AJ1-11095, a potent and highly selective type-II JAK2 inhibitor, shows enhanced therapeutic efficacy as compared with type-I JAK2 inhibitor ruxolitinib in models of myeloproliferative neoplasms (MPNs)

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Background

Therapy with JAK2 inhibitors **(JAK2i)** is a standard of care for myelofibrosis (MF). All FDA-approved JAK2i are **type-I inhibitors**, and while these agents provide symptomatic relief and reduce splenomegaly, they all fail to effectively eliminate the mutant MPN clone, and many patients discontinue type-I JAK2i treatment within 3-5 years of starting therapy. Type-I JAK2i bind the **active conformation** of JAK2, which can result in disease persistence due to continued JAK2 activation through heterodimerization with JAK1 or TYK2 **(Figure 1)**.[1] Recent studies have shown that **type-II JAK2i**, which bind the ***inactive* JAK2 kinase conformation**, can overcome type-I JAK2i persistence and enhance therapeutic efficacy in MPN animal models.[2] However, until now, the development of type-II JAK2i has been limited by off-target toxicity and poor bioavailability. We investigated the pre-clinical activity of AJ1-11095, a potent, orally administered, highly selective type-II JAK2i which is the first type-II JAK2i to be tested in a multi-institutional Phase 1 clinical trial for MF patients refractory to type-I JAK2i (NCT06343805).[3]

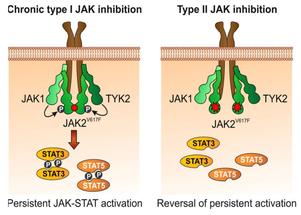


Figure 1: Type I vs. Type II JAKi and the rationale for Type II JAKi for the treatment of MPNs.[2]

Methods

A physics-based *in silico* computationally guided structure-based drug design (SBDD) tool was used to identify AJ1-11095.[4] SET2 sensitive ($SET2^{sens}$) and type I JAKi (ruxolitinib) persister lines ($SET2^{per}$) were cultured and generated using previous methods.[1] The $hMPL^{W515L}$ adoptive transfer model (Balb/c background) and the Cre-inducible human *SclCreER*;*JAK2*^{V617F};*GFP* model (C57B6/J background) were used for the *in vivo* disease-model efficacy studies using previously-established methods.[5-6] Kinome profiling was performed using KINOMEScan at standard 1uM ATP concentrations. Cytokine profiling was performed on isolated serum from treated mice using the Millipore 32-plex Luminex cytokine array kit.

Key Absorption, Distribution, Metabolism, Excretion Properties		AJ1-11095
JAK2 Cellular Activity	SET2-pSTAT5 IC_{50} (nM)	51
JAK Family Biochemical Selectivity (Fold over Kd)	Fold Selectivity over JAK1	780x
	Fold Selectivity over JAK3	>3840x
	Fold Selectivity over Tyk2	150x

AJ1-11095 displays potent JAK2 selectivity and minimal cross-reactivity

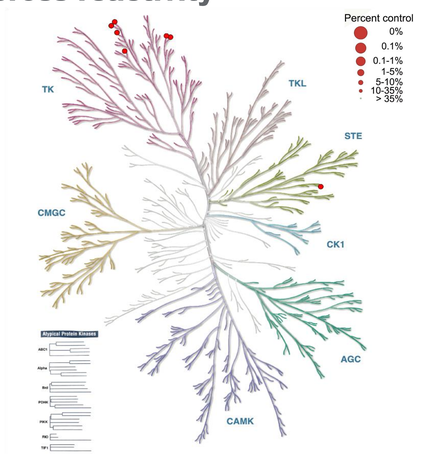
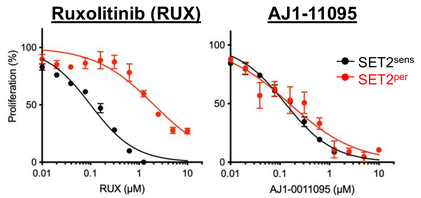


Figure 2: Kinome profiling of AJ1-11095 by KINOMEScan.

