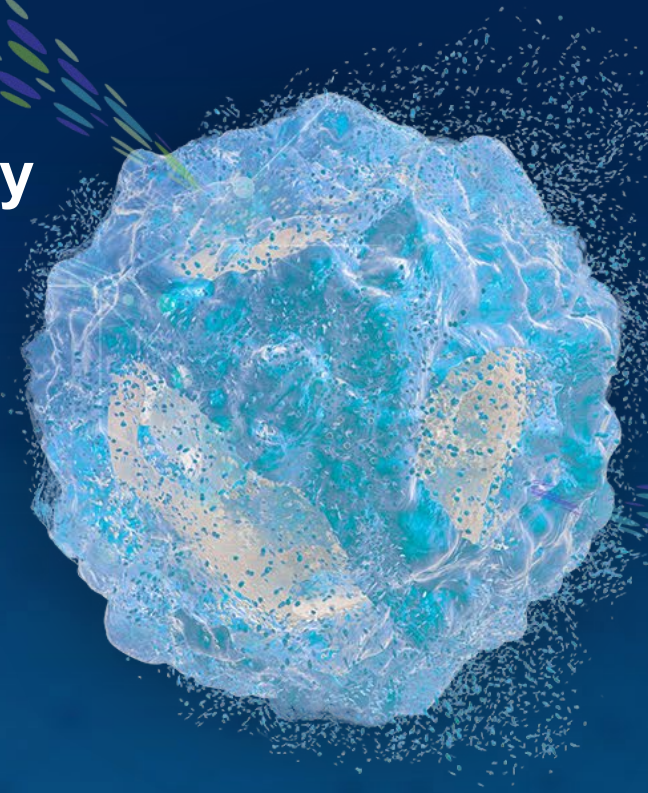
Discovery and preclinical characterization of orally bioavailable JAK2V617F mutant selective JH2 inhibitors with disease modification potential in myeloproliferative neoplasms



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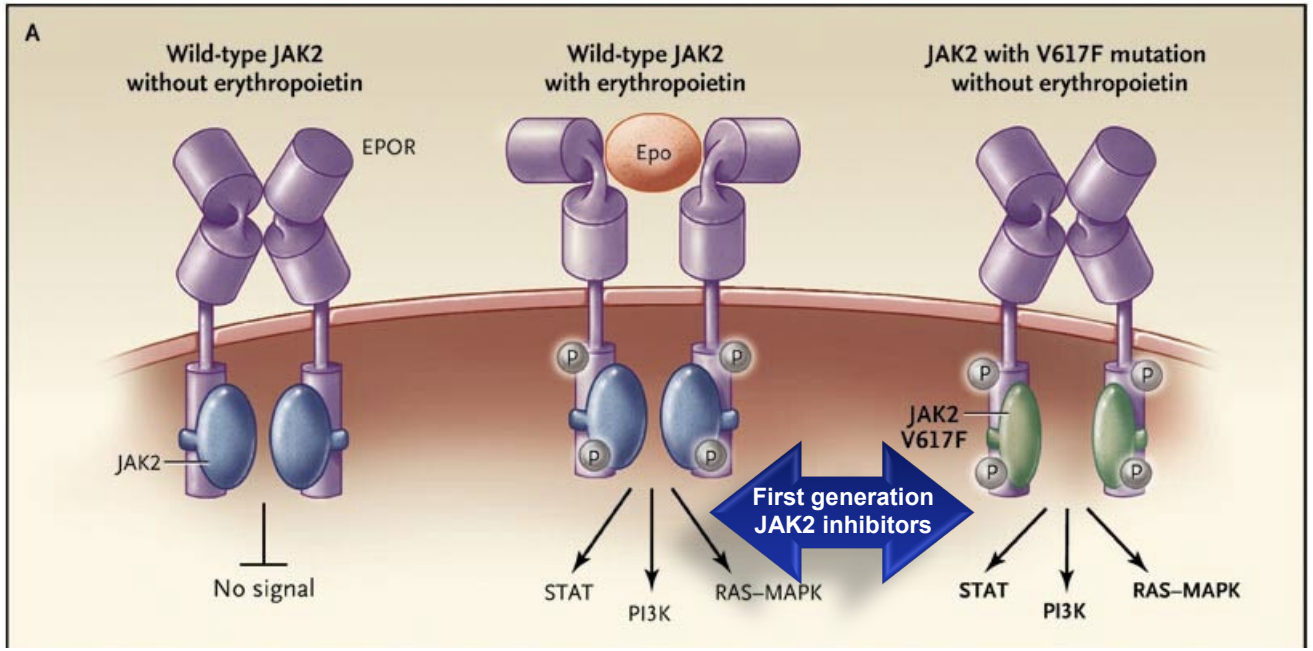
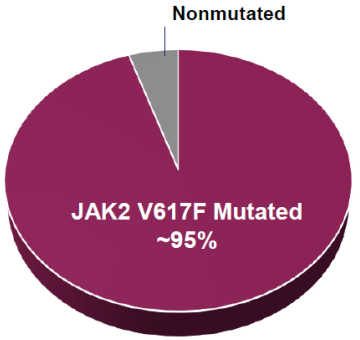
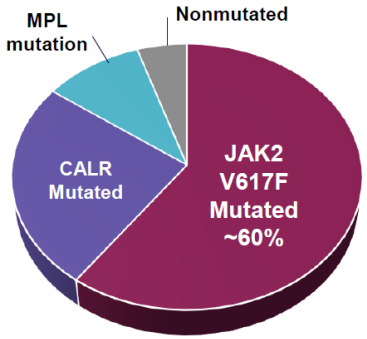
JAK2V617F is the Primary Driver Mutation Leading to Constitutive JAK2 Activation and Hyperproliferation in Patients With Myeloproliferative Neoplasms (MPN)



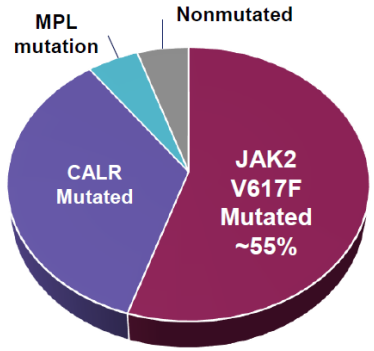
Campbell P.J. and Green A.R. N Engl J Med 2006;355:2452-2466



Polycythemia Vera (PV)



Essential Thrombocythemia (ET)

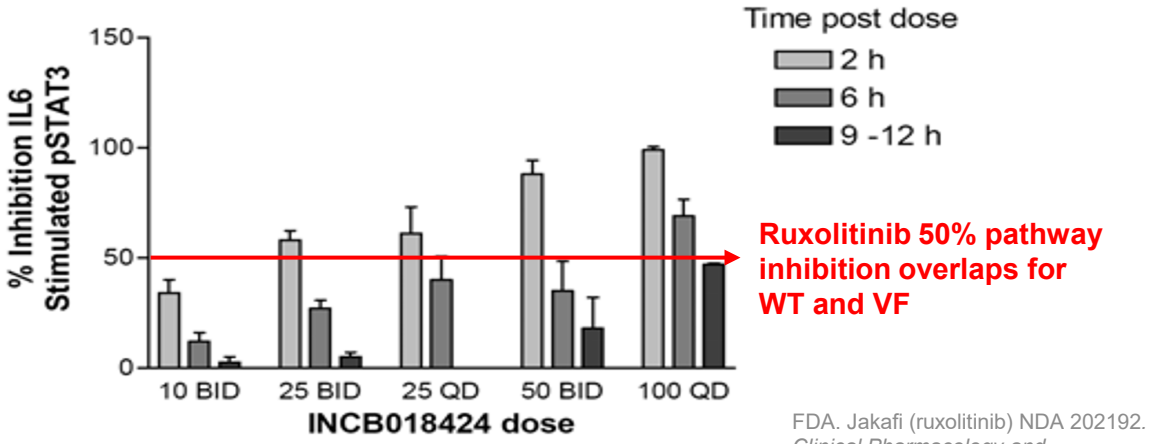


Primary Myelofibrosis (PMF)

