

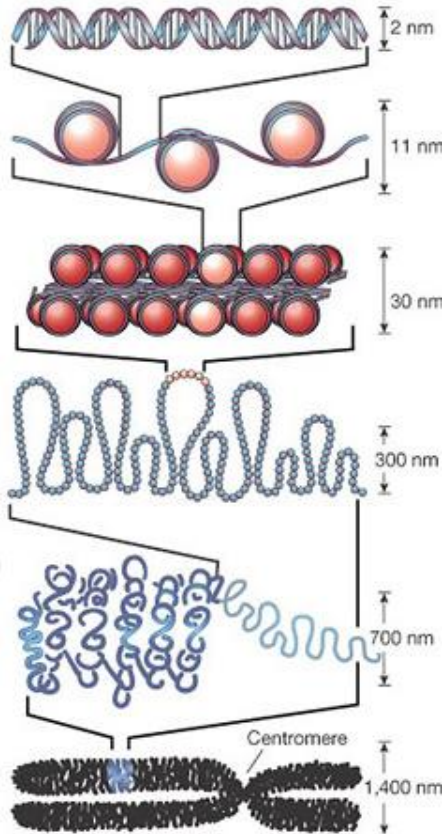
# Inhibition of HDACs and aminopeptidases is highly synergistic in Myeloma cells, disrupting the NF- $\kappa$ B signalling pathway

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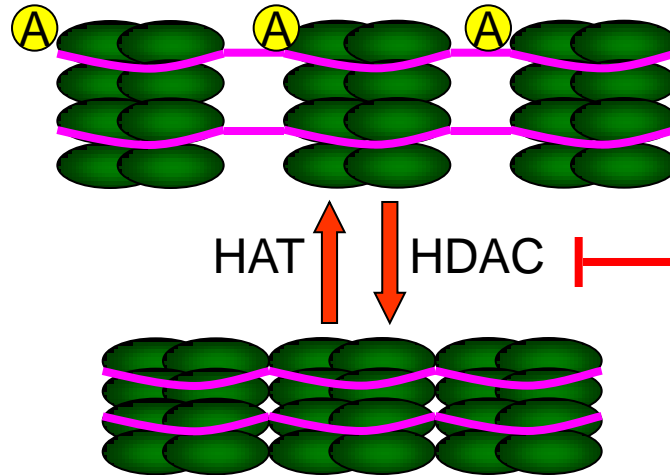
<sup>2</sup>Chroma Therapeutics Ltd, Abingdon, United Kingdom

# Histone Deacetylases (HDACs)



Active transcription

Repressed transcription



HDACs = family of 18 members, sub-divided into four classes: Class I (1,2,3, and 8), Class IIa+b (4,5,6, 7, 9, and 10), Class III (SIRT6), and Class IV (11).