AJ1-11095 overcomes type I JAKi persistence *in vitro*



	RUX	AJ1-11095
RUX^{sens}	96 nM	108 nM
RUX^{per}	1844 nM	240 nM
Fold shift	19x	2.2x

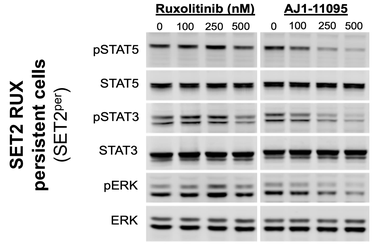
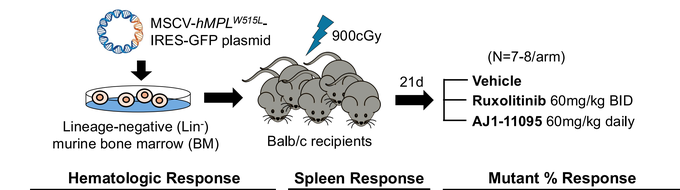


Figure 3: (Top panel) Cell viability of SET2 naive ($SET2^{sens}$; black) vs. persistence ($SET2^{per}$, red) cells cultured with ruxolitinib (RUX) vs. AJ1-11095. **(Bottom panel)** Western blot JAK/STAT signaling analysis of $SET2^{per}$ cells treated with ruxolitinib vs. AJ1-11095.

Pre-clinical efficacy of AJ1-11095 in the $hMPL^{W515L}$ adoptive transfer model[5]



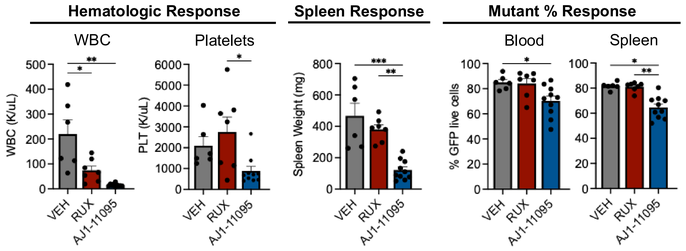


Figure 3: (Upper panel) Schematic of the $hMPL^{W515L}$ adoptive transfer model of myelofibrosis. **(Bottom panel)** Hematologic parameters (left), spleen volumes (middle), and mutant cell fraction (by GFP% flow) of vehicle (VEH) vs. ruxolitinib (RUX) vs. AJ1-11095 treated mice following the 3-week *in vivo* study.

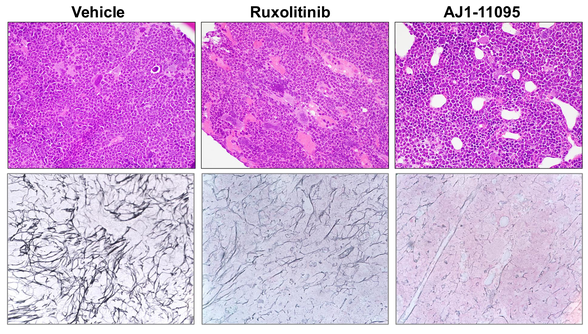


Figure 4: Histopathologic analysis of treated mice. (Upper panel) Bone marrow hemoxylin and eosin (H&E) staining and **(lower panel)** reticulin staining of $hMPL^{W515L}$ treated mice.