- Selectively targeting JAK2V617F offers disease-modifying potential in MPN
  - Potential for superior safety profile by sparing WT JAK2 in normal myelopoiesis
  - Potential for molecular responses through more rapid reduction in mutant allele

First Generation JAK Inhibitors Are Effective but Have Limitations

- First generation JAK2 inhibitors such as ruxolitinib are highly effective in reducing spleen size and symptoms in MPN, *but inhibit wild type and mutant JAK2 equally*
- Achieving >50% pathway inhibition with ruxolitinib requires high doses (≥ 25 mg BID) associated with Grade 3/4 thrombocytopenia and anemia, *thus limiting efficacy*
- A selective JAK2V617F inhibitor offers the potential for *disease modifying activity while sparing normal bone marrow function*
- An improved therapeutic index would enable higher dosing and potential for *more rapid disease modifying activity*



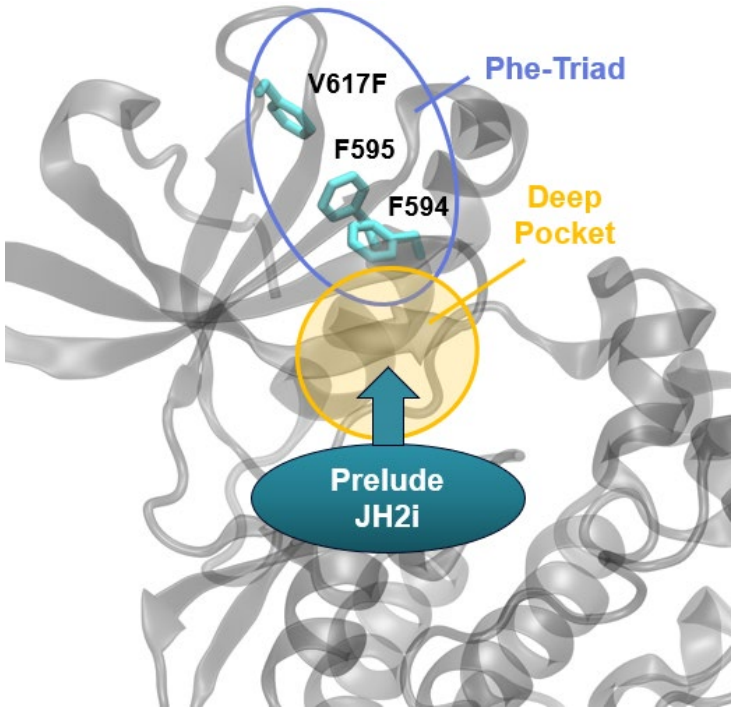
FDA. Jakafi (ruxolitinib) NDA 202192. *Clinical Pharmacology and Biopharmaceutics Review*

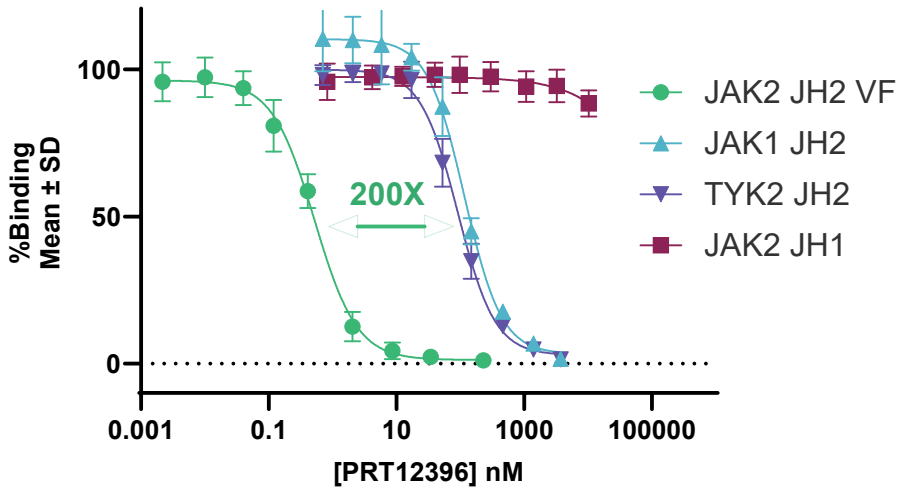
Table 3. Hematologic Adverse Events

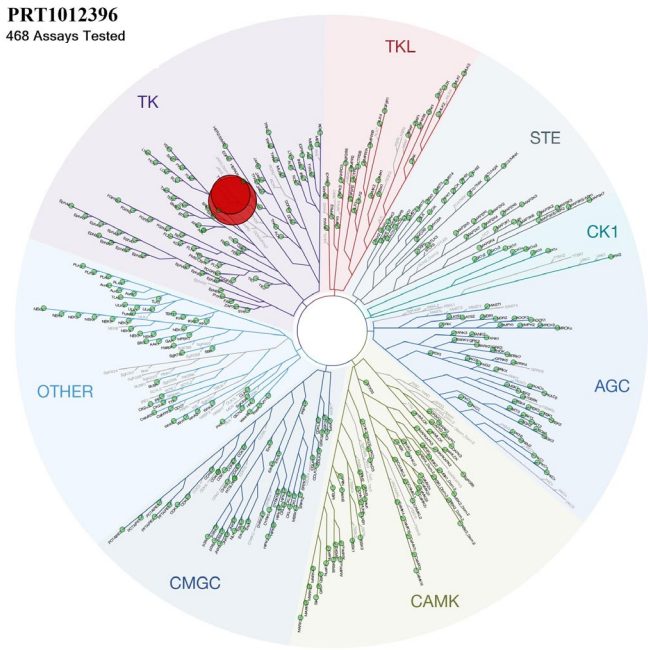
Variable	10 mg Twice Daily	15 mg Twice Daily	25 mg Twice Daily	50 mg Twice Daily
Thrombocytopenia — no./total no. (%)*				
Grade 3	3/29 (10)	1/35 (3)	11/47 (23)	3/5 (60)
Grade 4	0	0	3/47 (6)	1/5 (20)
New-onset anemia among patients who were transfusion-independent at baseline — no./total no. (%)†	3/19 (16)	2/24 (8)	8/30 (27)	0/2 (0)

Verstovsek S et al. *N Engl J Med.* 2010;363(12):1117–1127.