Felsenfeld and Groudine.  
Nature, 2003

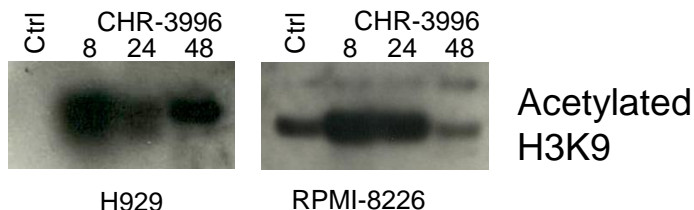
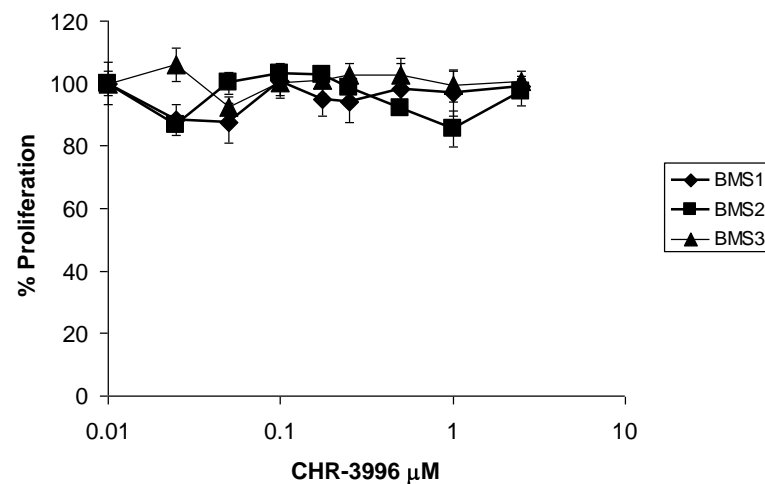
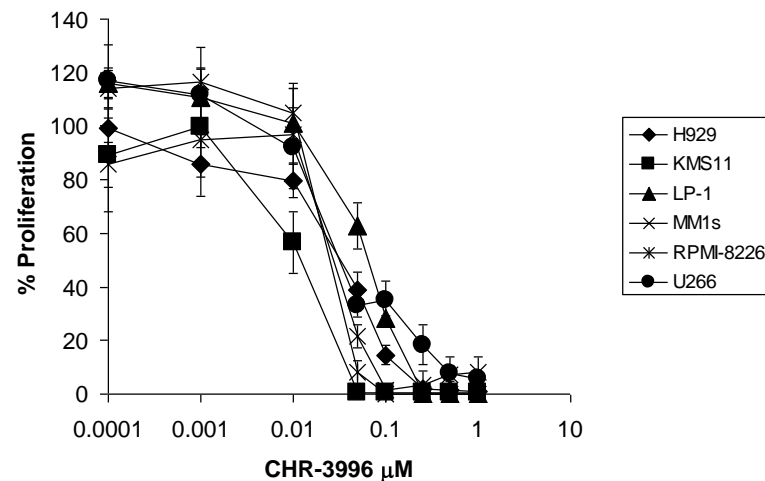
# CHR-3996: HDAC inhibitor (HDACi)

HDAC	IC <sub>50</sub> , nM		
	CHR-3996	SAHA	MGCD-0103
HDAC-1 (I)	3	30	100
HDAC-2 (I)	4	25	200
HDAC-3 (I)	7	150	2000
HDAC-5 (IIa)	200	3,000	>20,000
HDAC-6 (IIb)	2100	30	>20,000