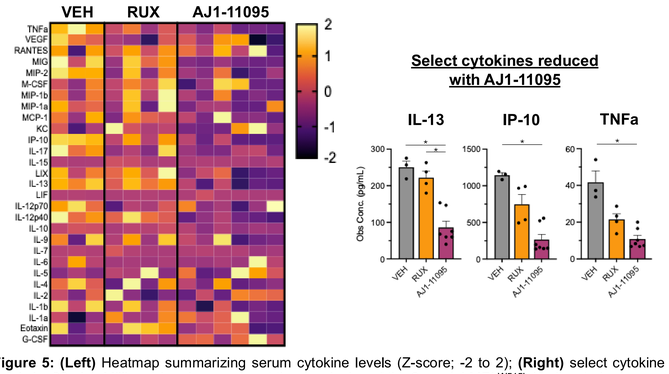
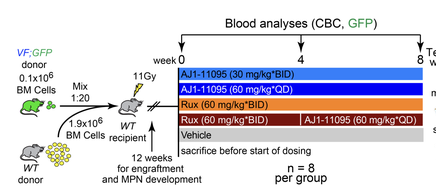
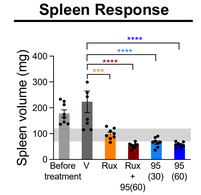


Figure 5: (Left) Heatmap summarizing serum cytokine levels (Z-score; -2 to 2); **(Right)** select cytokine levels by 32-plex Millipore Luminex assay at the conclusion of the 3-week $hMPL^{W515L}$ *in vivo* study.

Pre-clinical efficacy of AJ1-11095 in a *Jak2*^{VF} knock-in competitive transplant model[6]





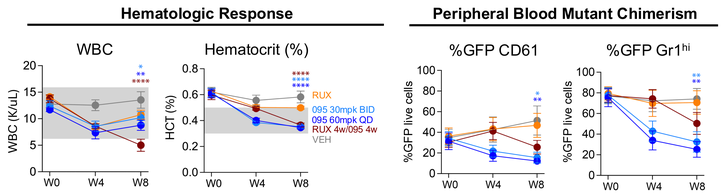


Figure 6: (Upper panel left) Schematic of the *Jak2*^{V617F} knock-in competitive transplant experiments. **(Upper panel right)** Weight trend of mice in response to treatment. **(Bottom panel)** Hematologic parameters (left) and spleen volumes (right) of vehicle (V) vs. ruxolitinib (Rux) vs. AJ1-11095 (both 30mpk BID and 60mpk QD) treated mice during the 8-week *in vivo* study. In a separate arm (maroon, above), a cohort of mice were treated initially with Rux for 4 weeks and then *switched* to AJ1-11095 for the completion of study.

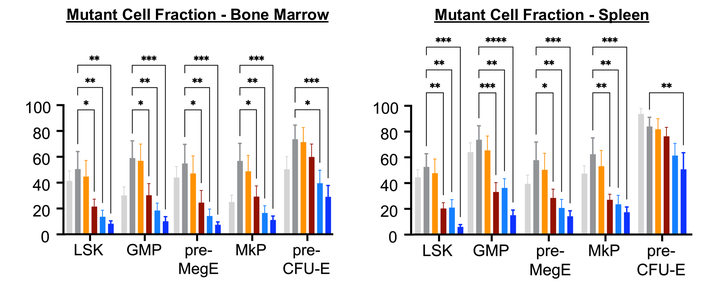


Figure 7: Frequencies of hematopoietic stem/progenitor cell (HSPC) populations in bone marrow **(Left panel)** and spleen **(Right panel).** LSK: lin-Sca1+cKit+; GMP: granulocytic-monocytic progenitor; MegE: megakaryocytic-erythroid progenitor; MkP: Megakaryocytic progenitor; CFU-E: colony forming unit – erythroid.

Conclusions

AJ1-11095, which is **currently being tested in a Phase 1 clinical trial** in patients with MF refractory to type-I JAK2i, is a potent, orally administered, highly selective type-II JAK2i that demonstrates **superior pre-clinical efficacy** compared to RUX, including reductions in mutant allele burden and reticulin fibrosis, with an enhanced safety profile compared to previous type-II JAK2i. These data further demonstrate the preclinical utility and clinical rationale for type-II JAK2i, including AJ1-11095, for the treatment of MPNs.

References

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Learn more about the AJ1-11095 phase 1 trial here: