class IIb (homologous to yeast protein Hda1, mostly cytoplasmic location, tissue-restricted expression)			
HDAC6	α-Tubulin, HSP90, HDAC11	Elevated in breast[B], CTCL; decreased in lung	KD decreased VEGF expression and decreased cell viability due to accumulation of misfolded proteins
HDAC10			KD of HDAC9/10 inhibited homologous recombination and increased sensitivity to DNA damage and decreased VEGF expression
Class IV (unknown yeast protein homology, cytoplasmic location, tissue-restricted expression)			
Class 11	HDAC6	Elevated in breast, renal, liver	KD induced apoptosis in colon, prostate, breast, and ovarian cancer lines

J Clin Invest. 2014 Jan;124(1):30-9. doi: 10.1172/JCI69738

HDAC6 differs from other HDACs and does not catalyze histones directly

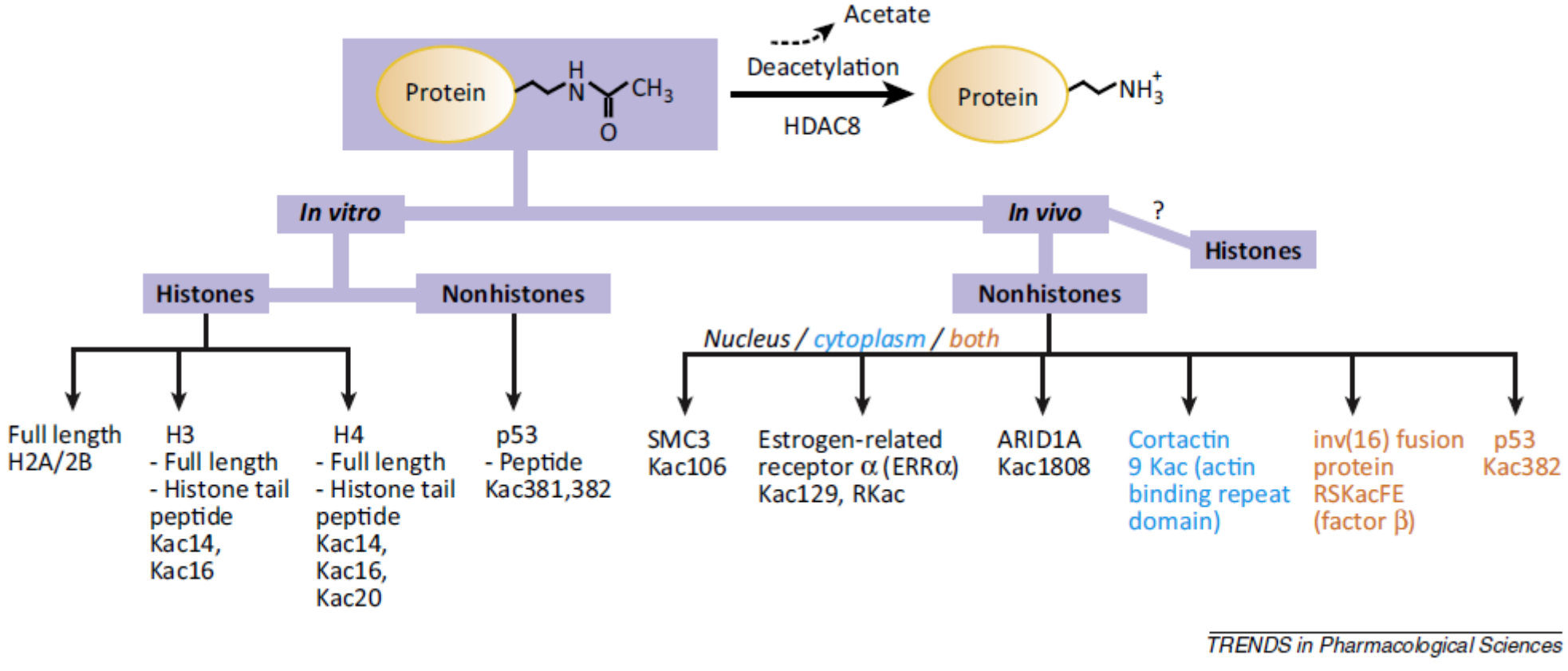
HDAC6 is localized exclusively in the cytoplasm in contrast to most HDACs which are transiently or permanently localized in the nucleus.

- HDAC6 does not catalyze histone deacetylation in vivo.
- Better drug target since there is no impact on DNA.
- HDAC6 has two main in vivo substrates:
  - **Alpha tubulin** is involved in cytoskeletal structural integrity and cellular motility
  - **Hsp90** (heat shock protein) helps client proteins fold properly and maintain function