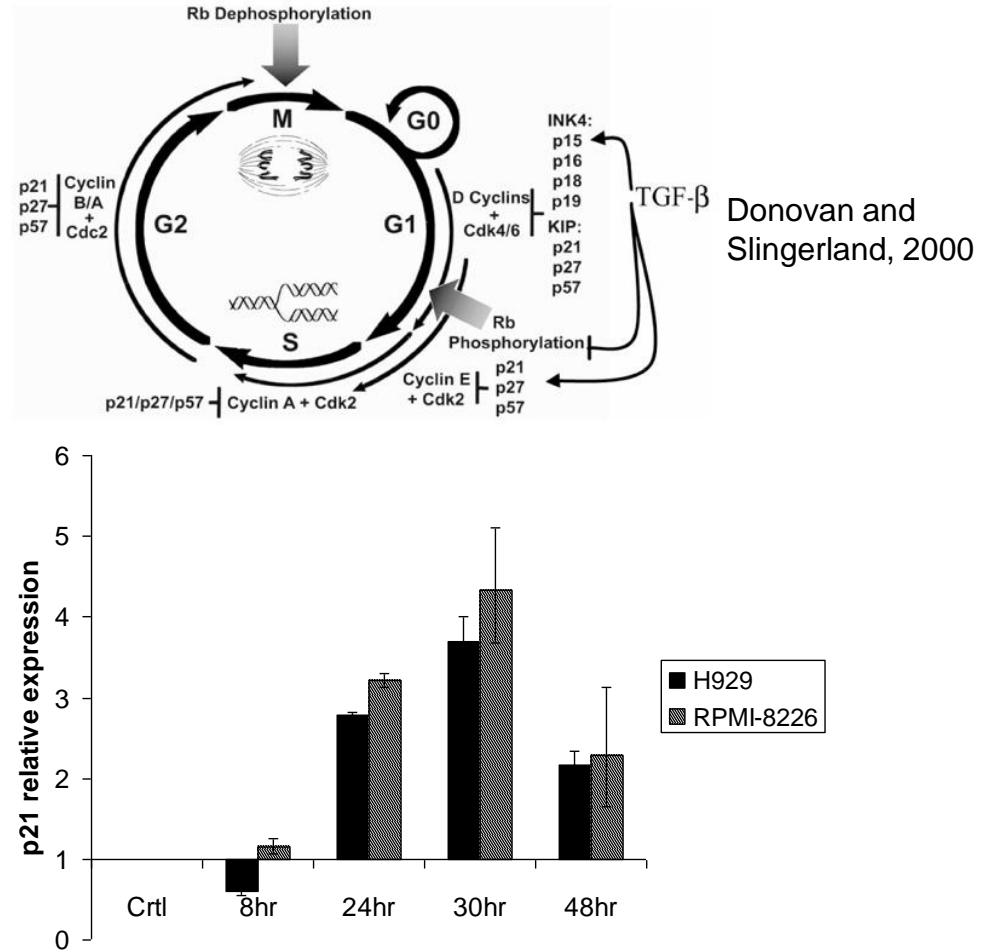
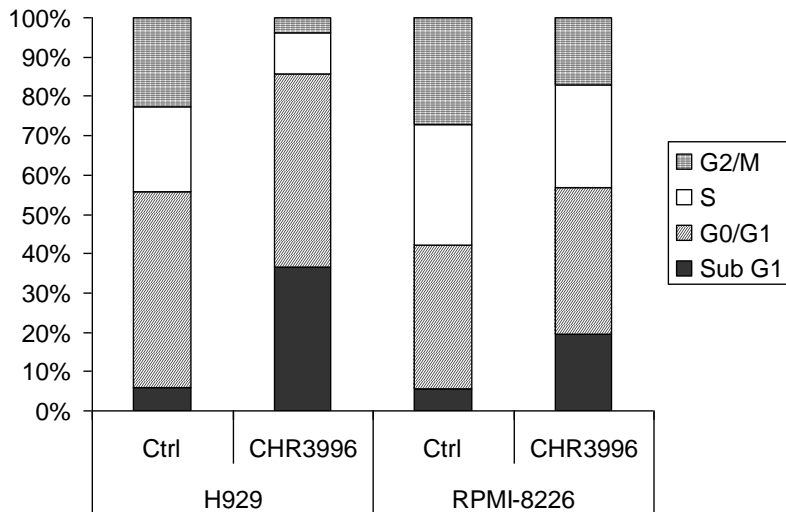
(Chroma Therapeutics Ltd.)

# CHR-3996 causes apoptosis in myeloma cells

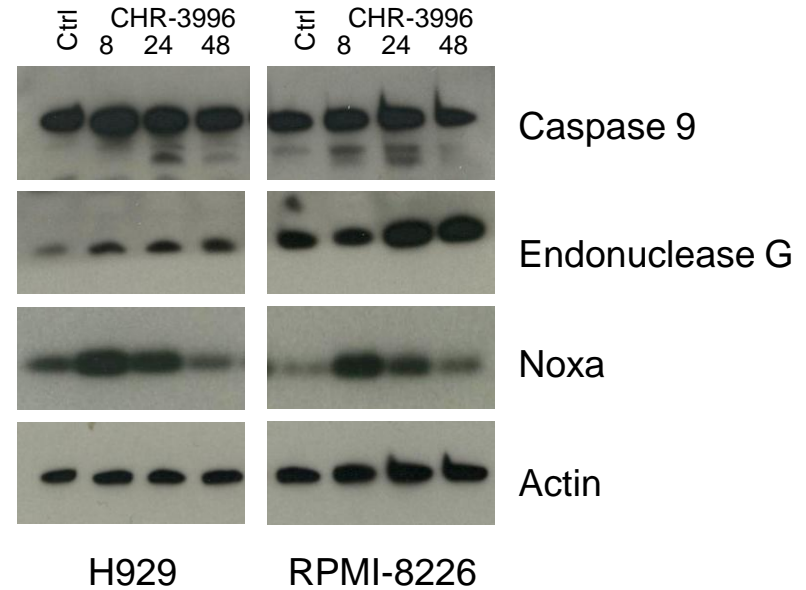
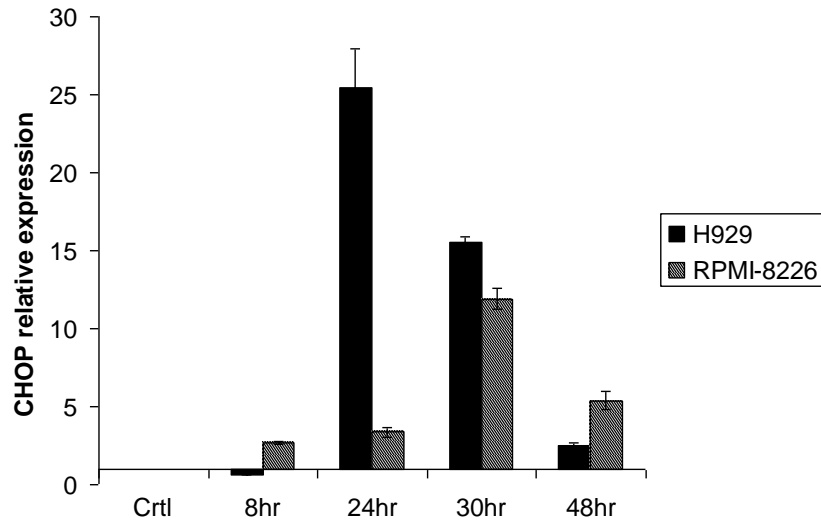
Cell line	CHR-3996 LC50 (nM)	SAHA LC50 ( $\mu$ M)	Na Valproate LC50 (mM)
H929	28.3	0.63	1.2
KMS11	9.3	0.23	0.38
LP-1	64.2	0.39	0.55
MM1s	33.1	0.63	1.10
RPMI-8226	33.0	0.45	0.64
U266	57.2	0.51	0.94
Patient cells	8	-	-



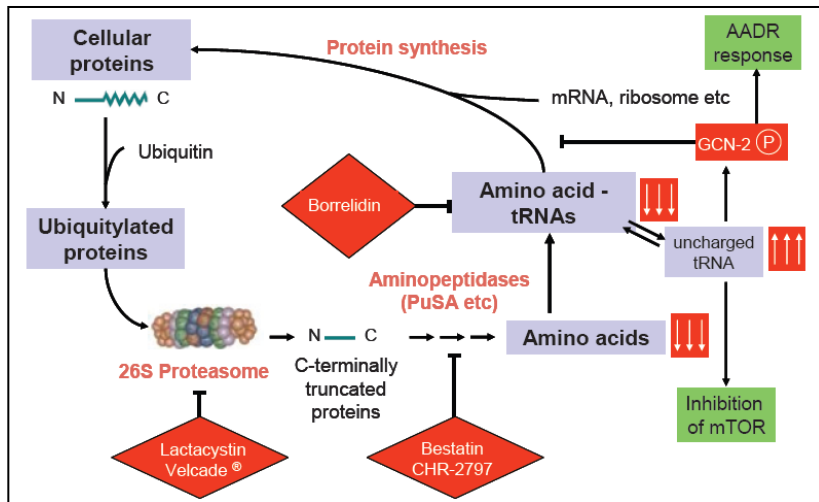
# CHR-3996 treatment promotes cell cycle arrest



# CHR-3996 up-regulates expression of pro-apoptotic proteins

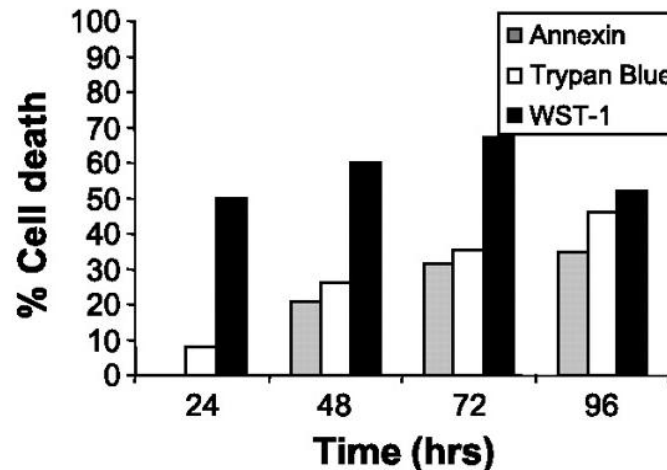
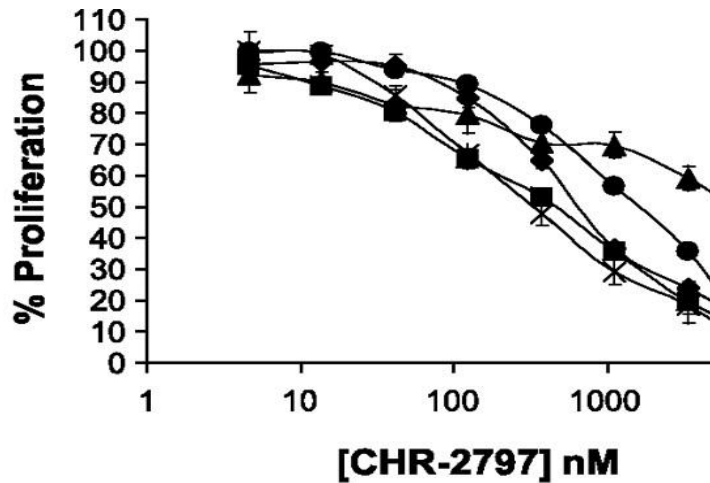


# CHR-2797 (Tosedostat)



(Chroma Therapeutics Ltd.)

CHR-2797, an aminopeptidase inhibitor (API), induces growth arrest and apoptosis in myeloma cells. There is activation of the unfolded protein response and autophagy pathways. Moore HE *et al*, 2008.



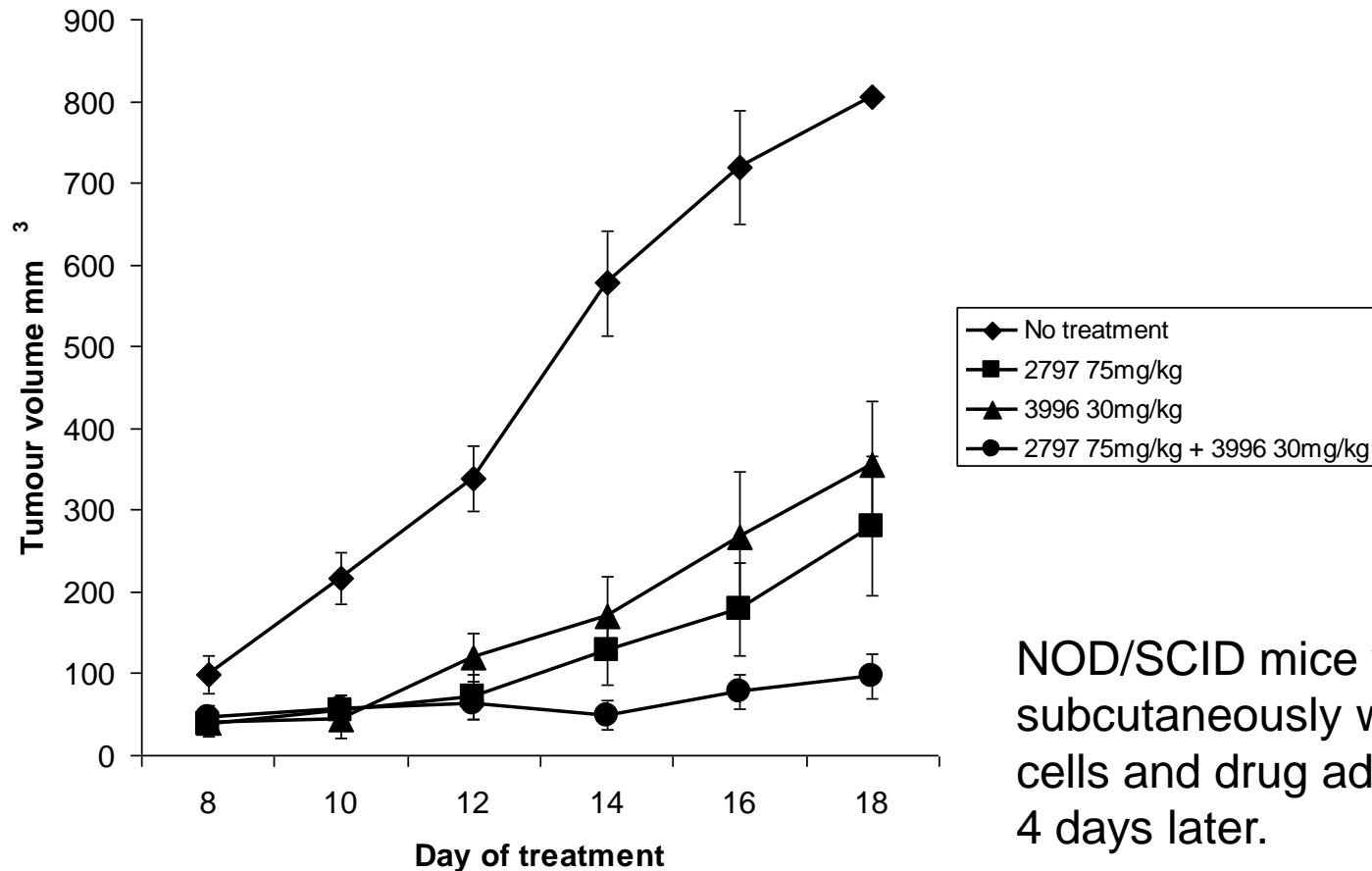
# HDAC inhibitors are highly synergistic with CHR-2797 *in vitro*

		HDACi + CHR-2797: concomitant	HDACi + CHR-2797: CHR-2797 24 hr prior
CHR-3996	H929	0.53	0.38
	RPMI-8226	0.36	0.19
SAHA	H929	0.68	0.24
	RPMI-8226	0.33	0.15
VA	H929	0.29	0.17
	RPMI-8226	0.14	0.15

CHR-2797 was combined with the HDAC inhibitors CHR-3996, SAHA and Sodium Valproate (VA) for 96 hours. The combination index was calculated by a 16-point Chou-Talalay analysis and the value for the 50% fraction stated.

A CI of <0.8 is defined as synergistic, 0.8-1.2 additive, and >1.2 antagonistic.

# CHR-3996 is synergistic with CHR-2797 *in vivo*



NOD/SCID mice were inoculated subcutaneously with  $2 \times 10^6$  H929 cells and drug administration started 4 days later.

# CHR-3996 affects key signalling pathways

## Cell cycle

eg Cyclins A/B1/D1/G2/E2/F, CDKN2B, (p15), CDKN1C (p57), CHK1, RB1, CDK1/3, Aurora kinase A&B.

## ER stress

eg IRE-1, ATF3/6, CHOP, TAO kinase 3.

## Apoptosis

eg Puma, AIF, Bim, CHOP, APAF, caspase 1, Bid, XIAP1, survivin.

CHR-3996 24 hours  
Affymetrix U133 plus 2.0 array  
(4583 genes)

## Wnt

eg GSK3 $\beta$ ,  $\alpha$ -catenin, Nemo like kinase.

## MAPK

eg MAPK6, Jun.

