198. Overcoming disease persistence in MPN
(Max-Planck Innovation)

Asset Overview

Product Type	small molecule
Disease Area	Oncology
Indication	Myeloproliferative neoplasms (MPN)
Current Stage	Lead Optimization
Target	JAK, YBX1
MoA	JAK inhibition, YBX1 inactivation
Brief Description	In combination with pharmacological JAK inhibition, YBX1 inactivation induces apoptosis in JAK2-dependent mouse and primary human patient cells, causing regression of the malignant clones in vivo, and inducing molecular remission. Pharmaceutical targeting of downstream pathways regulated by YBX1 (e.g. MEK-ERK-signaling) in combination with JAK-inhibitors in vivo resulted in eradication of JAK2-mutated clones in > 80 %. Consistent with these findings, direct pharmacologic targeting of YBX1 in combination with JAK-inhibitors abrogated ERK-phosphorylation, reduced proliferative capacity and enhanced induction of apoptosis in murine and human JAK2-mutated cells. Thus genetic and pharmacologic targeting of YBX1 or its dependent downstream effectors represents a novel therapeutic strategy to target JAK2-mutated clones and overcome disease persistence in JAK2-mutated cancers such as MPNs.
Intellectual Property	WO2021214303A1
Publication	Splicing factor YBX1 mediates persistence of JAK2-mutated neoplasms, Nature. 2020 Dec;588(7836):157-163. doi: 10.1038/s41586-020-2968-3. Epub 2020 Nov 25.
Inventors	Florian Heidel, Ashok KUMAR JAYAVELU, Matthias Mann

Highlights

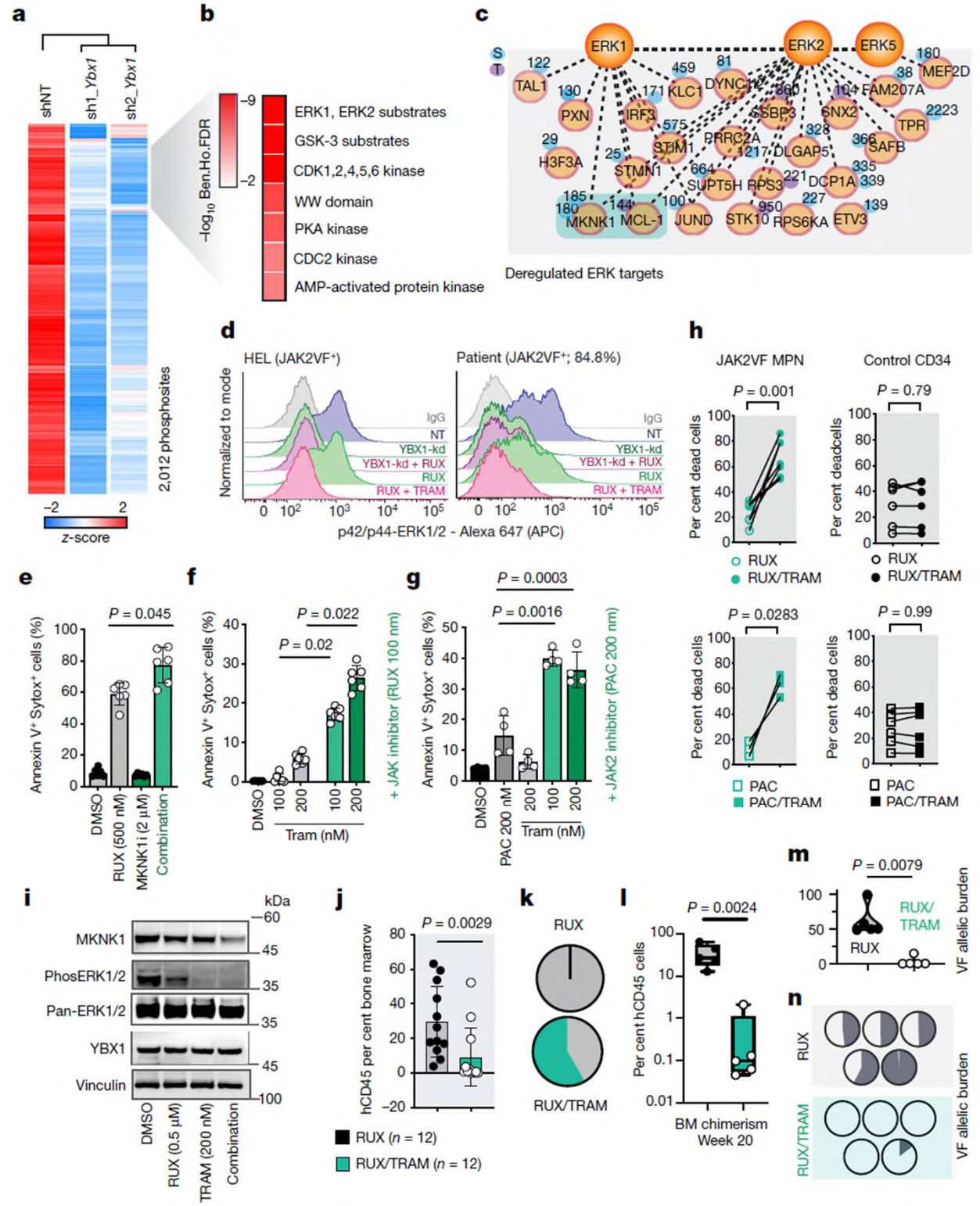
- Inactivation of YBX1, a post-translationally modifed target of JAK2, sensitizes cells that persist despite treatment with JAK inhibitors to apoptosis and results in RNA mis-splicing, enrichment for retained introns and disruption of the transcriptional control of extracellular signal-regulated kinase (ERK) signalling.
- In combination with pharmacological JAK inhibition, YBX1 inactivation induces apoptosis in JAK2-dependent mouse and primary human cells, causing regression of the malignant clones in vivo, and inducing molecular remission.
- This identifes and validates a cell-intrinsic mechanism whereby diferential protein phosphorylation causes splicing-dependent alterations of JAK2–ERK signalling and the maintenance of JAK2V617F malignant clones.

198. Overcoming disease persistence in MPN
(Max-Planck Innovation)

5TH KDDF GLOBAL C&D TECH FAIR

Key Data

Jak2-mutated clones are selectively vulnerable to inhibition of ERK signaling



198. Overcoming disease persistence in MPN
(Max-Planck Innovation)

Key Data

Jak2-mutated clones are selectively vulnerable to inhibition of ERK signaling

a, Unsupervised hierarchical clustering of 2,012 significantly downregulated phosphosites in *Ybx1*-targeted, *Jak2*-mutated mouse cells (two shRNAs) and non-targeting control (shNT). Averaged replicates (n = 4); heatmap shows z-scored log2-transformed phosphosite intensity. **b**, Kinase substrate motifs significantly downregulated in *Ybx1*-targeted JAK2VF cells (–log10 Benjamini–Hochberg FDR value). **c**, Network map: proteins assigned as ERK substrates (significantly downregulated phosphoserine (blue) and phosphothreonine (purple) residues, indicated by position). **a**, **c**, ANOVA, permutation-based FDR < 0.01. **d**, Representative flow cytometry histograms showing pERK levels in *JAK2*-mutated HEL (left) and primary patient cells (right) after RNAi (shNT; sh*Ybx1*) and/or drug treatment (500 nM JAK inhibitor (ruxolitinib, RUX) or 200 nM MEK/ERK inhibitor (trametinib, TRAM)). **e**–**g**, Pharmacologic inhibition of mouse JAK2VF cells with ruxolitinib alone or with MKNK1 inhibitor (CGP57380, MKNK1-i; 2 μM; n = 6, mean ± s.d.; **e**), ruxolitinib alone or with trametinib (100, 200 nM; n = 6, mean ± s.d.; **f**) or the JAK2 inhibitor pacritinib (PAC) alone or with trametinib (100, 200 nM; n = 4, mean ± s.d.; **g**), as indicated by percentage of apoptotic (annexin V+ Sytox+) cells. **h**, Proportion of dead cells in FACS-sorted CD34+ JAK2VF+ MPN bone marrow cells (n = 6) and non-malignant CD34+ controls (n = 6) treated with ruxolitinib (500 nM), pacrinitib (200 nM) or either plus trametinib (200 nM). **i**, Representative western blot (of n = 3) showing reduction of MKNK1 protein abundance in HEL cells following ruxolitinib + trametinib treatment. **j**, **k**, Xenograft model using NSGS mice transplanted with JAK2VF+ HEL cells and treated with ruxolitinib (n = 12) or ruxolitinib + trametinib (n = 12). **j**, Percentages of hCD45+ bone marrow cells (n = 12 per cohort; mean ± s.d.). **k**, Disease penetrance (%) in NSGS animals: hCD45 positivity (>1%, grey) or negativity (<1%, green) after ruxolitinib or ruxolitinib + trametinib treatment, respectively. **l**–**n**, Patient-derived xenograft (PDX) model to investigate primary JAK2VF+ bone marrow cells from three different patient donors in humanized mice (NSGW41). Recipient animals were treated with ruxolitinib (90 mg kg–1 twice a day) or ruxolitinib + trametinib (1 mg kg–1 once a day by gavage for 5 d every 4 weeks). **l**, Bone marrow analysis of human cell chimerism in week 20 (n = 5 per cohort). Box plots shows mean ± s.d. with minimum and maximum. **m**, Quantification of JAK2V617F allelic burden by pyrosequencing in sorted hCD45+ bone marrow cells of recipient animals. **d**–**m**, P values (two-tailed t-test. **n**, Pie charts of JAK2V617F allelic burden per individual for mice treated with ruxolitinib (grey) or ruxolitinib + trametinib (green). In **d**–**n**, n indicates independent biological replicates.