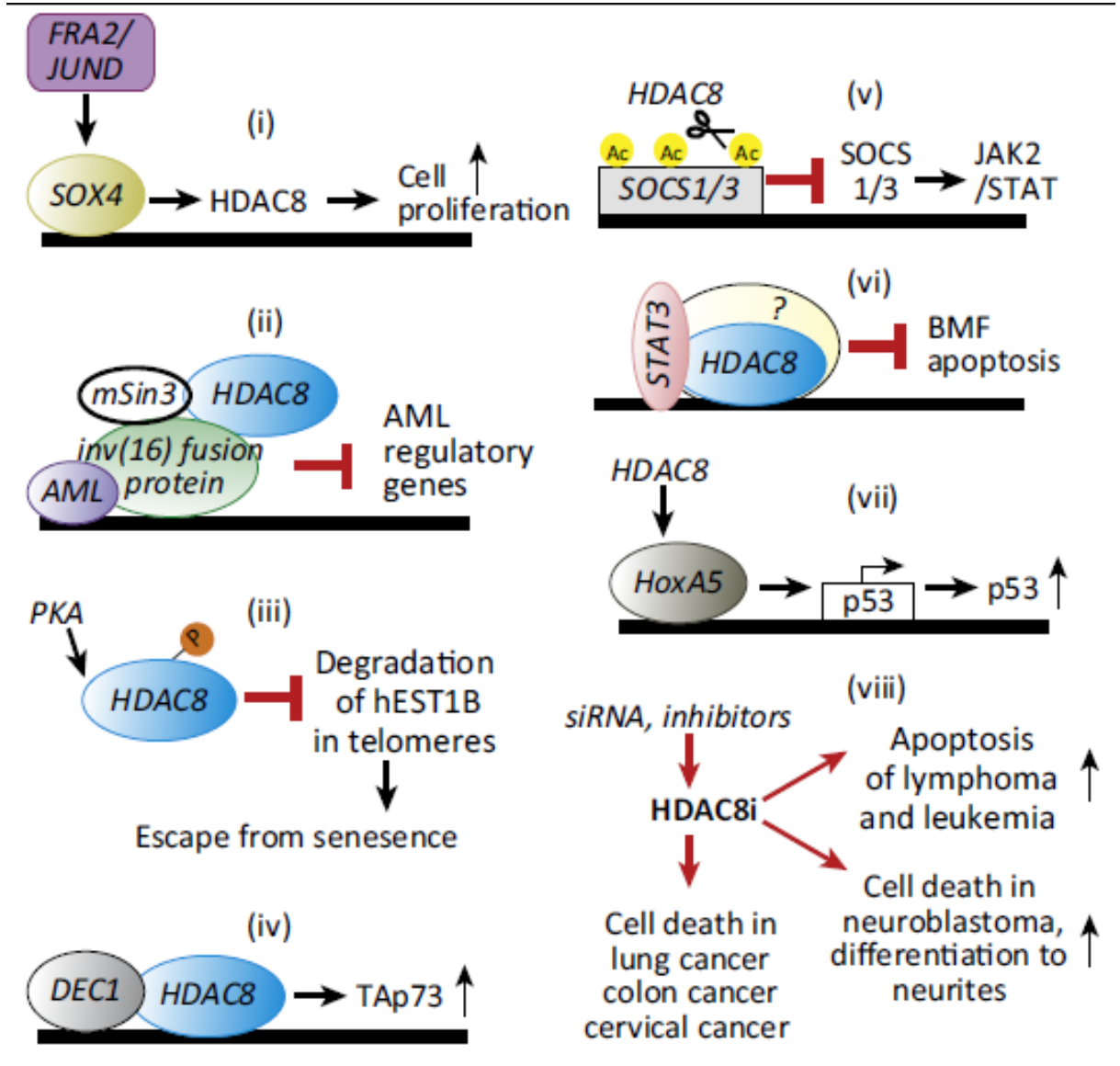
HDAC8: a novel multifaceted target for therapeutic interventions

HDAC8 diverged from other class I enzymes early in evolution, X-linked in humans, and its activity is independent of additional co-factors.



Trends Pharmacol Sci. *2015;36(7):481-92.*

Representative molecular interactions of HDAC8 with crucial proteins in cancer



Trends Pharmacol Sci. *2015;36(7):481-92.*

HDAC inhibitors

Drug	Inventor	Target	Indications	Status	Description
Panobinostat (LBH589, Farydak)	Norvatis	HDAC 1, 2, 3, 6	Multiple myeloma	FDA 2015	Approval for use in patients with multiple myeloma who had received at least 2 previous treatments, including bortezomib and an immunomodulatory agent.
Belinostat	TopoTarget	pan-HDACs	Peripheral T cell lymphoma (PTCL)	FDA 2014	
Vorinostat	Merck & Co Inc;	HDAC 1, 2, 3, 6	Cutaneous T cell lymphoma (CTCL)	FDA 2006	
Romidepsin	Celgene	pan-HDACs	CTCL, PTCL	FDA 2009	Cutaneous T-cell lymphoma (CTCL) patients who have received at least one prior systemic therapy.
Sodium Phenylbutyrate	Ucyclyd Pharma	pan-HDACs	urea cycle disorders	FDA 1996	
CG-745	CrystalGenomics	HDAC6	pancreatic cancer	FDA 2019	Orphan Drug Designation from the US FDA in Pancreatic Cancer

HDAC inhibitors (continued)

Drug	Inventor	Target	Indications	Status	Description
Ricolinostat (ACY1215)	Acetylon Inc;	HDAC 6 specific	Lymphoma, MM	Phase II	
ACY241	Acetylon	HDAC 6 specific	Stage III/Stage IV melanoma, NSCLC	Phase I	
Resminostat	4SC AG/Menarini	pan-HDAC	CRC, Lymphoma	Phase II	
Quisinostat	Johnson & Johnson ;	H3 , HDAC1,2	TCL	Phase II	
RG2833	Scripps Research Institute	HDAC1/3	Friedrick's Ataxia Temozolomide-resistant glioblastoma	Phase I	brain-penetrant inhibitor of HDAC
RGFP966		HDAC3	CTCL	Pre-clinical	brain-penetrant
BG45	Harvard Medical School	HDAC3	MM,TNBC	Pre-clinical	
PCI-34051	Pharmacyclics Inc	HDAC8	T cell lymphoma and GBM	Pre-clinical	
C149	Kyoto Prefectural University of Medicine	HDAC8	T cell lymphoma and GBM	Pre-clinical	

HDAC inhibitors (continued)

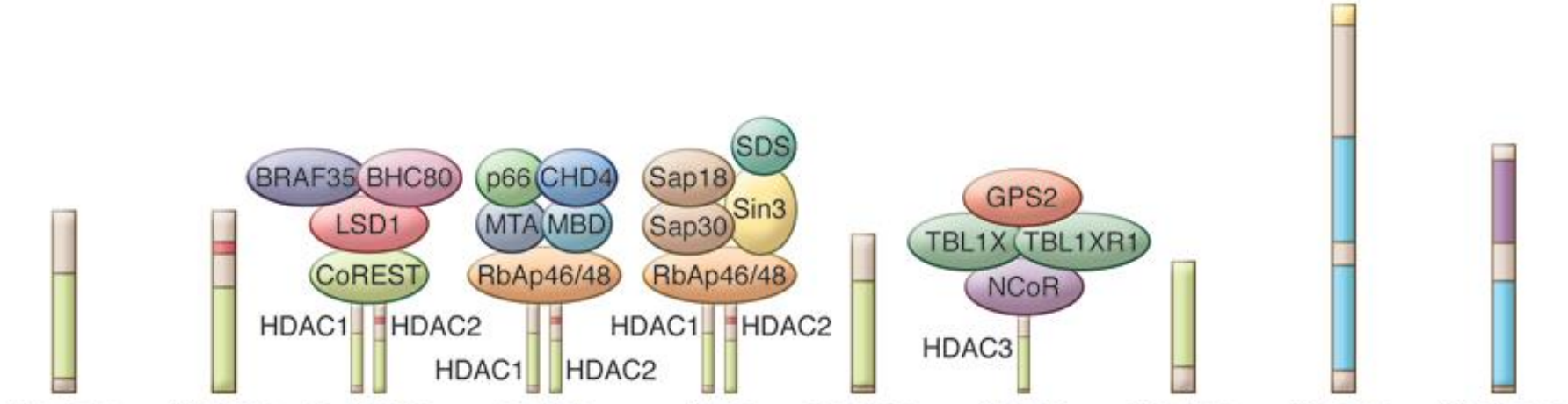
Drug	Inventor	Target	Indications	Status
Chidamide (Epidaza)	Chipscreen Biosciences (微芯生物)	Class I HDAC1, HDAC2, HDAC3, as well as Class IIb HDAC10	Chinese FDA for relapsed or refractory peripheral T-cell lymphoma (PTCL), and having orphan drug status in Japan	CFDA 2014
Abexinostat (PCI-24781)	Pharmacyclics	pan-HDAC	B-cell lymphoma, sarcoma	Phase II
Entinostat (MS275)	Syndax	class I HDAC1 and HDAC3	Hodgkin's lymphoma, advanced breast cancer, metastatic lung cancer, melanoma	Phase III
Mocetinostat	MethylGene	pan-HDAC	follicular lymphoma, Hodgkin's lymphoma and acute myelogenous leukemia	Phase II
KA2507	Karus Therapeutics	HDAC6	melanoma and/or other solid tumors	Phase I
NBM-BMX	NatureWise Biotech & Medicals	HDAC8	Malignant Neoplasm	Phase I

HDAC inhibitors (continued)

Drug	Inventor	Target	Indications	Status
Givinostat (ITF2357)	Italfarmaco	pan-HDAC	Relapsed/Refractory Hodgkin's Lymphoma, JAK2 mutant MF	orphan drug for JIA & Polycythemia in EU, Phase II
Pracinostat (SB939)	MEI pharma	Pan-HDAC	AML, T-cell lyphoma	FDA 2014
CHR-3996	Chroma Therapeutics	pan-HDAC	Solid Tumor	Phase I
AR-42	National Cancer Institute (NCI) Arno Therapeutics	pan-HDAC	MM, hematological malignancies	Phase I

HDAC inhibitors

The specificity of HDACi for HDACs and associated protein complexes



	HDAC1	HDAC2	CoREST	NuRD	Sin3	HDAC3	NCoR	HDAC8	HDAC6	HDAC10
Apcidin	++	++	++	++	++	++	++	–	–	–
Belinostat	++	++	++	++	++	++	++	–	++	–
Entinostat	++	++	++	++	–	++	++	+	–	–
Mocetinostat	++	++	++	++	–	++	++	–	–	–
Panobinostat	++	++	++	++	++	++	++	–	–	+
Abexinostat	++	++	++	++	++	++	++	–	++	++
PCI-334051	–	–	–	–	–	–	–	++	–	–
Romidepsin	++	++	++	++	++	++	++	–	–	–
Vorinostat	++	++	++	++	++	++	++	–	++	+
Trichostatin A	++	++	++	++	++	+	++	–	+	–
Valproic acid	++	++	++	++	–	+	+	–	–	–

J Clin Invest. 2014;124(1):30–39.

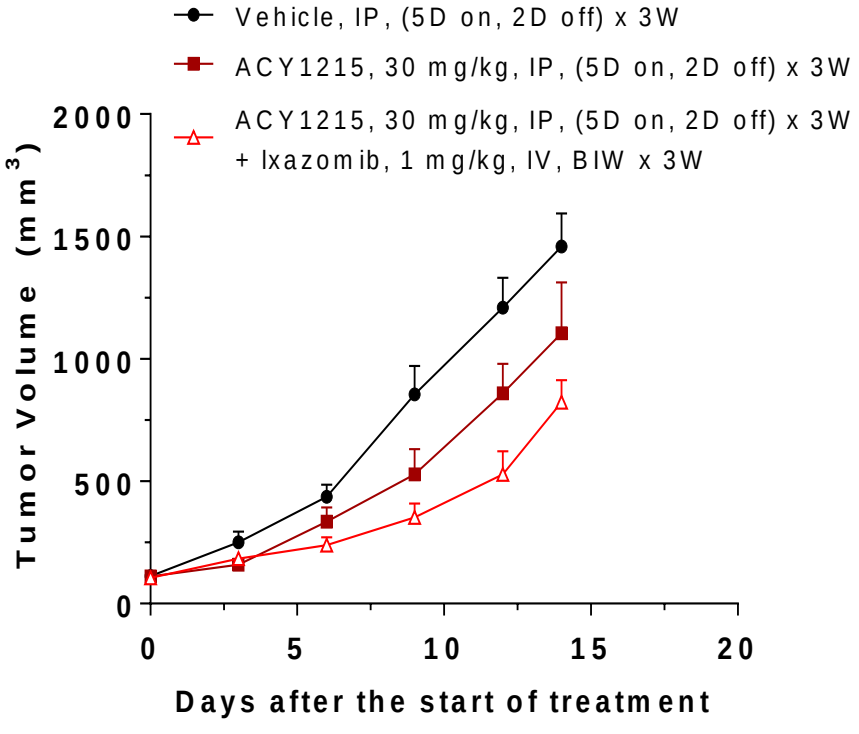
HDACs inhibitors in CDX models (1)