PRT12396 is an Allosteric JAK2V617F-Selective Pseudokinase (JH2) Inhibitor





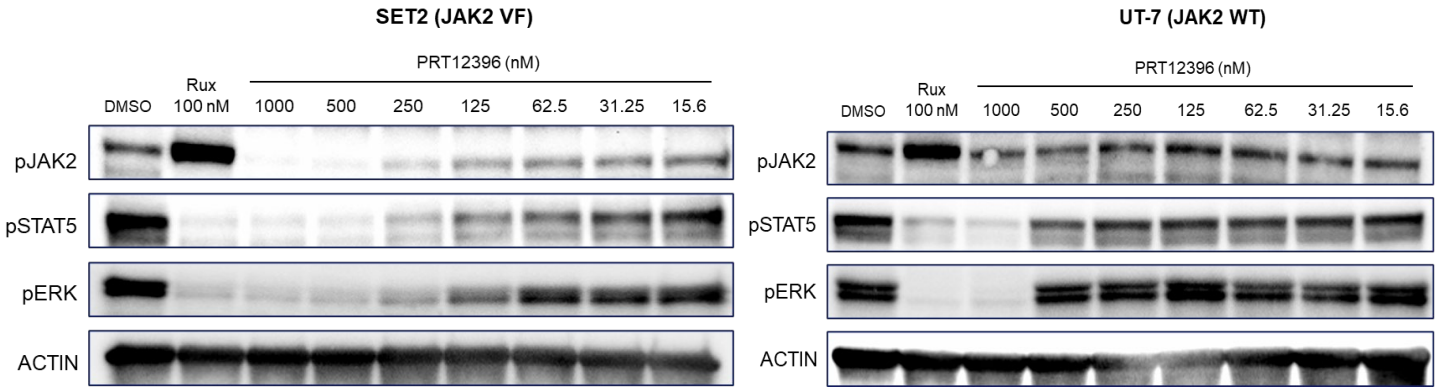


Tested in kinome binding assay at 100X JAK2VF IC_{50}

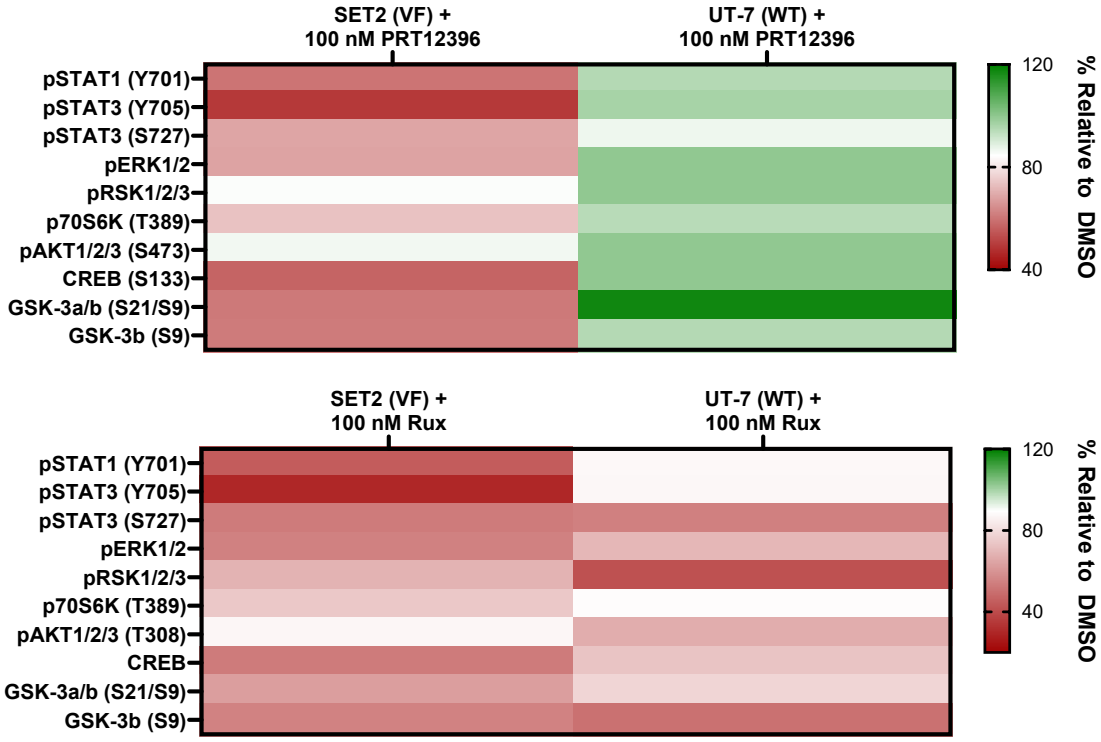
- Structure-Based Drug Design (SBDD) enabled identification of novel mutant-selective JAK2 JH2 inhibitors that bind into the “Deep Pocket” adjacent to the JAK2V617F-specific Phe-triad
- >200X selectivity over JAK1 and TYK2 and clean profile in KinomeSCAN™ panel of >450 kinases

PRT12396 Demonstrates Selective Inhibition of Aberrant Signaling in JAK2VF Cells

Dose-dependent inhibition of pJAK2 and STAT-MAPK signaling



Selective modulation of aberrant signaling in JAK2VF cells

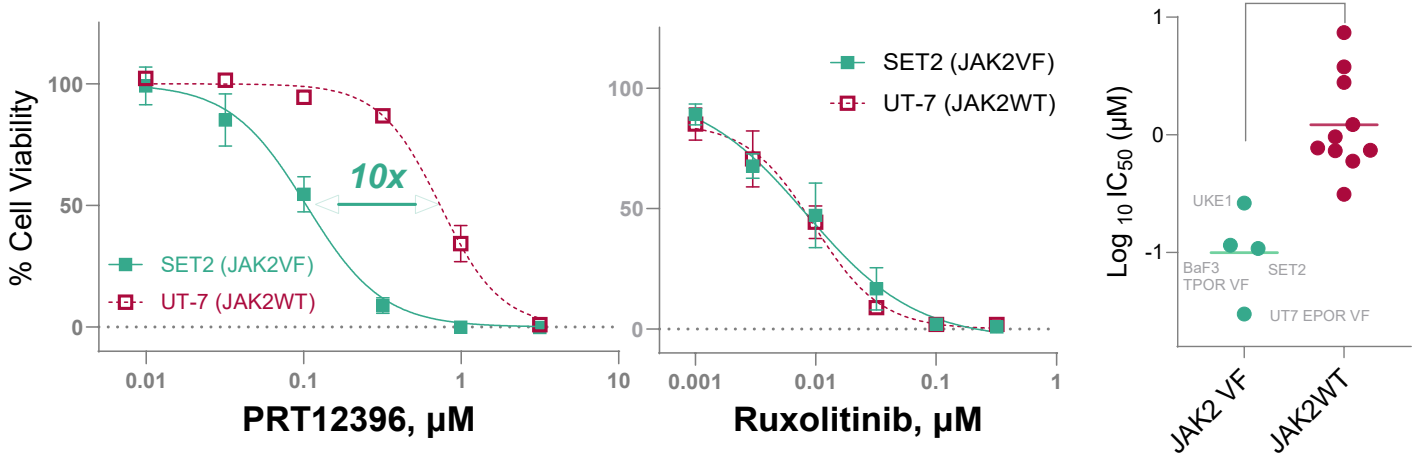


- PRT12396 selectively inhibits downstream STAT/MAPK/AKT signaling pathways in JAK2VF cells, with minimal effects in JAK2WT controls
  - Ruxolitinib exhibits non-selective inhibition in both WT and VF cells
- Dose-dependent suppression of JAK2 activation loop phosphorylation in JAK2VF cells
  - No paradoxical JAK2 hyperphosphorylation, unlike ruxoltinib

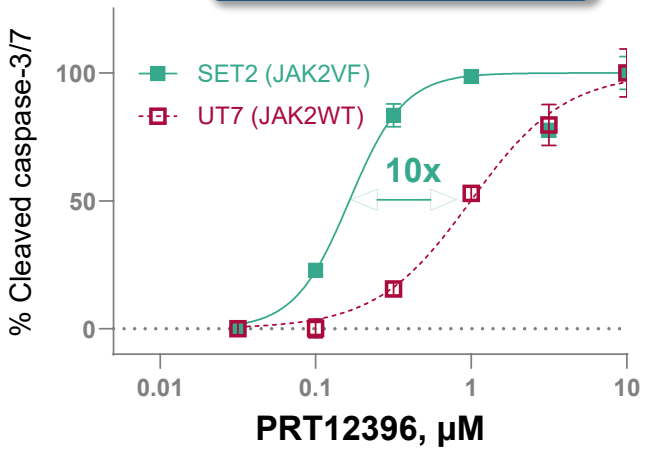
UT-7 cells stimulated with 50 ng/ml GM-CSF for 15 minutes prior to lysis
Phospho-protein abundance assessed using membrane-based sandwich immunoassay

PRT12396 Selectively Inhibits Mutant JAK2V617F in Cellular Assays Leading to Potent Anti-Proliferative Activity

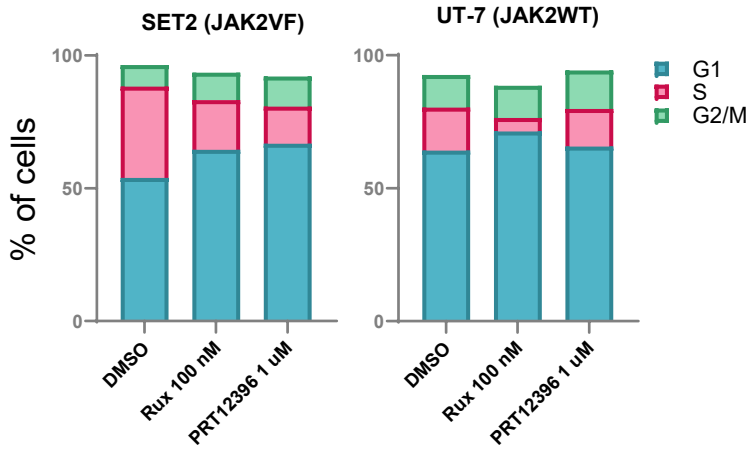
10x Selectivity in Anti-proliferation



Potent Apoptosis



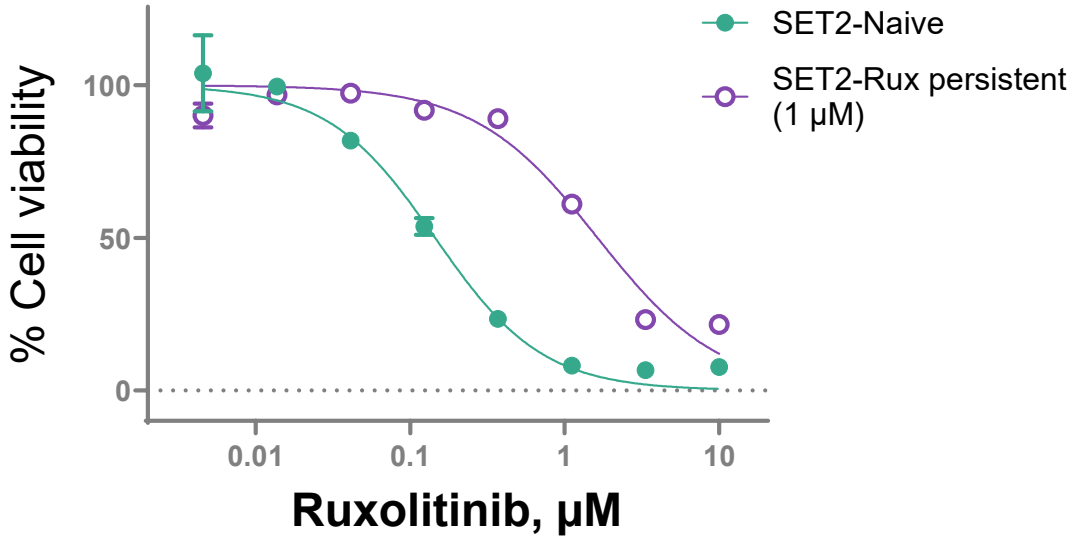
Selective G1/S Arrest

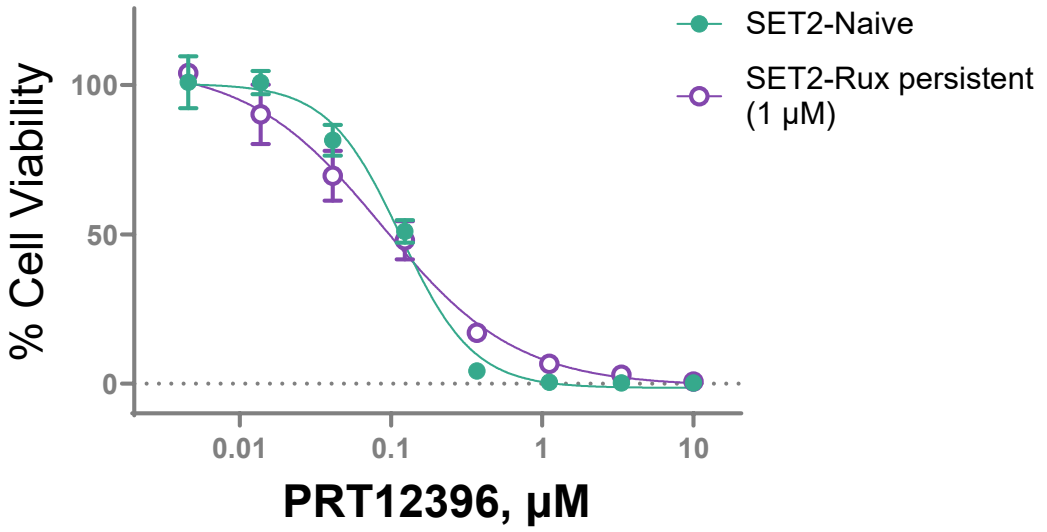


- 10X selective anti-proliferative activity in JAK2VF cell lines
- Selective G1/S cell cycle arrest in JAK2VF cells, with minimal effects in JAK2 WT cells
- Potent and selective induction of apoptosis in JAK2VF cells
- Ruxolitinib does not show separation between JAK2VF and WT cells in either cell-cycle or anti-proliferative effects

Cell viability was assessed in a 7-day CellTiter-Glo® assay
Cell cycle analysis was conducted at 24 hours by EdU/DRAQ5™ incorporation
Apoptosis was measured at 48 hours by Caspase-Glo® 3/7 assay

PRT12396 Maintains Activity in Models of Ruxolitinib-Persistence



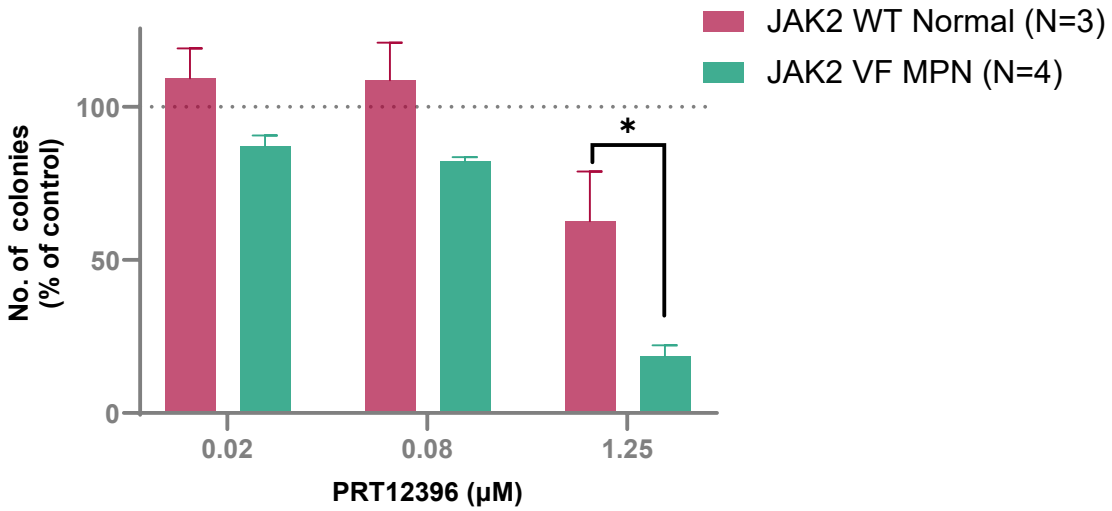


Ruxolitinib persistent cells were generated by culturing SET2 cells in increasing concentrations of ruxoltinib for 6-8 weeks

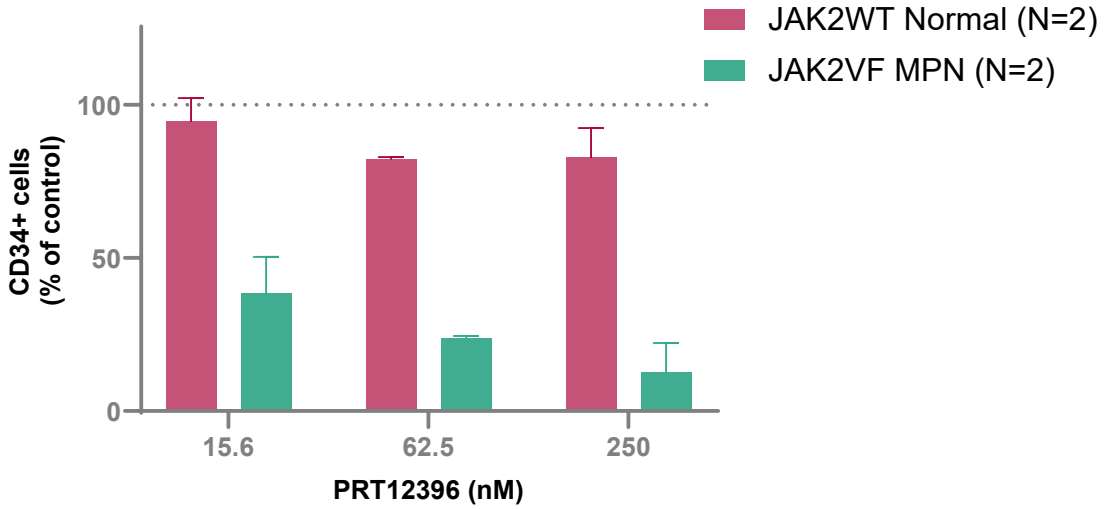
- PRT12396 demonstrates similar anti-proliferative activity in both SET2-naïve and Rux-persistent cells, suggesting *PRT12396 may address clinically relevant resistant mechanisms*

PRT12396 Selectively Inhibits Expansion and Proliferation of JAK2VF Primary MPN Cells

CFU Assay



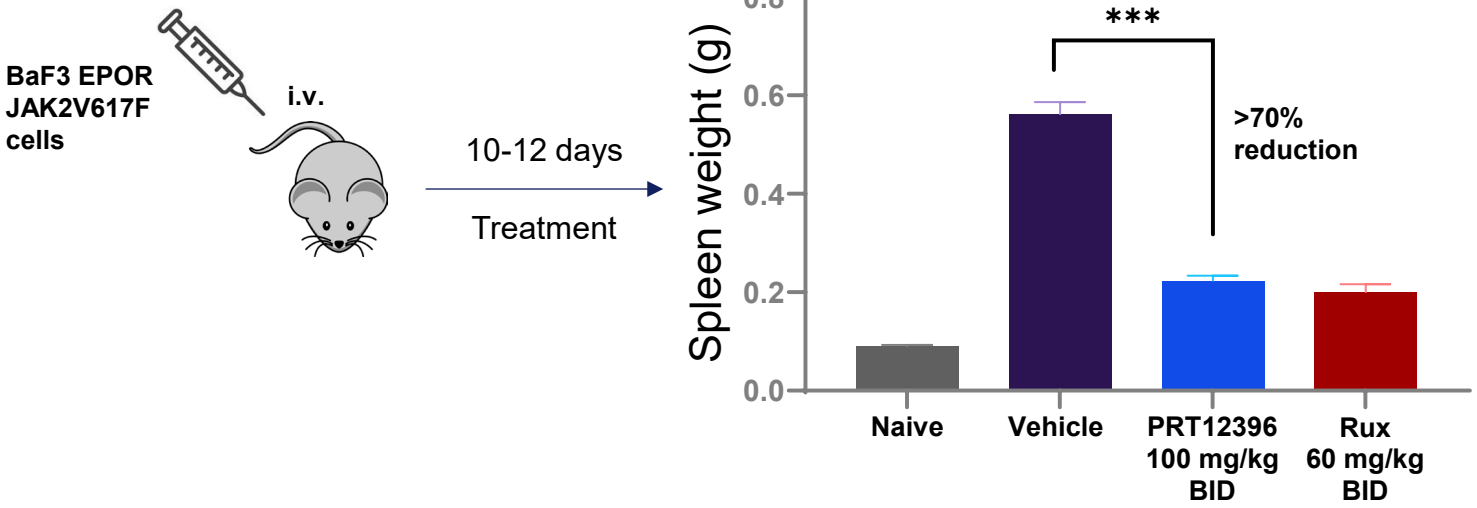
CD34⁺ ex vivo Proliferation Assay



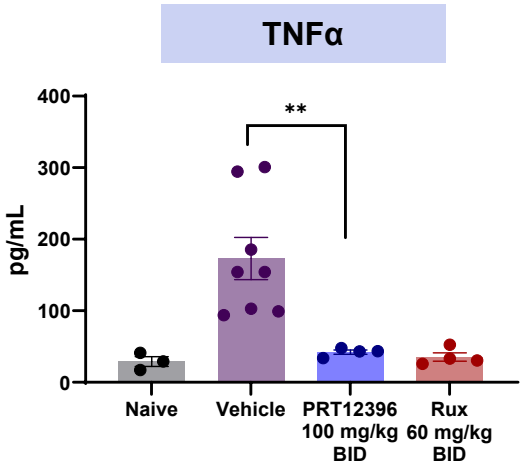
*p<0.05 by t-test

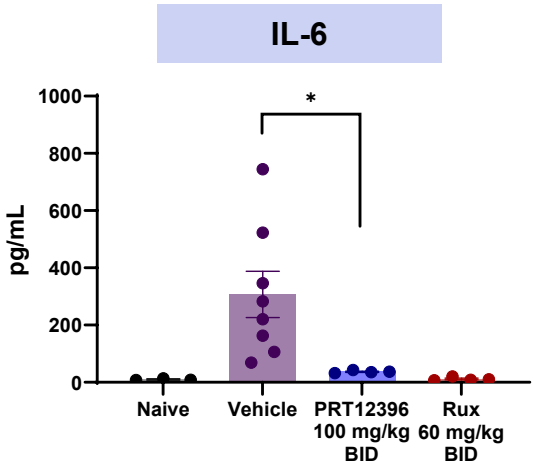
- PRT12396 demonstrates *selective and dose-dependent* inhibition of JAK2V617F MPN progenitor cells, in clonogenic and CD34⁺ proliferation assays
- Minimal impact on JAK2WT normal progenitors

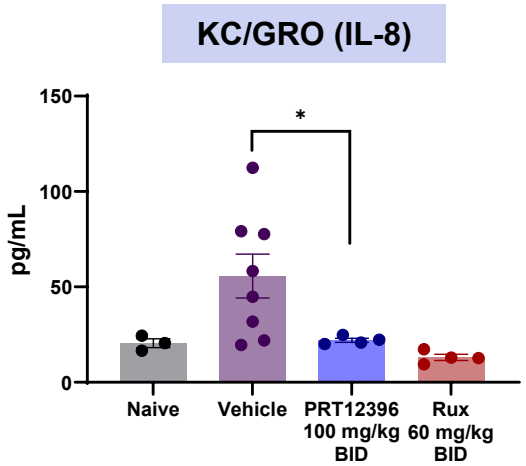
PRT12396 Shows Significant Efficacy, Comparable to Ruxolitinib, in BaF3-EPOR-JAK2VF Model

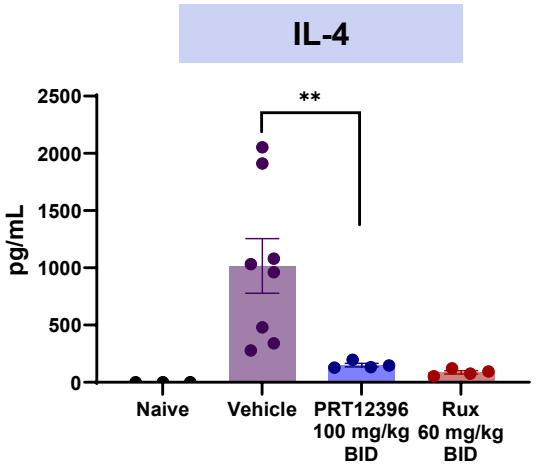


- Significant reduction in splenomegaly at well-tolerated doses
- Normalization of pathogenic cytokines



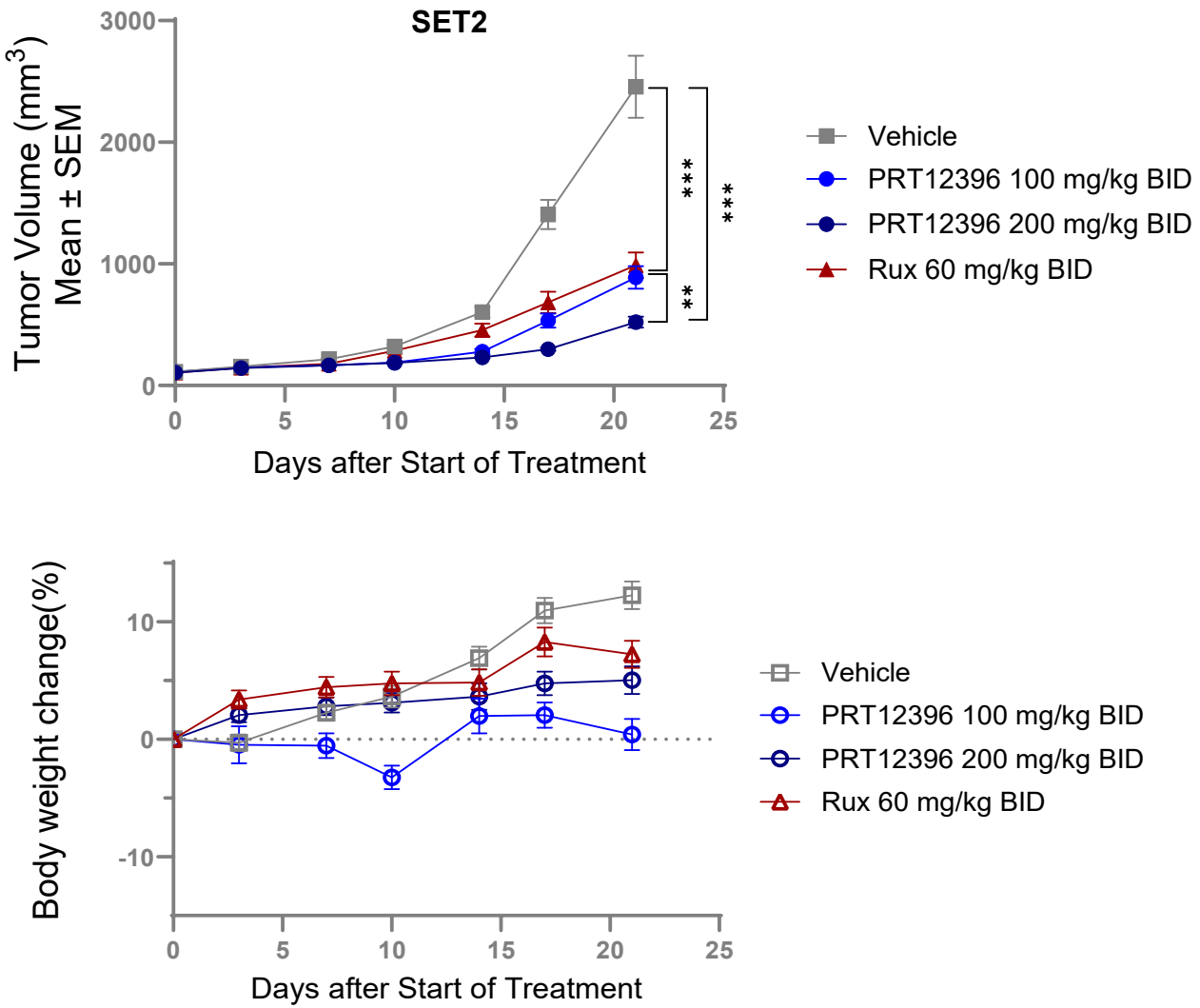




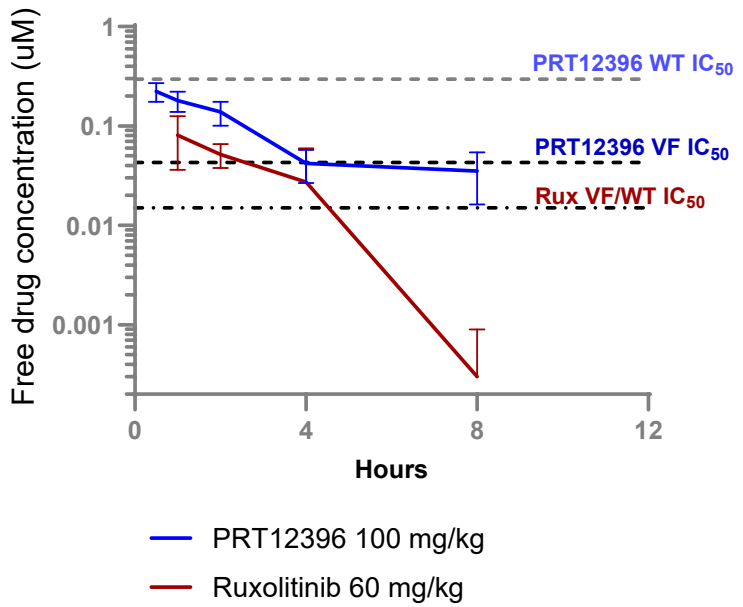


*p<0.05, **p<0.01, *** p<0.001 by Mann-Whitney U test

PRT12396 Demonstrates Significant Tumor Growth Inhibition in JAK2VF SET2 Xenograft Model



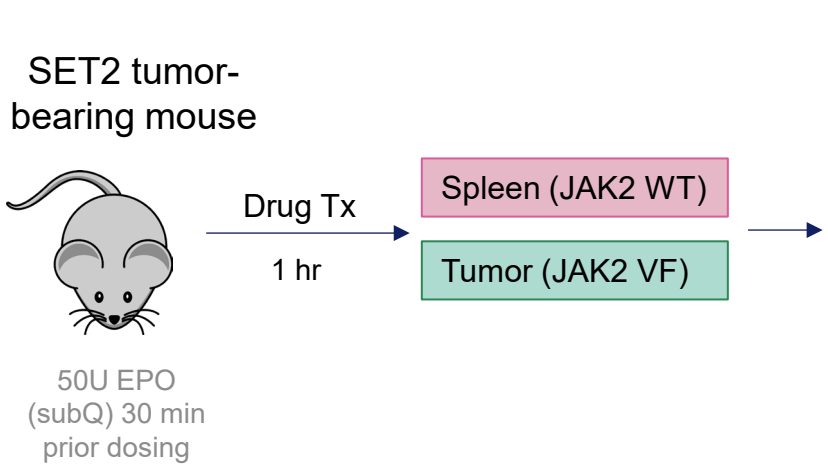
- Significant TGI (>80%), *superior to ruxolitinib*, observed at well-tolerated doses
- 4-6 hours plasma coverage > VF proliferation IC_{50} is associated with efficacy, similar to ruxolitinib

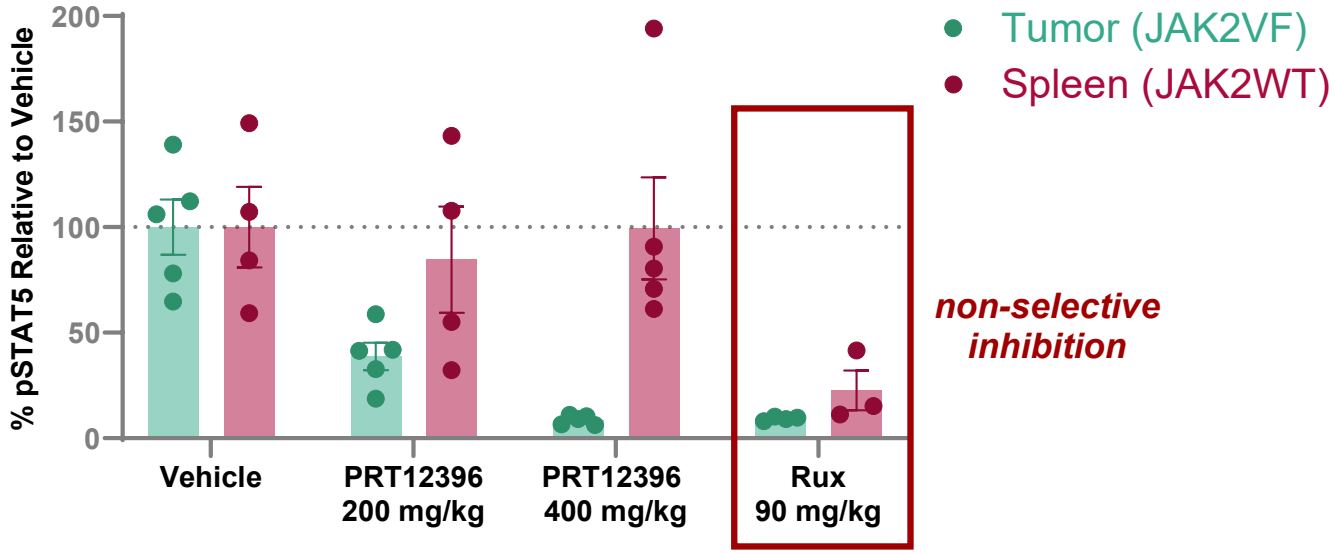


***p<0.01, *** p<0.001 by Mann-Whitney U test*

Dotted lines represent protein adjusted proliferation IC_{50} values from SET2 (VF) and UT-7(WT) 7-day proliferation assay

PRT12396 Exhibits Selective Inhibition of Mutant JAK2V617F *In Vivo*



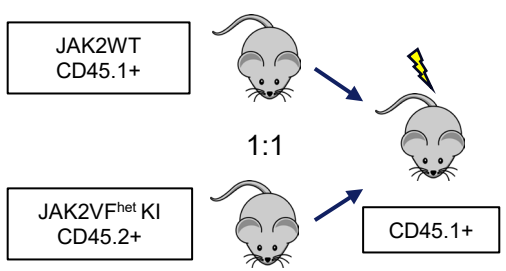


- Dose-dependent inhibition of pSTAT5 in JAK2VF tumor tissue, with minimal effects in WT spleen tissue
- Ruxolitinib shows *non-selective* pSTAT5 inhibition in both VF and WT tissue

Superior Efficacy to Ruxolitinib in a JAK2V617F Knock In Mouse Model

Key features that recapitulate human MPN

- Elevated blood counts
- Iron deficiency
- Splenomegaly
- Elevated inflammatory cytokines



