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The Second Generation Type II JAK2 inhibitor, AJ1-10502, Demonstrates Enhanced Selectivity,Improved Therapeutic Efficacy and Reduced Mutant Cell Fraction Compared to Type I JAK2 inhibitors in Models of Myeloproliferative Neoplasms (MPNs)

Shivam Rai[1], *Jan Stetka*[1], *Marc Usart*[1], *Hui Hao-Shen*[1], *Young Park*[2], *Anthony Martinez-Benitez*[2], *Matthew Wereski*[2], *Emily Guzzardi*[2], *Remie Houston*[2], *Sonali Persaud*[2] *Hailey Ramzan*[2], *Alan Futran*[3], *Charley Xu*[3], *Jeremy Greenwood*[3], *Sayan Mondal*[3], *Craig Masse*[4], *Ross Levine*[2], *Radek Skoda*[1], *Andrew Dunbar*[2]

[1]University Hospital Basel and University of Basel; [2]Memorial Sloan Kettering Cancer Center; [3]Schrodinger Inc.; [4]Ajax Therapeutics

Introduction

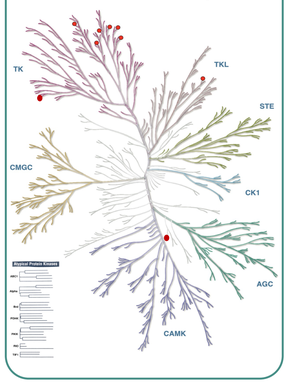
- Current type I JAK2 inhibitors including ruxolitinib (Rux) and fedratinib bind the active conformation of the kinase domain and improve symptoms and outcomes in MPNs; however, mutant allele burden remains essentially unchanged, and efficacy wanes over time. Moreover, sustained JAK/STAT signaling in the setting of type I inhibition plays a critical role in the MPN cell persistence (1).
- Type II JAK2 inhibitors bind the inactive conformation of the kinase domain , overcome Rux persistence in vitro and reduce *Jak2*VF allele fraction in vivo, suggesting an improved approach to targeting JAK2 with enhanced clinical efficacy and potential disease modification (2).
- Current type II JAK2 inhibitors, including CHZ868, are limited by lack of kinome specificity and off-target toxicity. We therefore sought to develop novel type II JAK2 inhibitors with improved potency and selectivity.
- Computational free energy perturbation and structure-activity relationship based methods were used to identify lead type II JAK2 inhibitor compounds (3). This work, followed by Absorption, Distribution, Metabolism and Excretion modeling led to the development of AJ1-10502.

Potency, Selectivity, and Drug-Like Properties of AJ1-10502

		AJ1-10502
JAK2 binding assay†	JAK2 Kd (nM)	0.70
JAK2 cellular activity	SET2-pSTAT5 IC_{50} (nM)	64
JAK family biochemical selectivity (fold over Kd)	fold selectivity over JAK1	271x
	fold selectivity over JAK3	1857x
	fold selectivity over TYK2	60x
Protein binding	Human PPB (% bound)	96
	Mouse PPB (% bound)	98
Human in vitro stability	Cl_{int} (mL/min/kg) hepatocytes	14
Mouse PK (IV) 1 mg/kg*	Cl_{obs} (mL/min/kg)	20
Mouse PK (PO) 60 mg/kg*	$T_{1/2}$ (h)	2.6
	C_{max} (ng/mL)	14967
	F (%)	62
	AUC (h*ng/mL)	30864
Physical properties	Solubility (µM) (pH = 7.4)	74

†JAK2-JH1 (Y1007/1008F) Type II binding assay
*PK study conducted in C57/BL6 mice. AJ1-10502 IV formulation: 10% DMSO/60% PEG400/saline; PO formulation: 20% HPβCD/saline (pH=4)

AJ1-10502 revealed potent JAK2 selectivity with minimal kinase cross-reactivity



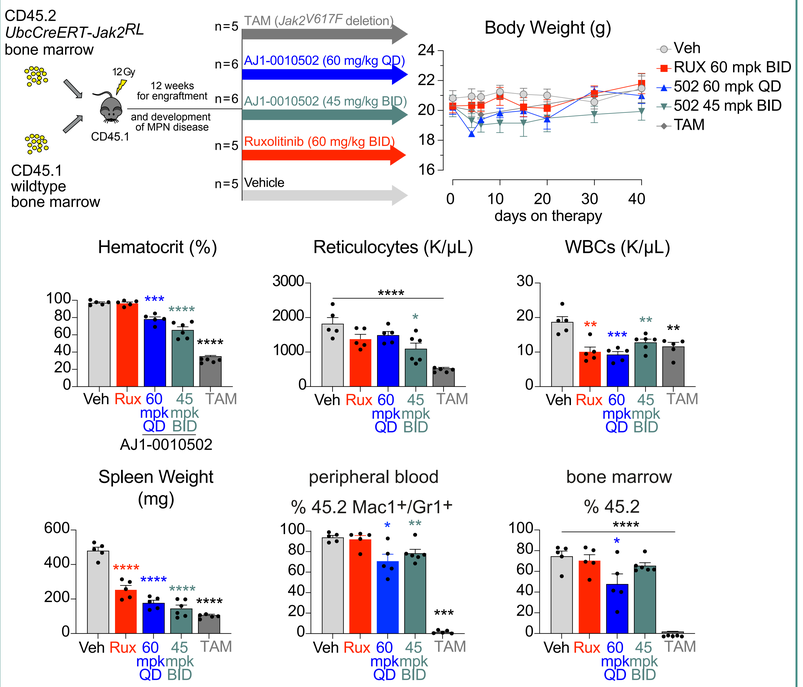
- SET-2 cells were made persistent by culturing at increasing rux concentrations (up to 700 nM)
- **AJ1-10502 retains activity against rux persistent SET-2 cells**