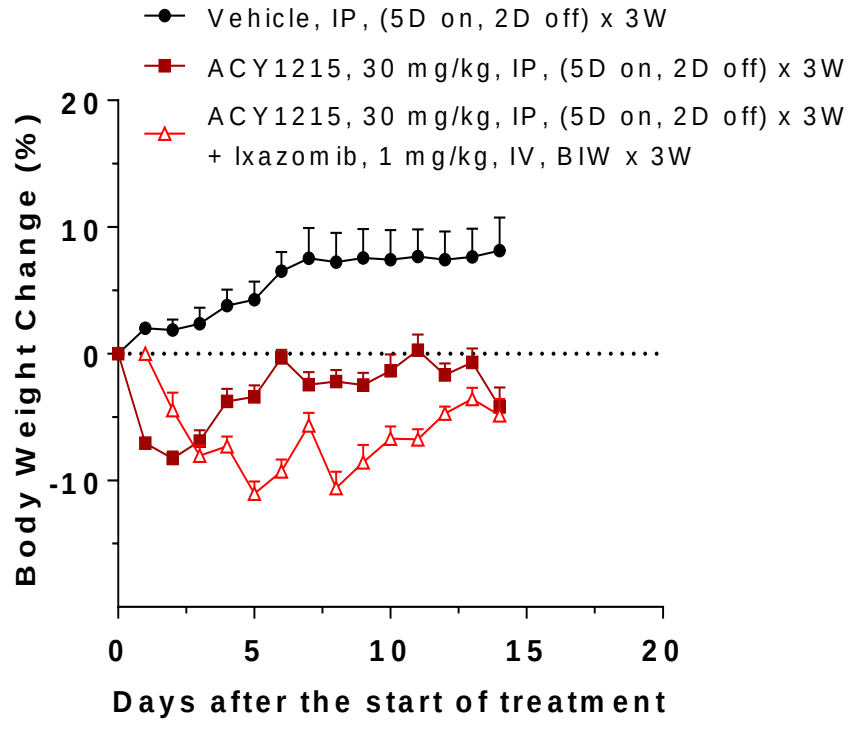
Cancer type	Model ID	Growth curve	Drugs tested	Dosage
Multiple myeloma	MM.1S	Yes	ACY-1215, Ixazomib,	ACY1215, 30 mg/kg, 20 µl/g, IP, 5D on, 2D off Ixazomib, 1 mg/kg, IV, 10 µl/g, BIW
Multiple myeloma	MM.1S	Yes	ACY-1215, Ixazomib,	ACY1215, 7.5 mg/kg, IP, 5D on, 2D off ACY1215, 15 mg/kg, IP, 5D on, 2D off Ixazomib, 4 mg/kg, PO, 10 µl/g, BIW ACY1215, 30 mg/kg, IP, 5D on, 2D off Bortezomib, 0.1 mg/kg, IP, 5D on, 2D off
Multiple myeloma	MM.1S	Yes	ACY-1215, Ixazomib,	ACY1215, 30 mg/kg, 20 µl/g, IP, 5D on, 2D off Ixazomib, 2/4 mg/kg, PO, 10 µl/g, BIW
Multiple myeloma	RPMI8226	Yes	ACY-1215, Ixazomib, Bortezomib	ACY1215, 30 mg/kg, IP, 5D on, 2D off Ixazomib, 0.75 mg/kg, IV, 10 µl/g, BIW Bortezomib, 0.5 mg/kg, IV, 10 µl/g, BIW
Lymphoma	Mino	Yes	ACY1215. Ibrutinib	ACY1215, 30 mg/kg, IP, QDx 21 Ibrutinib, ,25 mg/kg, PO, QDx 21

HDACs inhibitors in CDX models (2)

Cancer type	Model ID	Growth curve	Drugs tested	Dosage
Leukemia	MV4-11	Yes	ACY-1215, Panobinostat	ACY1215, 50 mg/kg, IP, 5D on, 2D off Panobinostat, 10 mg/kg, IP, 5D on, 2D off Panobinostat, 20 mg/kg, IP, 5D on, 2D off
Multiple myeloma	NCI-H929	Yes	ACY-241, Pomalidomide,	ACY-241, 50 mg/kg, 10 µl/g, IP, QD Pomalidomide, 1 mg/kg, 10 µl/g, IP, QD