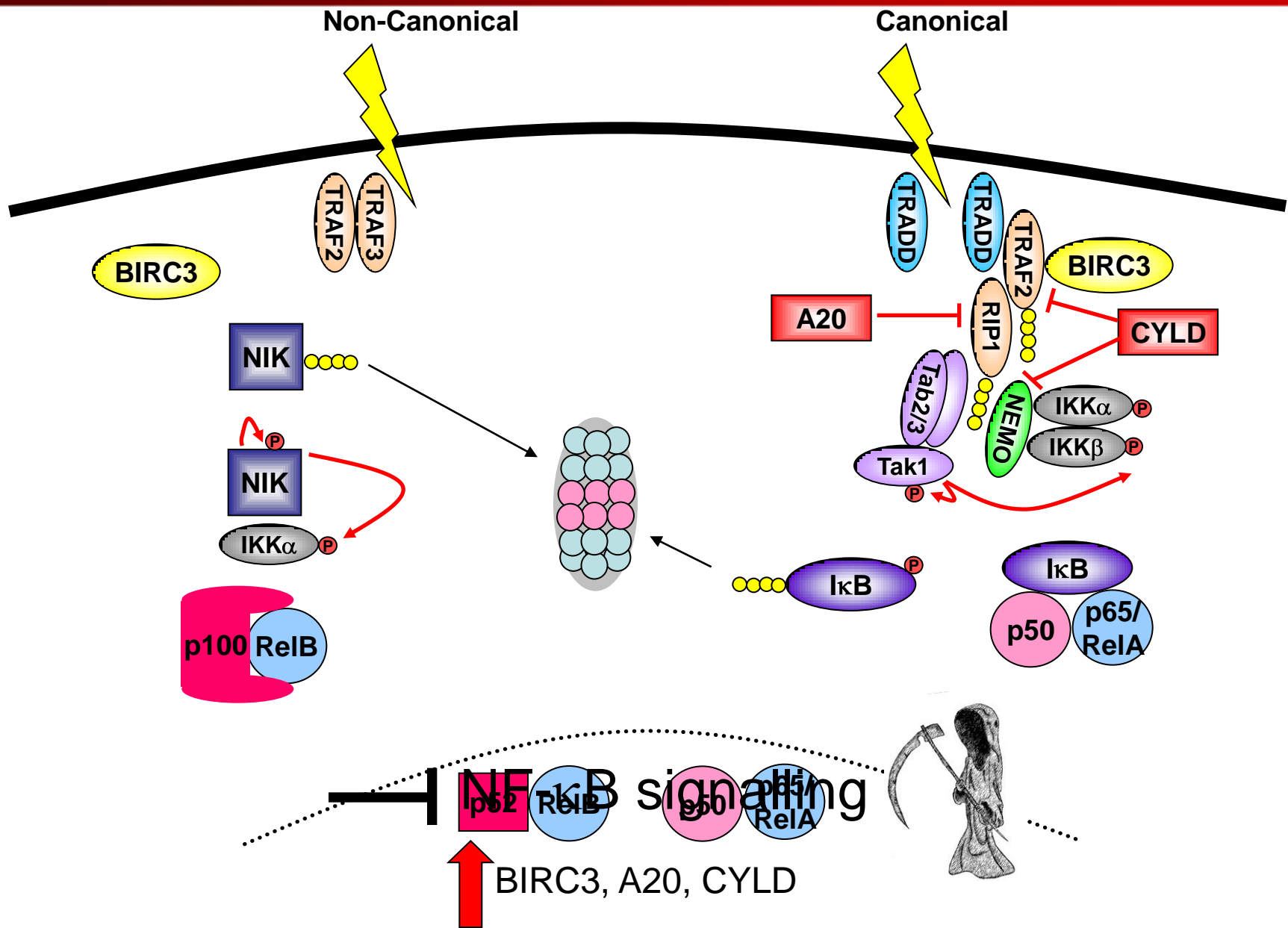
## p53

eg mdm2, PCNA, sestrin 1-3.

# The combination of CHR-3996 with -2797 increases expression of NF- $\kappa$ B negative regulators

	CHR-3996	CHR-2797 and -3996
BIRC3	23.51	110.62
A20	2.76	14.64
I $\kappa$ B $\alpha$	-	4.93
CYLD	3.8	4.16

# Combining CHR-2797 and-3996 activates NF-κB and initiates a negative feedback loop



# Summary

- CHR-3996 is an HDAC inhibitor that induces apoptosis of myeloma cells at low concentrations.
- It causes cell cycle arrest and up-regulation of CHOP and pro-apoptotic proteins.
- It is highly synergistic *in vitro* and *in vivo* with aminopeptidase inhibitor CHR-2797.
- The combination of these agents leads to rapid activation of NF- $\kappa$ B pathways and a subsequent large up-regulation of negative regulators of NF- $\kappa$ B signalling BIRC3, A20 and CYLD.

# Clinical development

- CHR-2797 (tosedostat) has completed a Phase I trial in haematological malignancies and demonstrates activity. A Phase II trial will commence soon.
- CHR-3996 is nearing completion of a Phase I trial in solid tumours.
- A clinical trial of the combination of the two compounds will begin soon in myeloma patients.

# Acknowledgements



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