KRONOS·BIO

Poster #1691

p300 catalytic inhibition selectively targets IRF4 oncogenic activity in multiple myeloma

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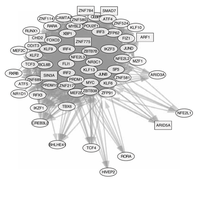
Background and rationale for targeting IRF4 in multiple myeloma (MM)

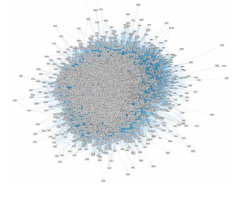
- Multiple myeloma (MM), an incurable plasma cell malignancy, is addicted to oncogenic transcription factor (TF) IRF4 signaling.
- Lineage TFs like IRF4 are difficult to drug but represent high value oncology targets owing to the depth and context selectivity of their genetic dependency.
- Using a transcription regulatory network (TRN) based approach to integrate high dimensional multi-omic dataset, we mapped the regulatory interactions that govern MM identity and defined the key regulatory activity of IRF4 and its druggable co-factors.
- This unbiased analysis identified p300 lysine acetyl transferase (KAT) function as the nearest selective neighbor cofactor of IRF4 for therapeutic intervention, with more potent and selective effects than historically observed with p300/CBP bromodomain inhibitors.
- We developed KB528 a highly selective inhibitor of the KAT domain of p300 and its close homolog CBP, and demonstrate how p300-KAT inhibition specifically perturbs IRF4 MM signaling pathways.
- We show that selective downregulation of IRF4 by p300 KAT inhibition preferentially inhibits the growth of MM *in vitro, ex vivo* and *in vivo*, establishing rationale for further therapeutic development.

MM transcription regulatory network (TRN) shows p300 and IRF4 as highly interconnected codependencies

1. Identify core MM TFs using core regulatory circuitry (n=65)



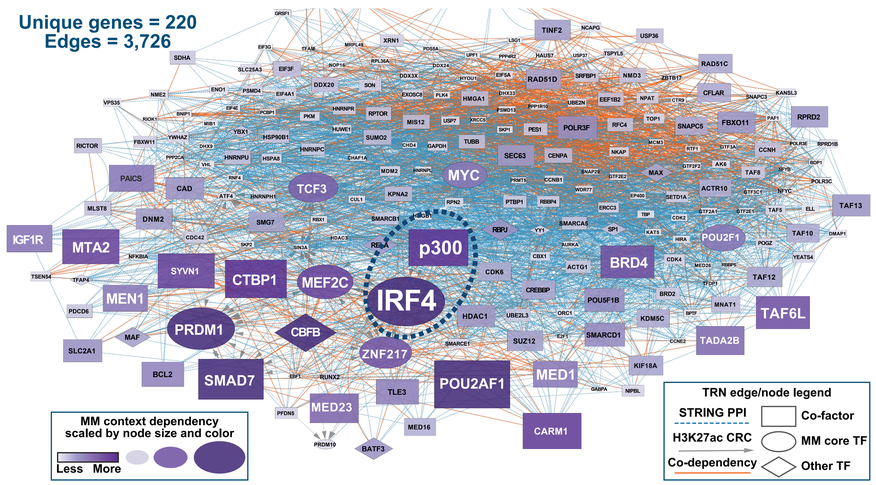
2. Expand to include predicted PPIs using STRING database (n= 4,325)



3. Refine by potential genetic context dependency using DepMap (n=503)

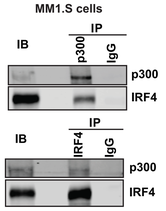
4. Refine for edges connected to a core MM TF and organize network using spring edge weight

Unique genes = 220
Edges = 3,726

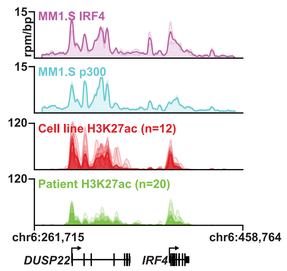


5.Experimental evidence of IRF4 and p300 regulatory interactions

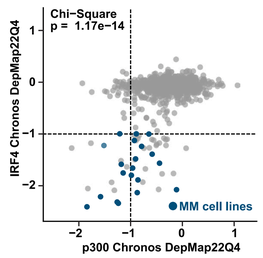
p300 and IRF4 physical interaction by co-immunoprecipitation



p300 and IRF4 binding at IRF4 locus

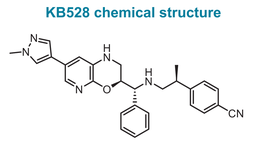


Genetic co-dependency of p300 and IRF4

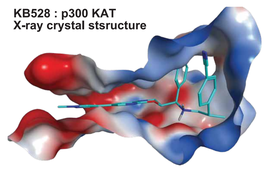


KB528: a novel and selective p300 KAT inhibitor

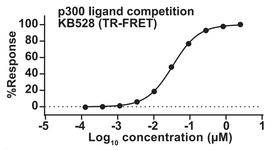
KB528 chemical structure



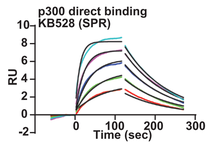
KB528 : p300 KAT X-ray crystal stsructure



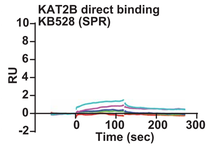
p300 ligand competition KB528 (TR-FRET)



p300 direct binding KB528 (SPR)

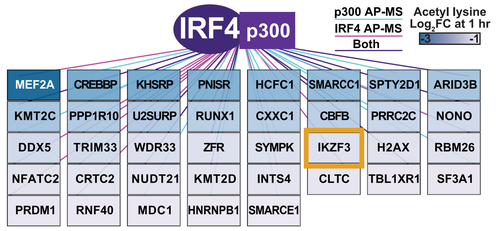


KAT2B direct binding KB528 (SPR)

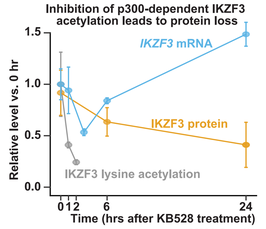


MW	465 Da
TR-FRET competition on p300	10 nM
Enzymatic activity on p300	102 nM
Enzymatic activity on other KATs PCAF, HAT1, MYST2/3/4, TIP60, GCN5L2	>10 μM
SPR K_d on p300	82 nM
SPR K_d on KAT2B	>2.5 μM
Cell target engagement (H3K27ac)	20 nM
IRF4 mRNA modulation IC_{50}	42 nM
MYC mRNA modulation IC_{50}	38 nM
Cell proliferation (MM1.S)	68 nM

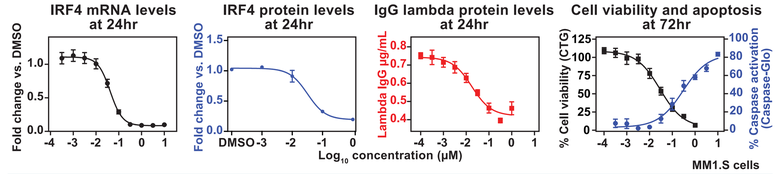
AP-MS reveals a p300 and IRF4 shared interactome and p300 acetylates key MM TRN components



Inhibition of p300-dependent IKZF3 acetylation leads to protein loss

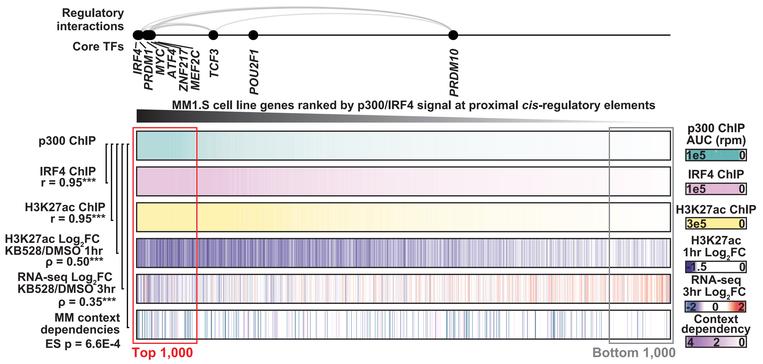


KB528 collapses IRF4 levels and activity inducing apoptosis

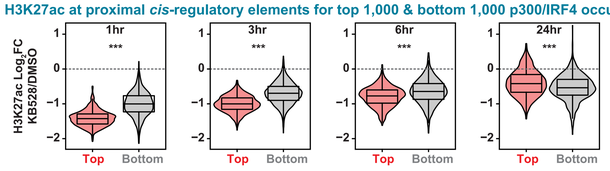


p300/IRF4 occupancy drives selective changes in chromatin and gene expression upon p300 KAT inhibition with KB528