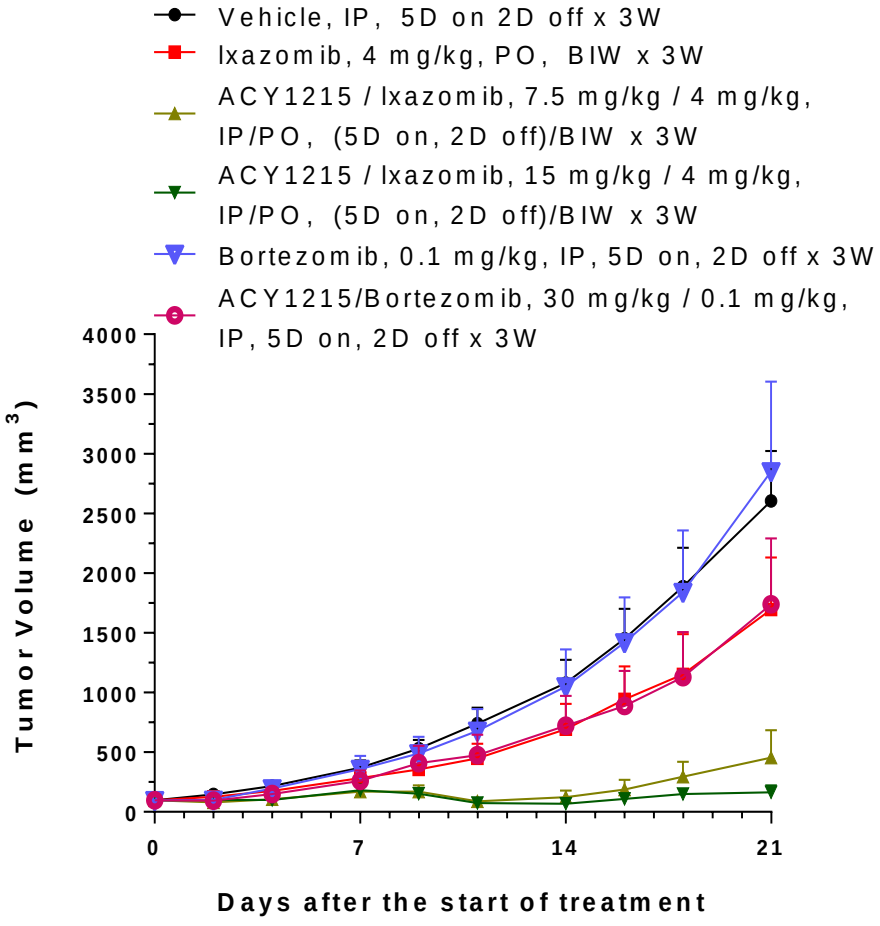
HDAC inhibitors in MM.1S

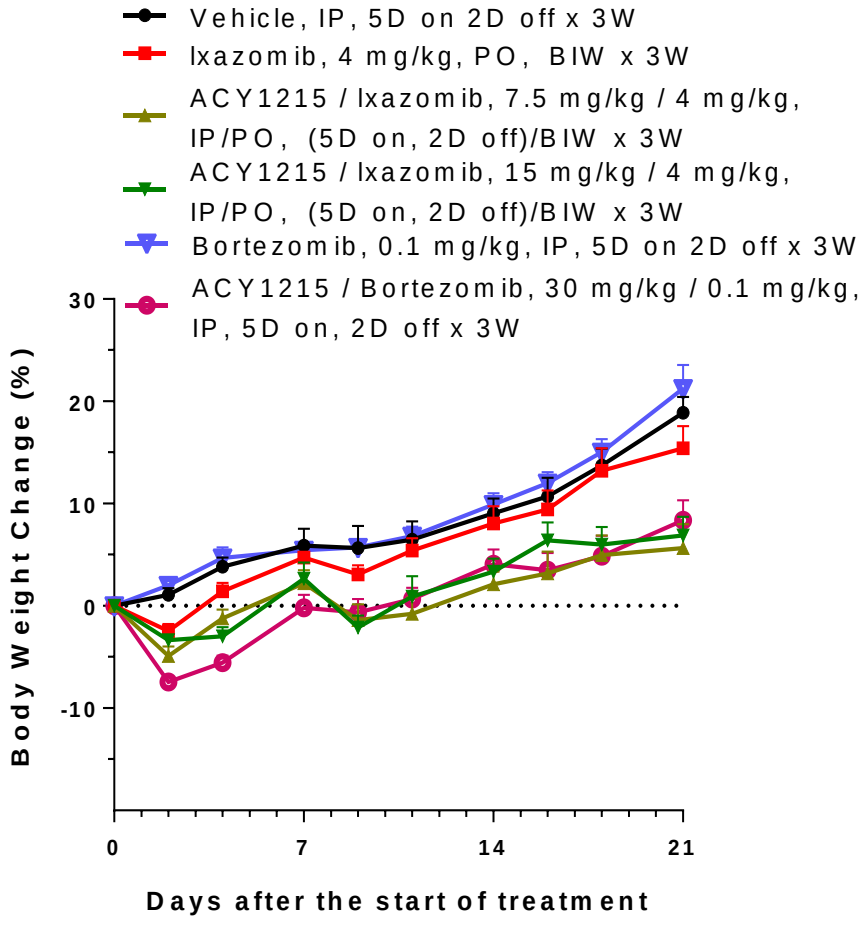




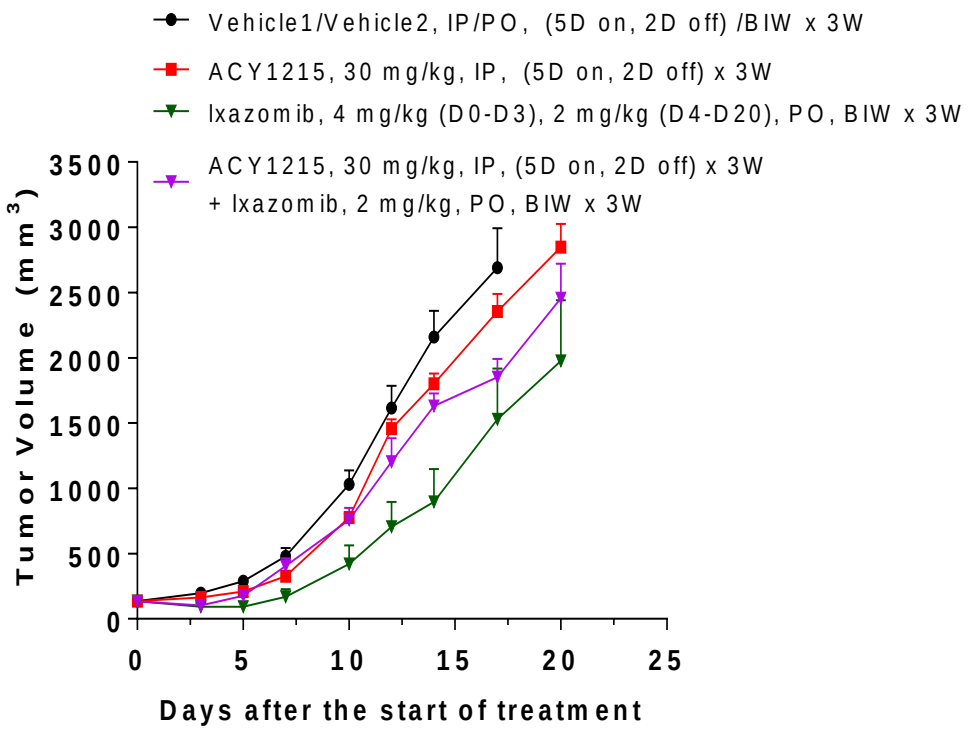
Note: Dosing suspended (mice with BW loss~10-13%) in combo group.

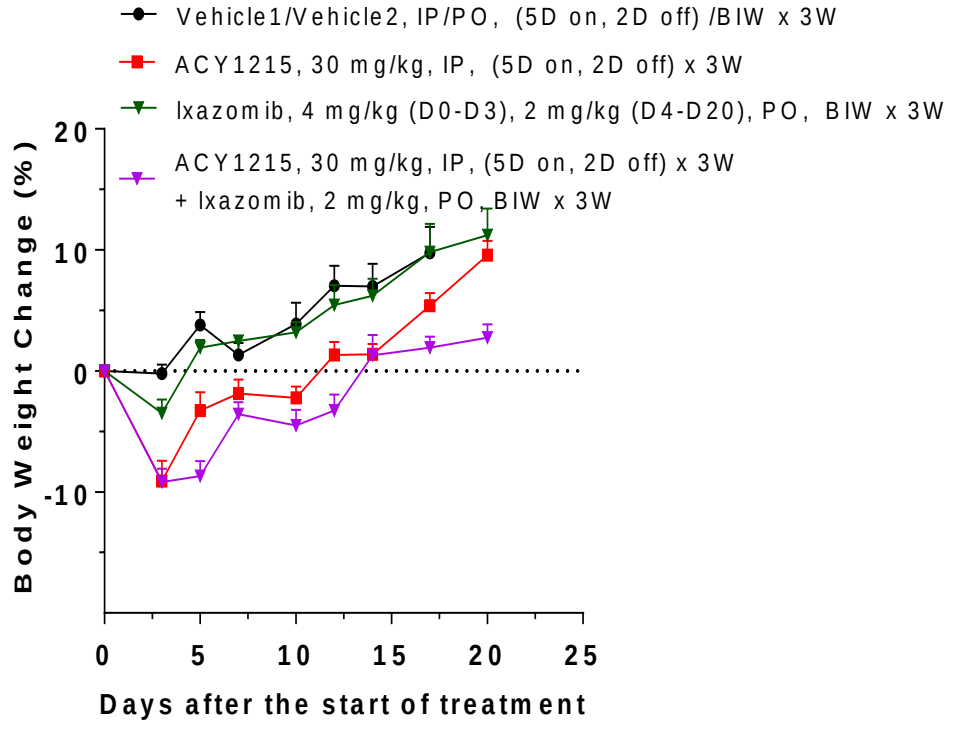
Anti-tumor efficacy of HDACi in MM.1S xenograft model



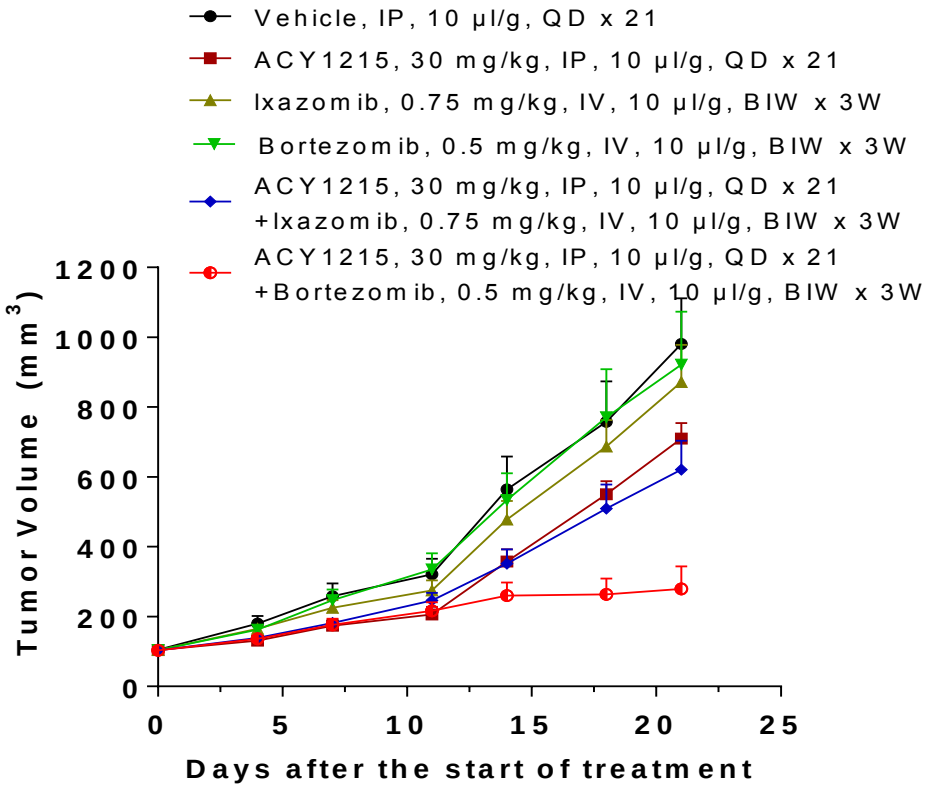


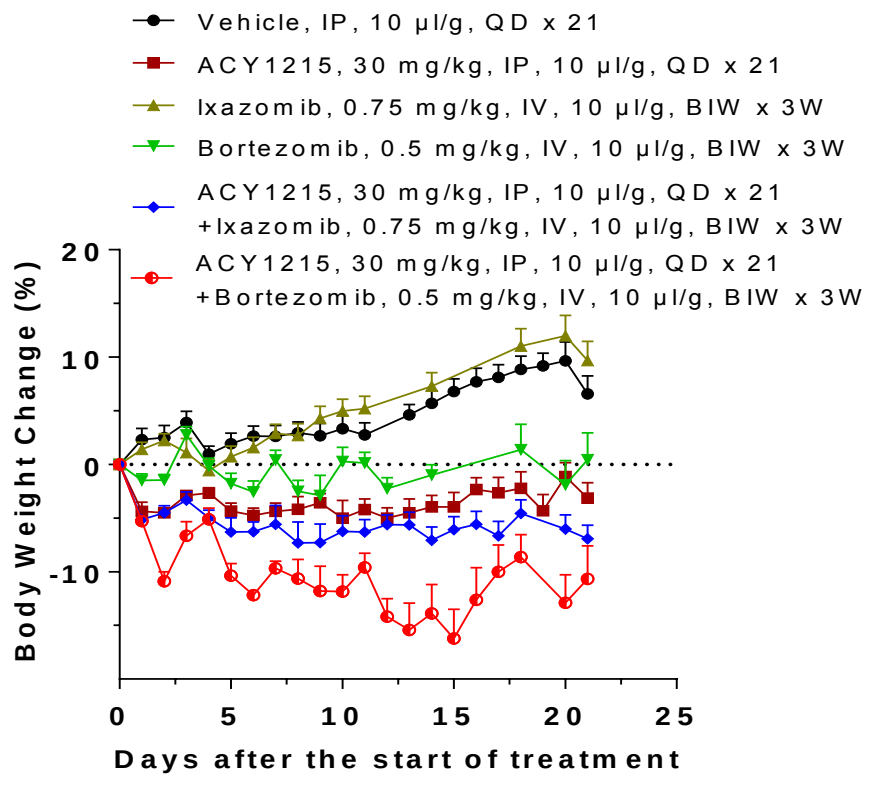
Anti-tumor efficacy of HDACi + Ixazomib in MM.1S model





Anti-tumor efficacy of HDACi + proteosome in RPMI-8226 model

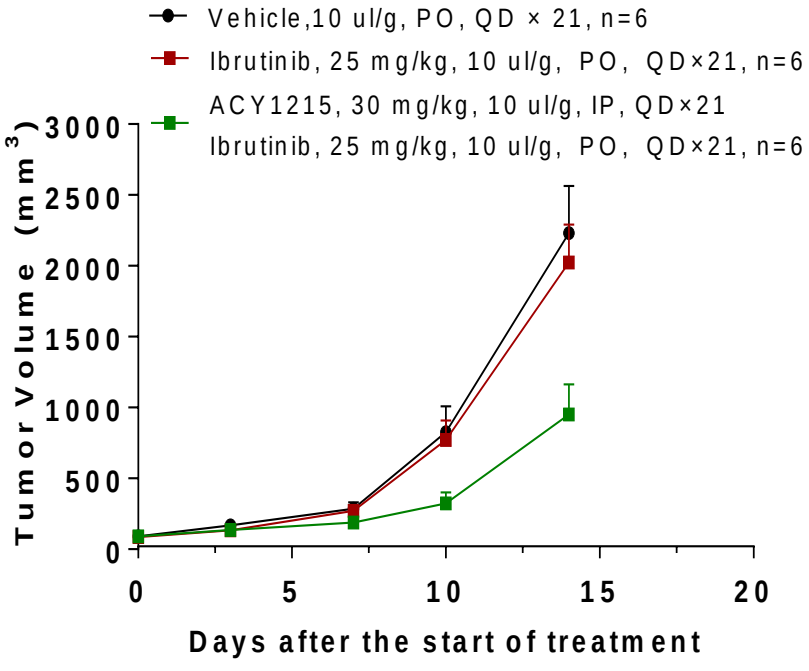


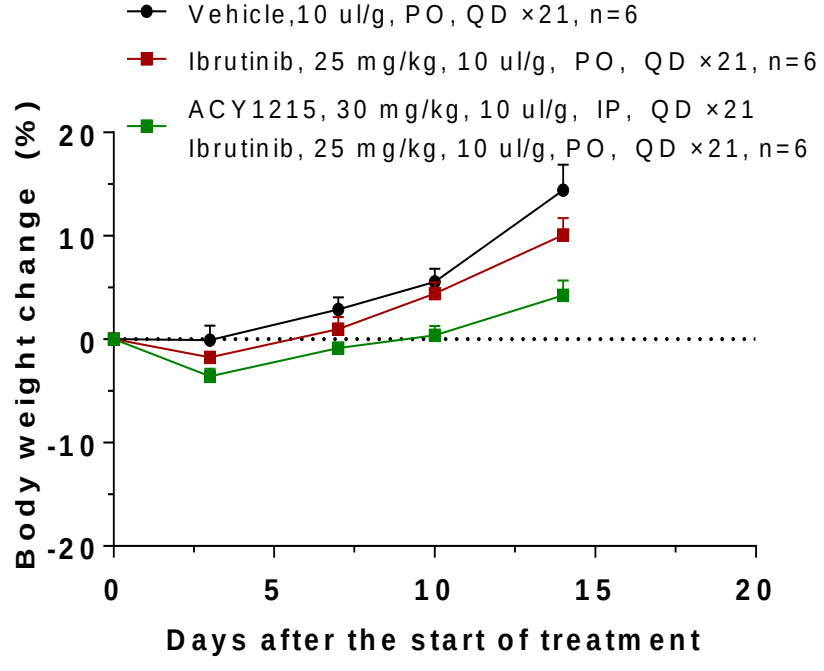


Note:

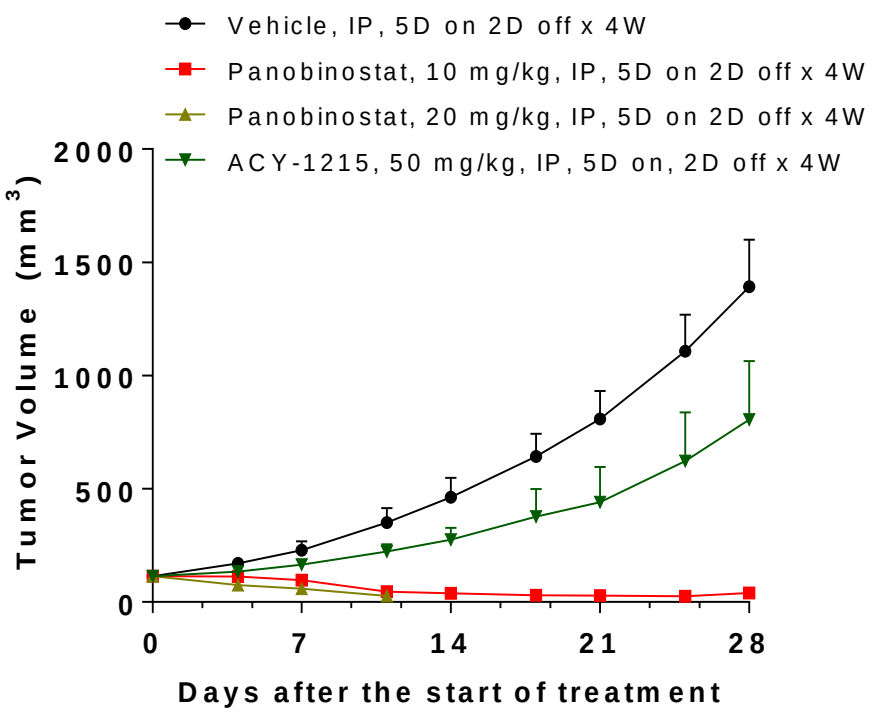
1. Dosing suspended (mice with BW loss ~10-13%) in ACY1215 single and combo groups.
2. N = 8 for each group. 3 mice died in ACY1215 and bortezomib combo group at D9, D13, and D16. Diarrhea were observed in this group.

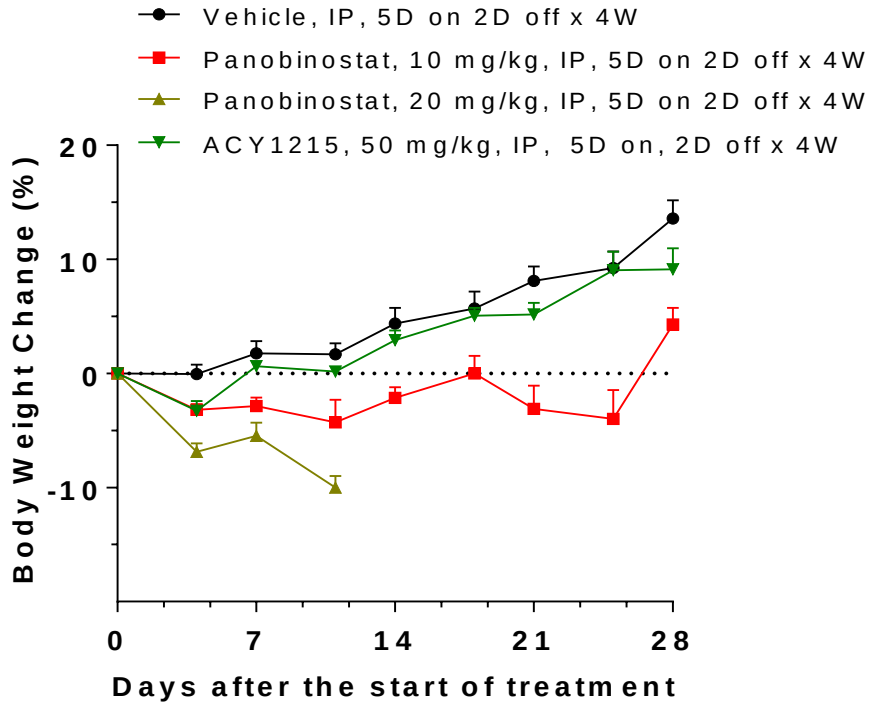
Anti-tumor efficacy of HDACi + BTKi in Mino xenograft model



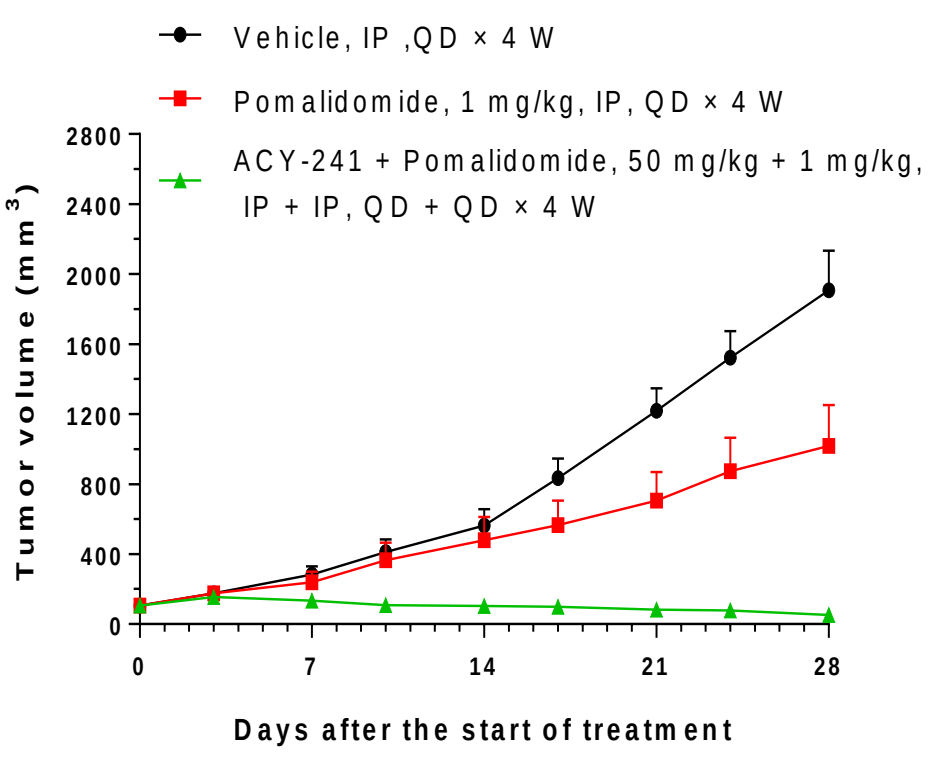


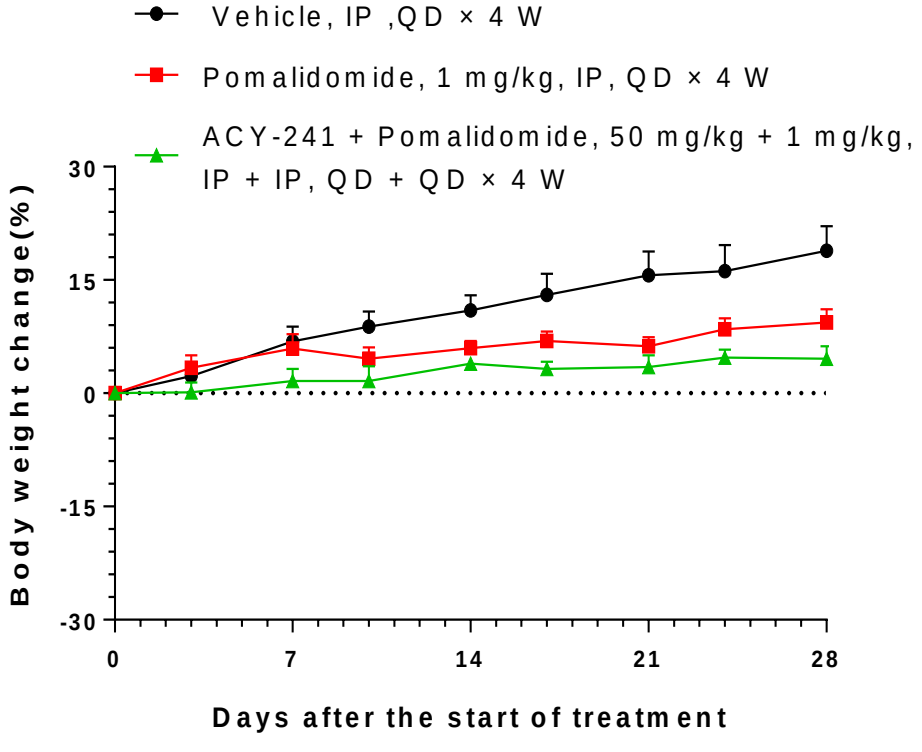
Anti-tumor efficacy of HDACi in MV4-11 xenograft model





Anti-tumor efficacy of HDACi in NCI-H929 xenograft model





OUR COMMITMENT

Improving Health. Making a Difference.

For questions and requests, please email to info_onco@wuxiapptec.com

ONCO

WuXi

https://onco.wuxiapptec.com

Mobile App