Compound	SET2 Naive IC_{50} (nM)	SET2-Rux-Persistent IC_{50} (nM)	Persistent/Naive (fold)
Ruxolitinib	86	723	8.4
CHZ868	80	161	2.0
AJ1-10502	**379**	**930**	**2.5**

Compound	GM-CSF-pSTAT5 IC_{50} (nM) (JAK2)	IL2-pSTAT5 IC_{50} (nM) (JAK1/3)	IL12-pSTAT4 IC_{50} (nM) (TYK2/JAK2)
Ruxolitinib	48	14	33
AJ1-10502	**77**	**1609**	**678**

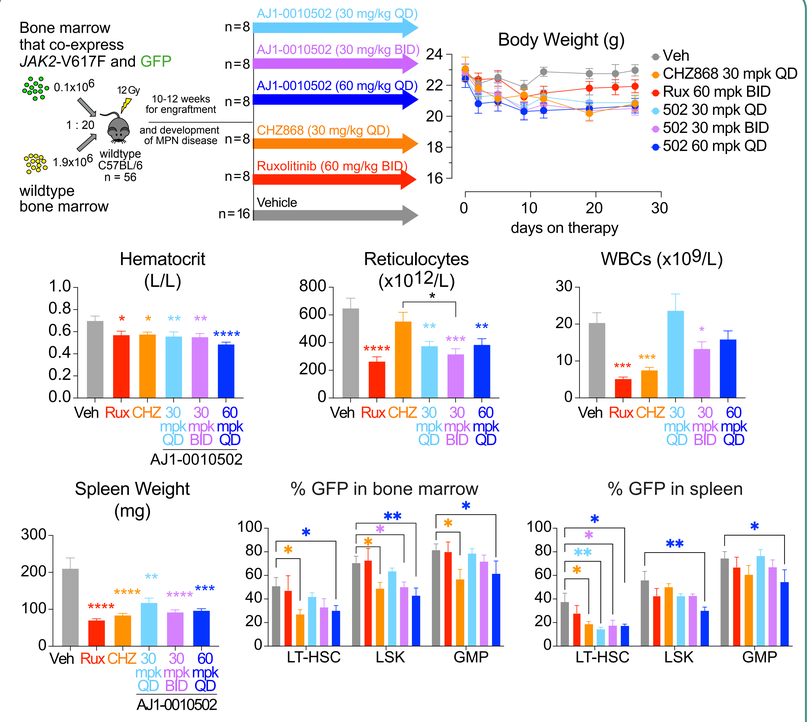
AJ1-10502 Functional selctivity in human PBMCs: **Better selectivity for JAK2 compared to Rux**

AJ1-10502 reduces blood counts, splenomegaly and mutant allele burden in two different mouse models of MPN



We evaluated AJ1-10502 *in vivo* using a novel dual Dre/Cre-recombinase *Jak2*VF knock-in/knock-out model allowing for the comparison of JAK inhibitors to *Jak2*VF genetic deletion. AJ1-10502 demonstrated reductions in leukocytosis comparable to that of Rux but dose-dependent improvements in hematocrit, reticulocytes and spleen weights superior to Rux, with reductions in spleen weights on par to that of Jak2VF deletion.

Critically, we observed significant reductions in peripheral blood and bone marrow mutant allele fraction with AJ1-10502 not observed with Rux, including within Mac1+Gr1+ myeloid cell fractions.



We tested the in vivo efficacy of AJ1-10502 in a separate series of competitive transplants using a Cre-inducible human JAK2-V617F transgenic mouse line. Consistent with the phenotypes observed in the DreCre MPN mouse model, we found reduced leukocytosis, hematocrit, reticulocytes and spleen weights in mice treated with AJ1-10502.

Most importantly, AJ1-10502 caused significant reductions in mutant cell fraction within the hematopoietic stem (HSC) compartment of bone marrow and spleen, including among granulocyte macrophage progenitor (GMP) and long-term HSCs (LT-HSCs).

References

1. Koppikar *et al*. Heterodimeric JAK-STAT activation as a mechanism of persistence to JAK2 inhibitor therapy. Nature. 2012 Sep 6;489(7414):155-9. doi: 10.1038/nature11303.
2. Meyer *et al*. CHZ868, a Type II JAK2 Inhibitor, Reverses Type I JAK Inhibitor Persistence and Demonstrates Efficacy in Myeloproliferative Neoplasms. Cancer Cell. 2015 Jul 13;28(1):15-28. doi: 10.1016/j.ccell.2015.06.006
3. Wang *et al*. Accurate and reliable prediction of relative ligand binding potency in prospective drug discovery by way of a modern free-energy calculation protocol and force field. J Am Chem Soc. 2015 Feb 25;137(7):2695-703. doi: 10.1021/ja512751q

Conclusion

- AJ1-10502 is a potent, selective type II JAK2 inhibitor with enhanced efficacy compared to Rux and improved safety profile compared to previous non-selective type II JAK2 inhibitors.
- Most importantly, AJ1-10502 demonstrated superior reductions in peripheral blood and bone marrow mutant cell fraction in two different mouse models of MPN suggesting the potential for disease modification.
- These data demonstrate the preclinical utility of AJ1-10502 and provide a strong rationale for the clinical development of selective type II JAK2 inhibitors for MPN patients.