2-week engraftment

Vehicle

PRT11758 po 100 mg/kg BID

Ruxolitinib po 60 mg/kg BID

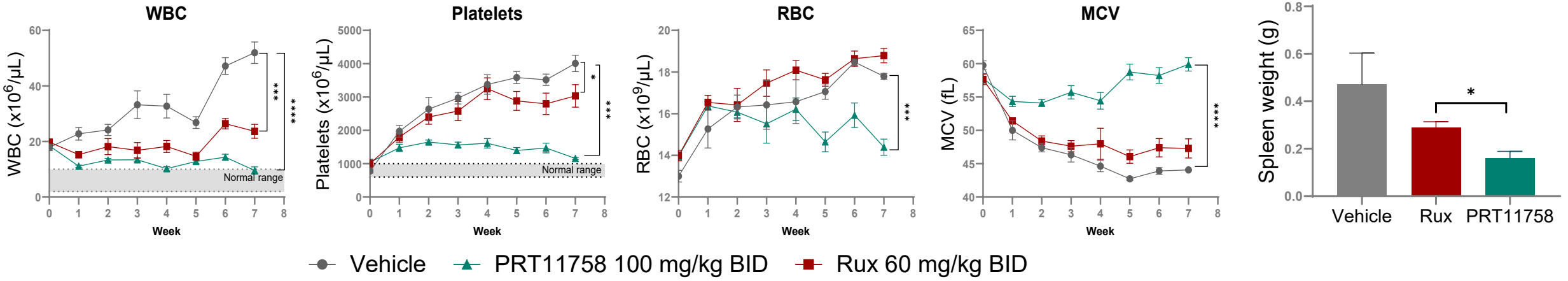
7 weeks

Weekly CBCs

At termination
- Spleen weights
- Cytokine analysis
- Flow cytometry

PRT11758 is a preclinical tool compound with ~5X JAK2VF selectivity

Marty C et al. *Blood*. 2010;116(5):783-7



*p<0.05 ,**p<0.01, *** p<0.001 by t-test

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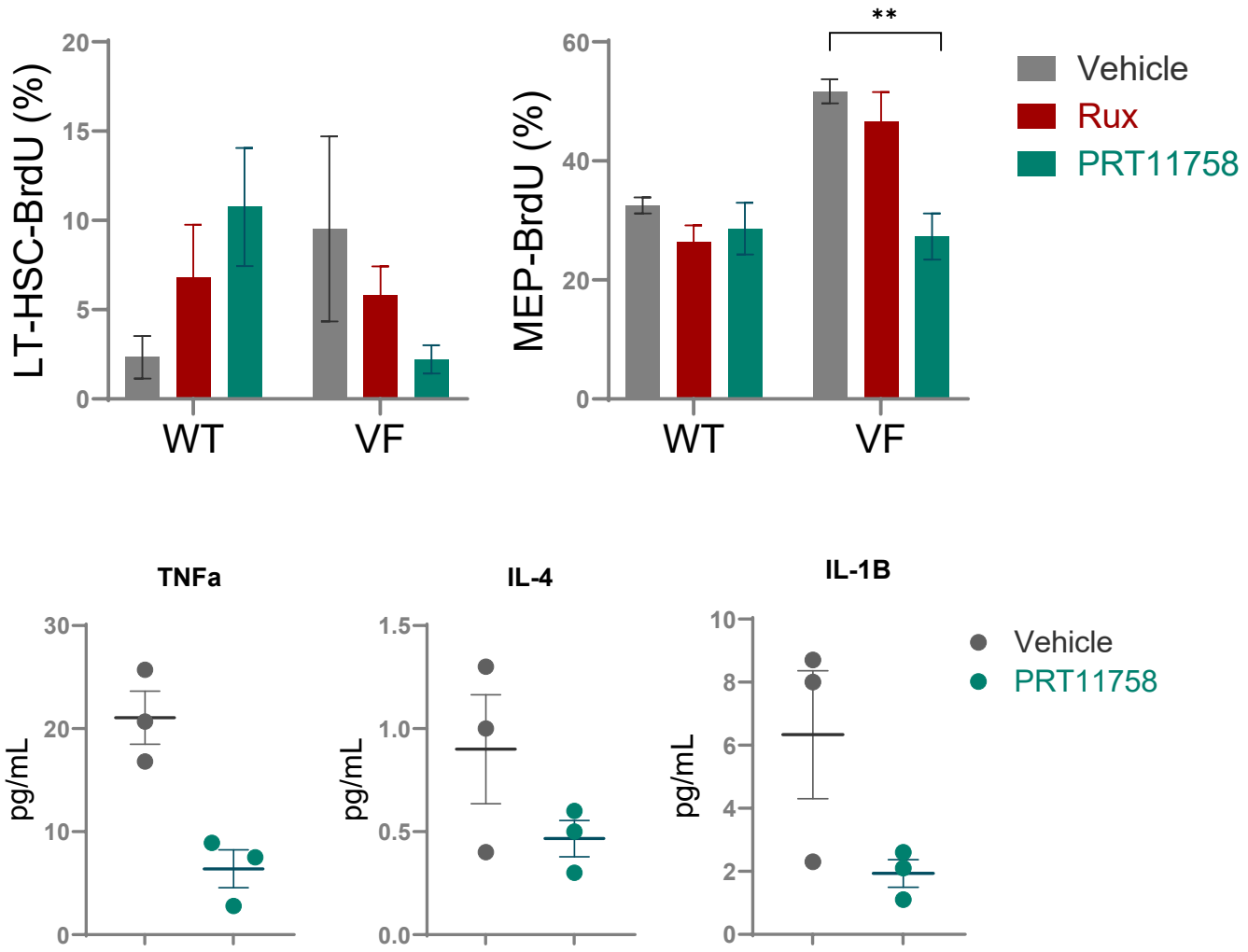
- Normalization of WBCs and platelets without cytopenias
- Decrease in RBCs without a drop in MCV, suggesting improvement of iron deficiency
- Significant reduction of splenomegaly

Selective Effects on JAK2VF Stem and Progenitor Cells in JAK2VF Knock in Model



- Selective decrease in proliferation of JAK2VF long-term-HSC and megakaryocyte-erythroid progenitor populations
- Minimal effects on JAK2WT stem and progenitor cells
- Reduction in key inflammatory cytokines

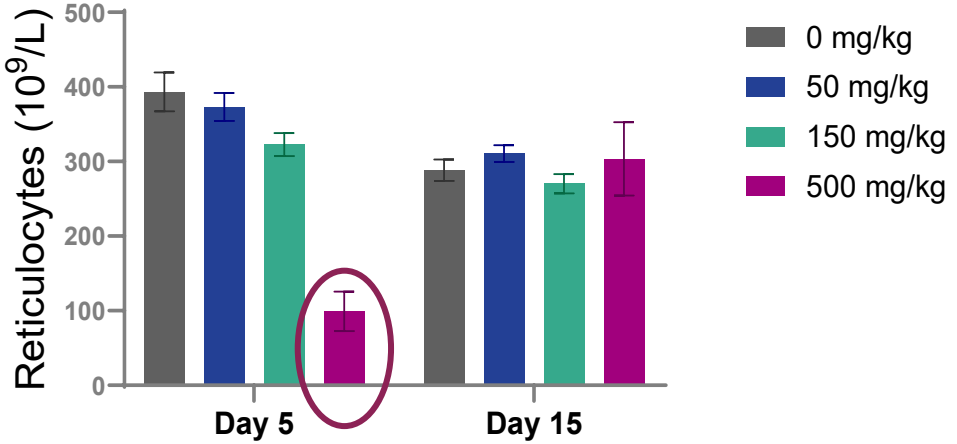
***p<0.01 by t-test*

Stephane Giraudier, Duanya Liu (INSERM)

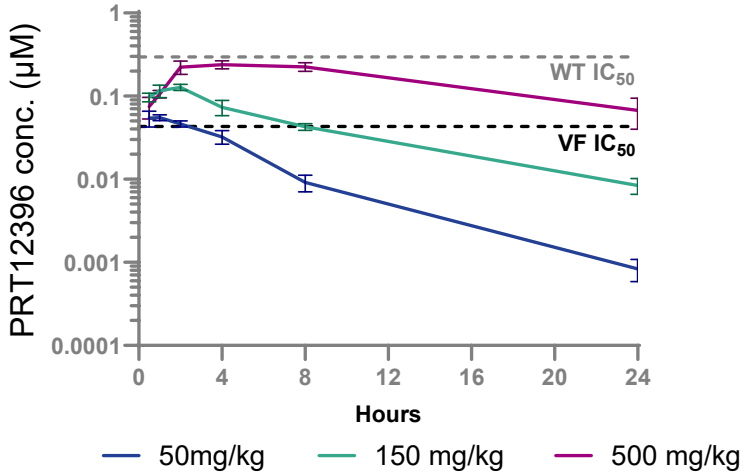
PRT12396 Demonstrates Significant Therapeutic Window *In Vivo*

- No evidence of WT JAK2 inhibition in 2-week rat toxicology study at low and mid doses that provide efficacious exposures
  - Reduction in neutrophils and reticulocytes observed *only at high dose*, consistent with plasma exposure approaching JAK2WT IC_{50}
- AUC exposures required for efficacy are *10X lower* than those associated with bone marrow suppression (reticulocyte inhibition)

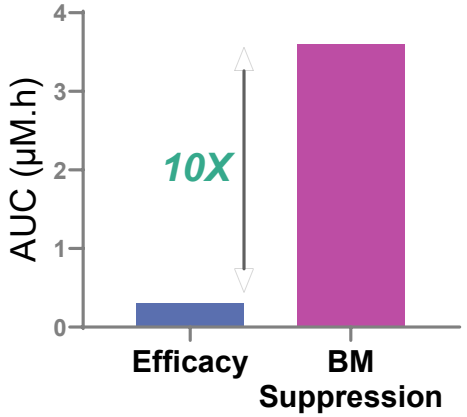
Reticulocyte Inhibition Only at High Dose, with Full Recovery by Day 15



Plasma Exposures at Low/Mid Doses Remain Below WT JAK2 IC_{50}



Efficacy AUC is 10X Lower Than Toxicity AUC



Dotted lines represent proliferation IC_{50} values from SET2 (VF) and UT-7 (WT) 7-day proliferation assay

Summary: PRT12396 is a JAK2V617F-Selective JH2 Inhibitor with Disease Modifying Potential in Myeloproliferative Neoplasms

- ✓ Selectively inhibits JAK2V617F activity in a cellular context while preserving wild type JAK2-mediated cytokine signaling
- ✓ Demonstrates robust preclinical activity in multiple preclinical MPN models, superior to ruxolitinib
- ✓ Selectively inhibits proliferation of JAK2VF stem and progenitor cells *in vitro* and *in vivo*
- ✓ Well tolerated in toxicological studies with minimal effects on hematologic parameters
- ✓ Phase 1 study in MPN patients expected to be initiated in 1H 2026

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