Top p300/IRF4 occupied genes (including key MM TRN TFs) are selectively downregulated by KB528

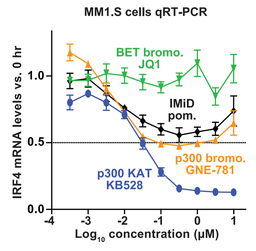


Change in H3K27ac at proximal *cis*-regulatory elements for top 1,000 & bottom 1,000 p300/IRF4 occupied genes

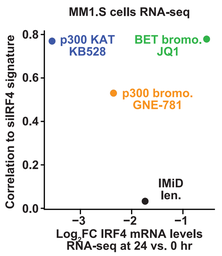


Preferential IRF4 downregulation by KB528 p300 KAT inhibition

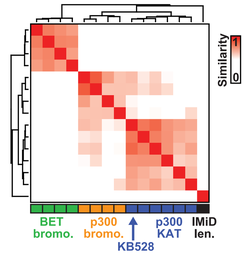
KB528 fully downregulates IRF4 mRNA at 24 hr



KB528 is selective against IRF4 signature

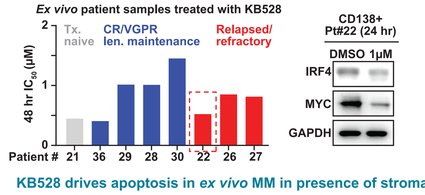


p300 KAT and bromo. inhibitors show differentiated viability profiles

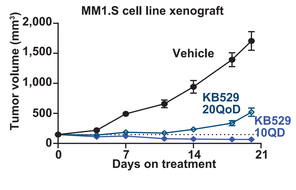


p300 KAT inhibition is efficacious *ex vivo* and *in vivo* and synergizes with standard of care mechanisms

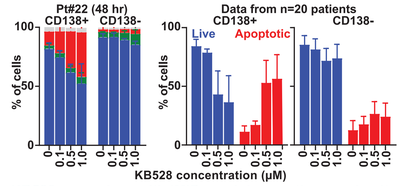
KB528 treatment downregulates IRF4 and selectively leads to apoptosis across primary patient MM



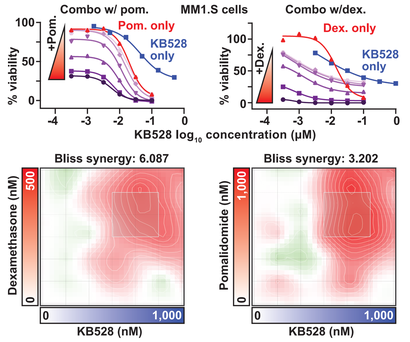
KB529 treatment downregulates IRF4 and is efficacious *in vivo*



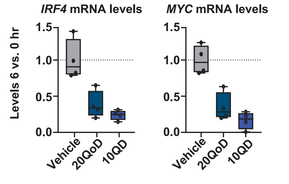
KB528 drives apoptosis in *ex vivo* MM in presence of stroma



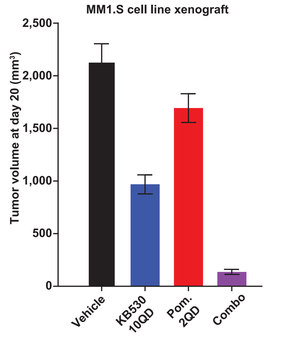
KB528 synergizes with IMiDs and dexamethasone *in vitro*



IRF4 mRNA levels and MYC mRNA levels



KB530 synergizes with IMiDs *in vivo*



p300 KAT inhibition as a therapeutic strategy for MM

- We developed KB528, a novel selective p300 KAT inhibitor to target p300 - a druggable, context-specific MM dependency with a functional relationship to the oncogenic TF IRF4.
- p300 KAT inhibition preferentially modulates the IRF4 TRN at the chromatin, transcriptional and post-translational levels and is distinct from other mechanisms including IMiDs, p300 bromodomain and BET bromodomain inhibition in its selectivity towards the IRF4 TRN.
- p300 KAT inhibition synergizes with therapeutic mechanisms targeting orthogonal pathways, including IMiDs and glucocorticoid agonists.
- p300 KAT inhibition is efficacious *ex vivo* and *in vivo* and has broad activity on MM cells across genotypes and independent of prior lines of therapy.
- These data motivated the development of KB-9558, Kronos Bio's p300 KAT inhibitor development candidate, which is currently undergoing IND enabling studies for a Phase 1 clinical trial in relapsed/refractory MM.