In Vitro and *In Vivo* Screening Platform for Discovery of JAK2 Inhibitors

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Abstract

Background: The JAK-STAT signaling pathway, activated by cytokine receptors, plays a central role in regulating cell proliferation, differentiation, apoptosis, and immune responses. JAK2, a key kinase in this pathway, transduces signals from hematopoietic growth factor receptors such as thrombopoietin (TPO), erythropoietin (EPO), and granulocyte-macrophage colony-stimulating factor (GM-CSF). Dysregulation of JAK2 is implicated in the pathogenesis of myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), myelofibrosis (MF), and essential thrombocythemia (ET).

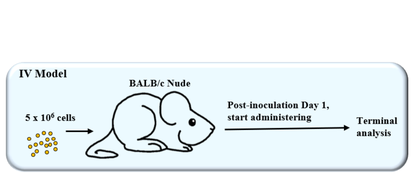
Methods: An *In vivo*, a murine model of JAK2-driven myeloproliferative disease was established by intravenous transplantation of JAK2-activated cell lines into BALB/c nude mice. Disease progression was monitored using bioluminescent imaging. Terminal analyses included evaluation of splenomegaly, serum biochemistry (ALT and AST), and splenic histopathology (hematoxylin and eosin staining). For *in vitro* analysis, a panel of cell lines was treated with increasing concentrations of JAK inhibitors (Ruxolitinib, Fedratinib, and Tofacitinib) to evaluate compound efficacy. Inhibition of downstream JAK2-STAT5 signaling was quantified using an α-LISA for phosphorylated STAT5 (p-STAT5). Dose-response profiles were generated, and IC_{50} values were calculated to determine the relative sensitivity of the cells to each agent.

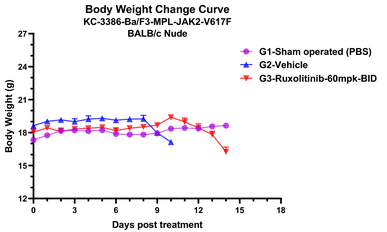
Results: *In vivo*, our model mice displayed progressive increases in bioluminescent signal, marked splenomegaly and hepatomegaly, decreased survival, and elevated serum ALT/AST levels. Ruxolitinib treatment significantly suppressed bioluminescent signal progression, reduced hepatic and splenic tumor burden, and extended survival. *In vitro*, Ruxolitinib potently and dose-dependently inhibited JAK2-STAT5 signaling, with IC_{50} values confirming target sensitivity. The cell lines exhibited differential sensitivity to the JAK2 inhibitors Ruxolitinib, Fedratinib, and Tofacitinib.

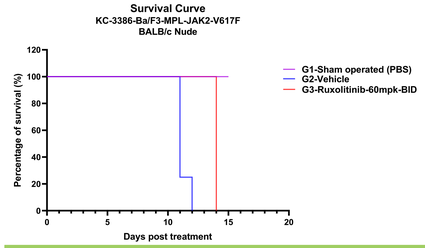
Cell ID	Cell name
KC-0153	Ba/F3 (IL3 dependent)
KC-1258	Ba/F3-ETV6-JAK3
KC-1479	Ba/F3-EPOR (EPO dependent)
KC-1568	Ba/F3-JAK2-V617F
KC-2073	Ba/F3-MPL-W515L
KC-2922	Ba/F3-MPL-W515R
KC-2924	Ba/F3-MPL-W515A
KC-3198	Ba/F3-EPOR-JAK2-WT (EPO-dependent)
KC-3783	Ba/F3-MPL-CALR-del61
KC-3385	Ba/F3-MPL-W515L-JAK2
KC-3914	Ba/F3-MPL-CALR-WT
KC-4672	Ba/F3-EPOR-JAK2-V617F-mJAK2-KO-GFP-Luc2
KC-4575	Ba/F3-MPL-W515L-JAK2-GFP-Luc2

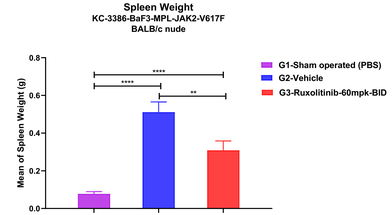
Cell ID	Cell name
KC-1270	Ba/F3-ETV6-JAK2
KC-1303	Ba/F3-ETV6-JAK1
KC-1480	Ba/F3-MPL(TPO dependent)
KC-1567	Ba/F3-EPOR-JAK2-V617F
KC-2880	Ba/F3-EPOR-JAK2-V617F-mJAK2-KO
KC-2923	Ba/F3-MPL-W515K
KC-3167	Ba/F3-JAK2-WT (IL3-dependent)
KC-3782	Ba/F3-MPL-CALR-del52
KC-3784	Ba/F3-MPL-CALRins5
KC-3386	Ba/F3-MPL-JAK2-V617F
KC-4378	Ba/F3-EPOR-JAK2-V617F-mJAK2-KO
KC-4678	Ba/F3-EPOR-JAK2-V617F-EGFP-Luc2
KC-4576	Ba/F3-MPL-JAK2-V617F-GFP-Luc2

JAK-Focused *In Vivo* Models

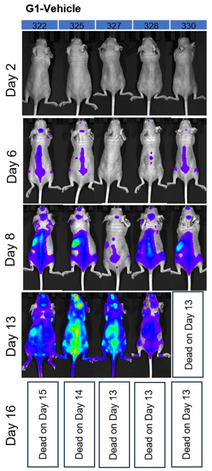


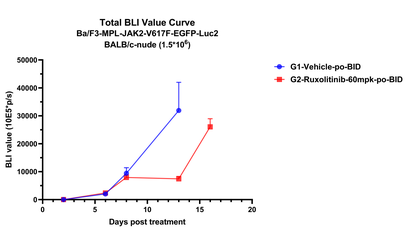


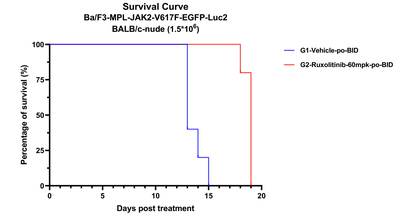




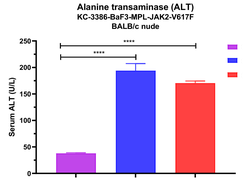
Ba/F3-MPL-JAK2-V617F-EGFP-Luc2

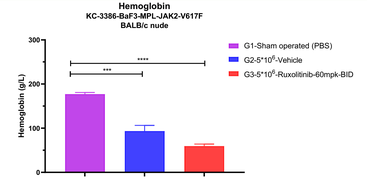




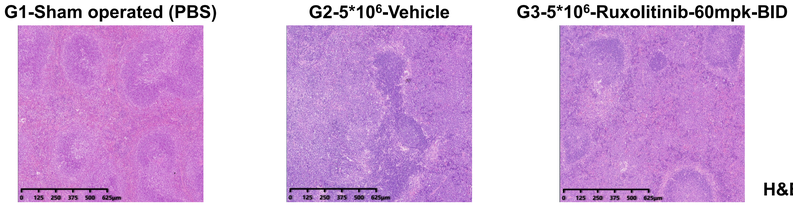


Blood biochemistry and blood routine testing

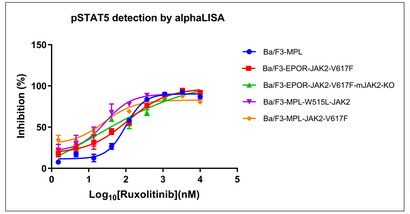




Histological analysis (spleen)



JAK-Focused Ba/F3 *In Vitro* Models and Benchmarking Data



Cell Line ID	Cell Line Name	Compound IC50 (nM)		
		Ruxolitinib	Fedratinib	Tofacitinib
KC-1303	Ba/F3-ETV6-JAK1	115.19	1899.84	253.46
KC-1270	Ba/F3-ETV6-JAK2	295.57	714.57	2802.34
KC-1258	Ba/F3-ETV6-JAK3	10000	1435.66	1446.42
KC-1568	Ba/F3-JAK2-V617F	39.75	459.19	153.2
KC-3167	Ba/F3-JAK2-WT (IL3-dependent)	163.86	940.09	424.1
KC-1567	Ba/F3-EPOR-JAK2-V617F	181.87	1105.36	946.47
KC-2880	Ba/F3-EPOR-JAK2-V617F-mJAK2-KO	46.2	543.66	193.96

Cell Line ID	Cell Line Name	Compound IC50 (nM)		
		Ruxolitinib	Fedratinib	Tofacitinib
KC-2073	Ba/F3-MPL-W515L	89.73	886.15	423.01
KC-2922	Ba/F3-MPL-W515R	72.85	736.55	478.38
KC-2923	Ba/F3-MPL-W515K	149.37	1110.17	1050.99
KC-2924	Ba/F3-MPL-W515A	148.84	1182.42	1021.51
KC-3782	Ba/F3-MPL-CALR-del52	268.05	1314.88	1788.12
KC-3783	Ba/F3-MPL-CALR-del61	185.21	1152.2	1652.09
KC-3784	Ba/F3-MPL-CALRins5	197.92	1167.66	1548.13
KC-3914	Ba/F3-MPL-CALR-WT	TBD	TBD	TBD

10 100 1000 (nM)

Cell Line ID	Cell Line Name	Compound IC50 (nM)		
		Ruxolitinib	Fedratinib	Tofacitinib
KC-3198	Ba/F3-EPOR-JAK2-WT (EPO dependent)	57	772.28	495.03
KC-3386	Ba/F3-MPL-JAK2-V617F	251.66	1053.46	1899.68
KC-3385	Ba/F3-MPL-W515L-JAK2	98.03	914.57	712.47
KC-0153	Ba/F3 (IL3 dependent)	226.09	870.03	752.67
KC-1479	Ba/F3-EPOR (EPO dependent)	32.85	272.26	142.89
KC-1480	Ba/F3-MPL (TPO dependent)	144	746	TBD

Conclusion

An *in vivo* and *in vitro* screening platform for JAK2 inhibitors has been established, which plays a crucial role in optimizing treatment regimens for diseases with aberrant JAK2 activation.

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