

# Genetics and targeted therapy of MPNs –

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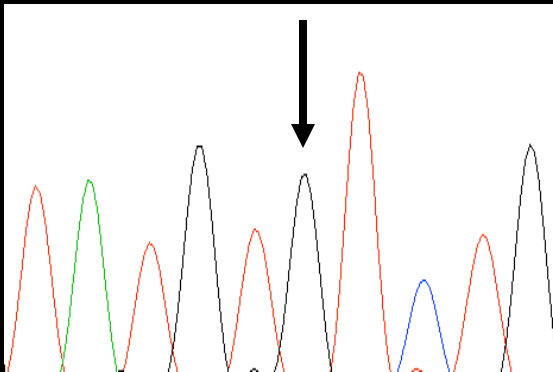
# Scientific Questions/Advances

- Are mutations which activate JAK2 a hallmark of all MPN patients?
- What do mutations which occur in concert with JAK2/MPL mutations do?
- What have we learned about JAK2 inhibitors?
- What novel therapies are of potential benefit for MPN patients?

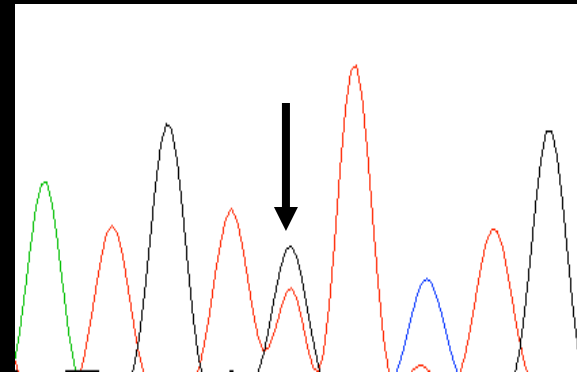
# JAK2V617F Mutations in MPN patients

No mutation in normal tissue

Heterozygous mutation in MPN Cell



G



G/T

## Valine->Phenylalanine Amino Acid Change

	JAK2 - Wildtype								JAK2 - Mutant							
DNA	tat	gga	gta	tgt	gtc	tgt	gga	gac	tat	gga	gta	tgt	ttc	tgt	gga	gac
Protein	Y	G	V	C	V	C	G	D	Y	G	V	C	F	C	G	D
					↑								↑			
					617								617			

- 90% of PV
- 60% of ET/PMF
- <10% of CMML/AML

\*James *et al.* Nature 2005  
 Levine *et al.* Cancer Cell 2005  
 Baxter *et al.* Lancet 2005  
 Kralovics *et al.* NEJM 2005

# JAK2V617F negative MPN

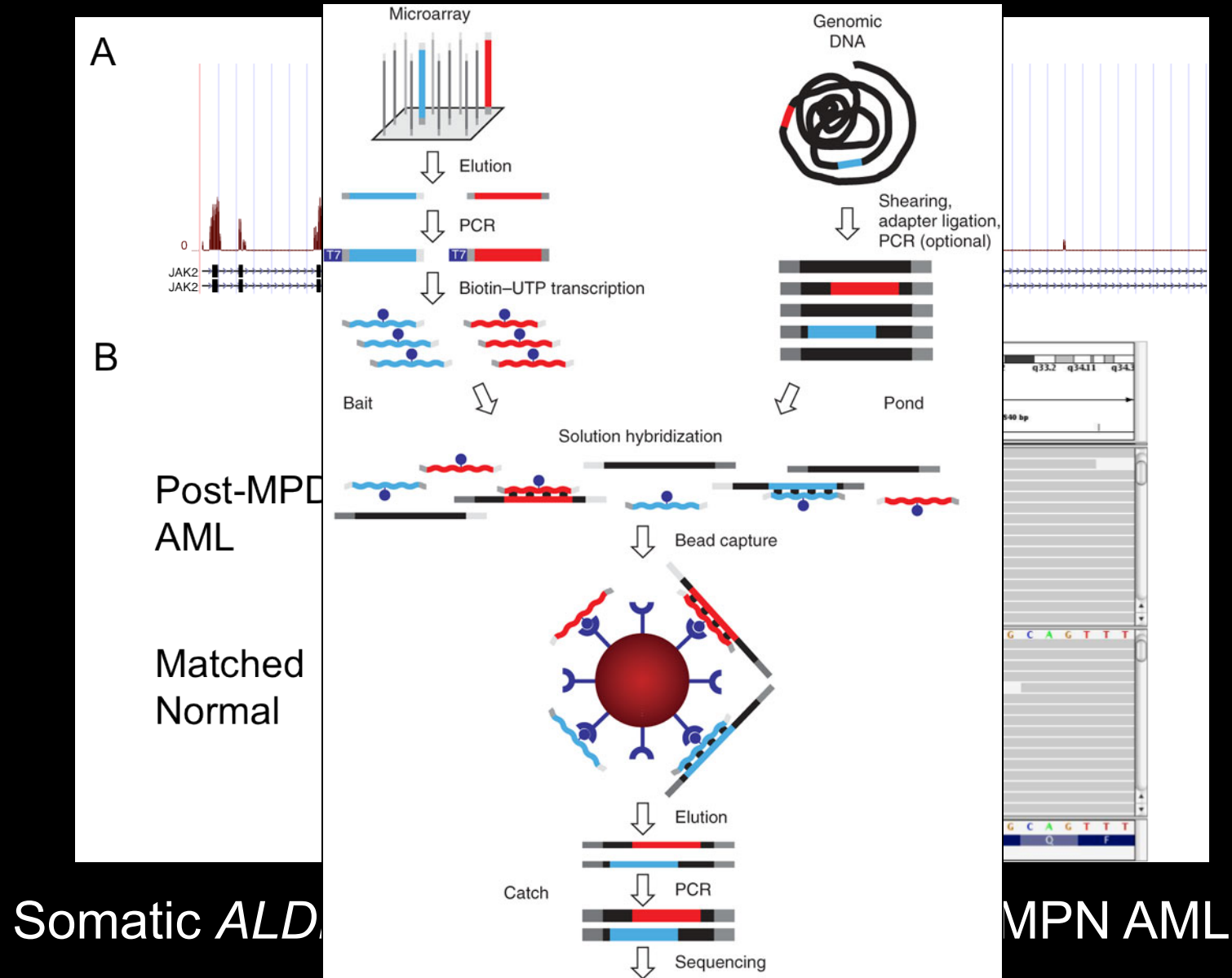
- JAK2V617F-negative PV
  - JAK2 exon 12 mutations
  - loss of function mutations in LNK, negative regulator of JAK2 (Oh et al Blood 2010)
- JAK2V617F-negative ET/PMF
  - MPL mutations in 10%
  - LNK mutations in <5%
- Somatic mutations have not been identified in 30-40% of MPN patients
  - Sequencing known genes in the JAK2 pathway has not provided the answer->how to proceed?

# Whole Exome Sequencing to identify MPN Alleles\*

- To date we have sequenced 40 exomes from MPN patients
  - 20 JAK2/MPL negative patients
  - 15 patients with myelofibrosis
  - 5 patients with MPN which transformed to leukemia
- We have sequenced members of 2 families with high penetrance MPN – try to find familial predisposition locus
- Complements efforts by Sanger/European group focusing on JAK2+ disease, PV/ET/PMF

\*Jay Patel, Ann Mullally, Ben Ebert (MPN Foundation Grant)

# Whole Exome Sequencing to identify MPN Alleles\*



\*Jay Patel

# Lessons from Exome Sequencing to Date

- Easy to generate data – much more difficult to accurately analyze it
- Recurrence/testing large number of samples will be key
- Functional studies will take months to years to find true “drivers” which cause MPN versus “passengers” along for the ride
- Many mutations may not be specific to MPN, but might be seen in MPN, MDS, AML
- We hope to find lesions with clinical significance
  - Novel therapeutic targets
  - Lesions which predict outcome to ensure we aggressively treat patients with poor prognosis and leave good prognosis patients alone
  - Lesions which occur at transformation to AML->prevent or treat leukemic transformation

# Are there cooperating somatic mutations?

- If most MPN patients are JAK2 positive, why do some people develop PV, or ET, or PMF?
- Perhaps it is the presence of a second mutation, which occurs in concert with JAK2, which determines the specific MPN?

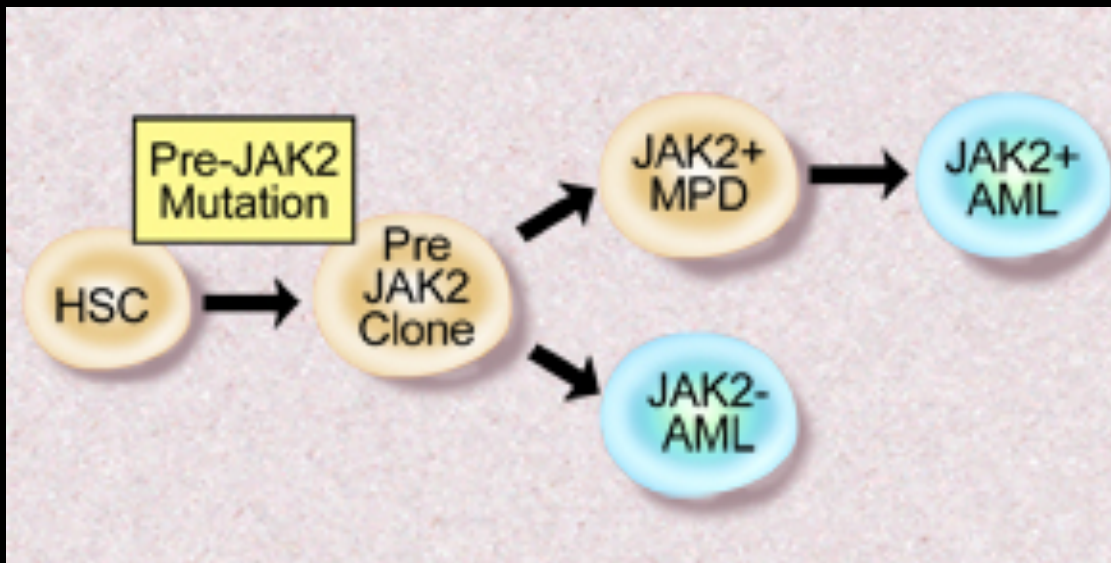


# Cooperating Mutations in MPN Patients

- Recent studies have identified somatic disease alleles which occur in concert with JAK2/MPL mutations
  - TET2 loss of function mutations in 10% of MPN patients
  - ASXL1 mutations in 8-10% of MPN patients
  - IDH1/2 mutations in 3-5% of MPN patients
  - EZH2 mutations in 10-15% of patients
- Same mutations are seen in MDS and AML patients->they do not explain the PV/ET/MF concondrum
- In some cases (TET2, IDH1/2) these mutations occur most commonly at progression to AML
- Limited functional data suggest these mutations affect the epigenetic state of MPN cells->affect the way DNA is packaged and which parts of it are used in MPN cells

# Leukemic Transformation of MPN

- Patients with PV, ET, and PMF are at high risk for transformation to AML  
-associated with a dismal prognosis
- Genetic/Epigenetic events which contribute to leukemic transformation are not known
- Approximately 50% of JAK2+ MPN patients transform to a JAK2-negative MPN\*



\*Campbell et al. Blood 2006  
Theocarides et al. Blood 2007

# Mutational Studies in post-MPN AML

	Gene	Mutational Frequency*
	JAK2	39.6% (21/53)
	TET2	28.3% (15/53)
→	SRSF2	18.9% (10/53)
	ASXL1	17.0% (9/53)
	IDH1/2	13.2% (7/53)
	TP53	11.3% (6/53)
	MPL	7.5% (4/53)
→	U2AF1	5.7% (3/53)
→	SF3B1	3.8% (2/53)
	K-Ras	3.8% (2/53)
→	RUNX1	3.8% (2/53)
→	N-Ras	3.8% (2/53)
→	DNMT3a	1.9% (1/53)
	PTEN	1.9% (1/53)
	PHF6	1.9% (1/53)
→	FLT3	1.9% (1/53)

**No mutations  
found in *c-KIT*,  
*EZH2*, or *WT-1***

→ Spliceosome mutations

→ Denotes frequently  
mutated in *de novo* AML

# TET2 Mutations, but not ASXL1 Mutations are Acquired at Leukemic Transformation

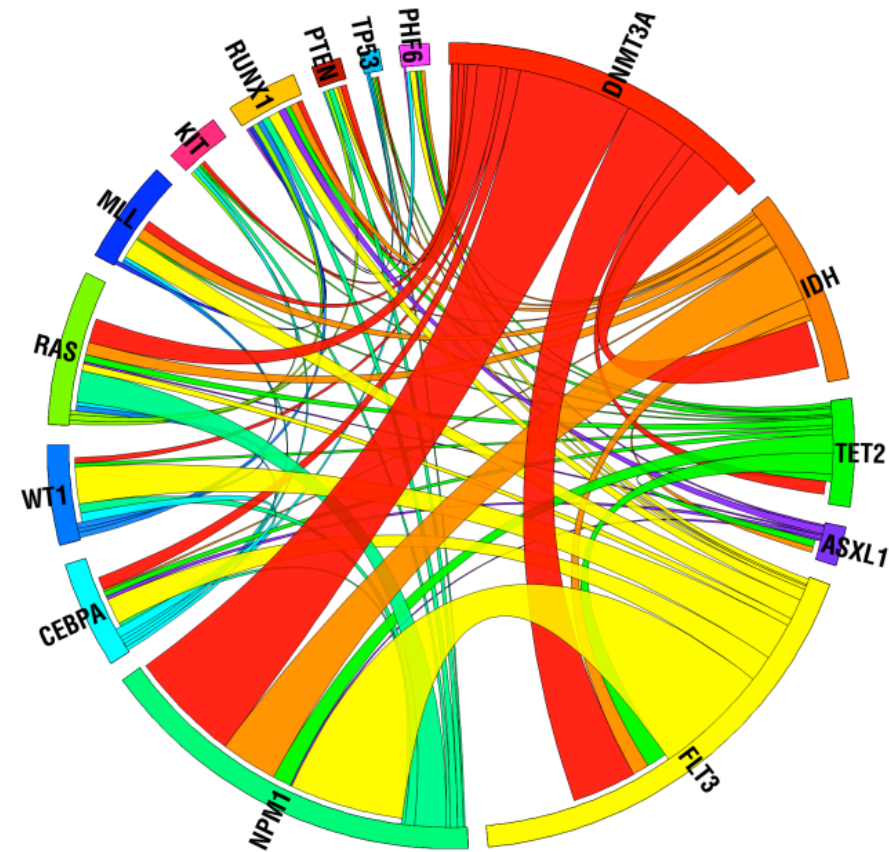
Sample	MPD	Genotype during MPN			Genotype during AML		
		JAK2	TET2	ASXL1	JAK2	TET2	ASXL1
1	MF	V617F	WT	Frameshift	V617F	R2000K	Frameshift
2	MF	V617F	WT	WT	WT	WT	WT
3	MF	V617F	WT	Q976X	WT	WT	Q976X
4	MF	V617F	WT	WT	V617F	E1234G	WT
5	MF	V617F	WT	WT	V617F	WT	WT
6	MF	WT	WT	WT	WT	WT	WT
7	MF	WT	WT	WT	WT	WT	WT
8	PV	V617F	WT	WT	V617F	N1641I	WT
9	PV	V617F	WT	C687X	V617F	WT	C687X
10	PV	V617F	WT	WT	V617F	WT	WT
11	PV	V617F	WT	WT	V617F	R1358C	WT
12	ET	WT	WT	WT	WT	G1256R	R404X
13	PV	V617F	WT	WT	WT	E1222G	WT
14	MF	V617F	WT	WT	WT	WT	WT
Frequency of mutation		78.6%	0%	21.4%	50%	42.9%	28.6%

ASXL1 25-Base  
pair deletion

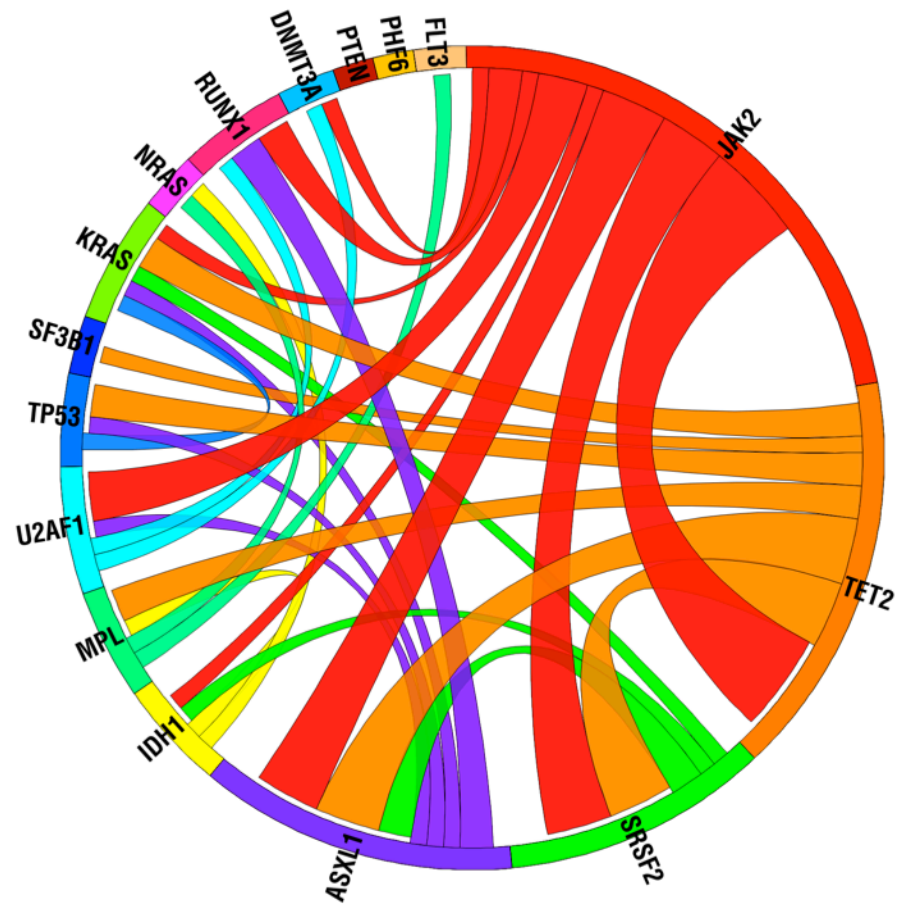
ASXL1 25-Base  
pair deletion

De Novo AML and AML after MPN are different diseases:  
Need different therapeutic approaches!

### De novo AML (ECOG 1900)



### Secondary AML from MPNs



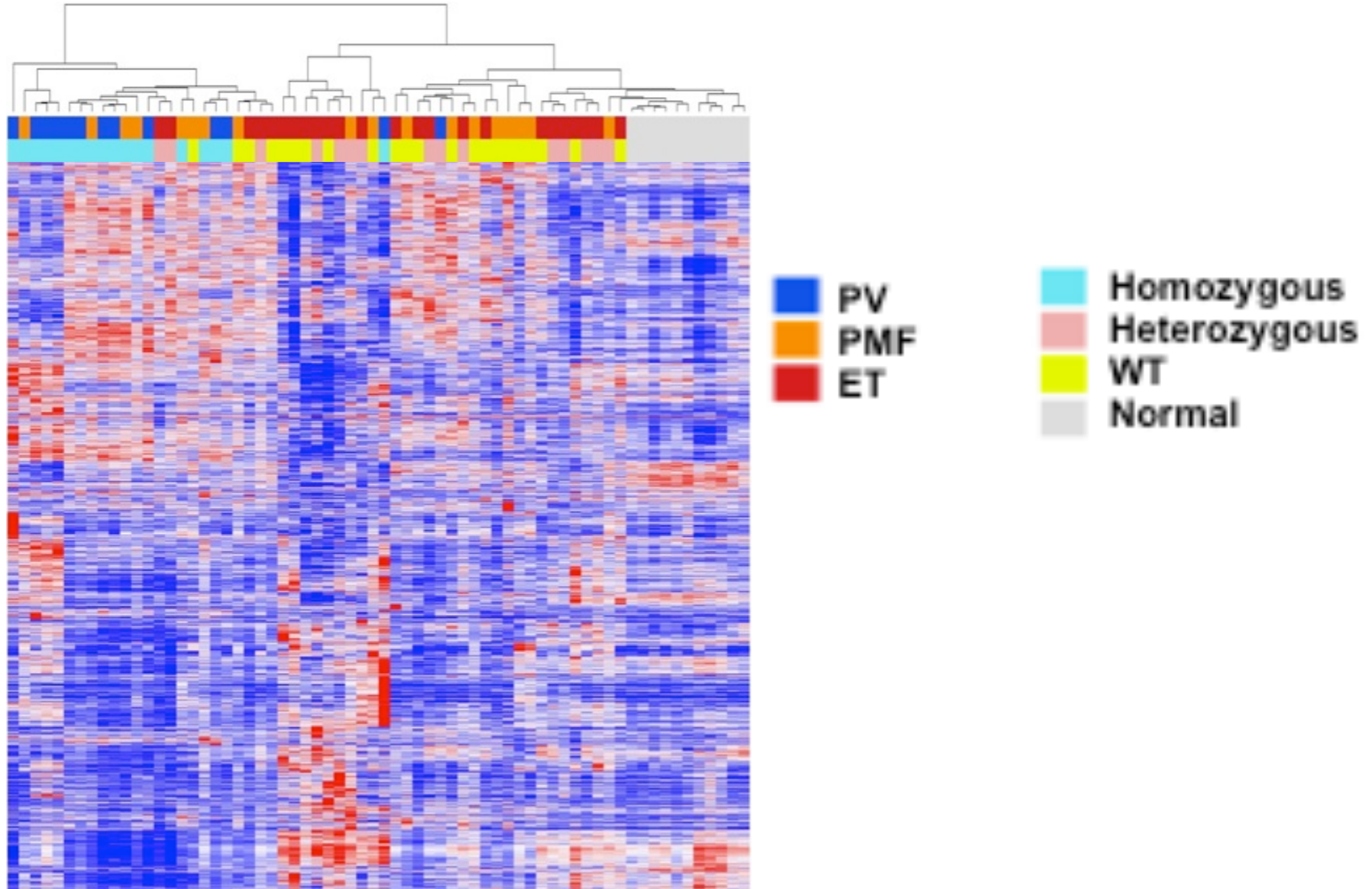
# What about gene expression – can we measure gene expression and learn something about pathogenesis of MPN\*

- Determine if there is a common genetic signature associated with MPN or with *JAK2V617F* mutations
- Identify genes which segregate with clinical phenotype
- Identify candidate genes in JAK2/MPL-negative MPN

\*Ben Ebert/Todd Golub

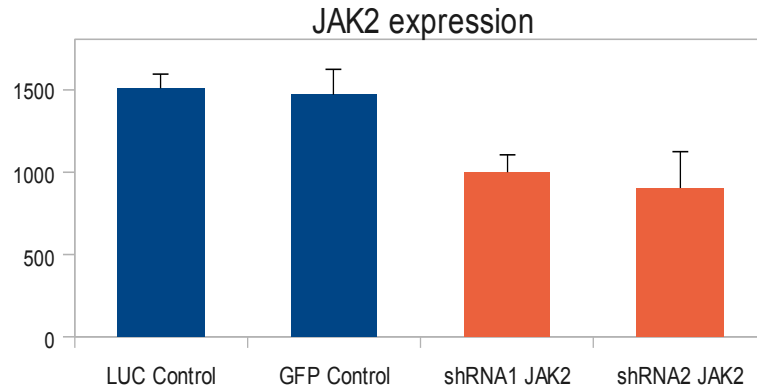


Gene Expression Profiling of MPN Samples clearly distinguishes MPN Patients from Normal Blood Cells, but does not distinguish based on disease or JAK2 status

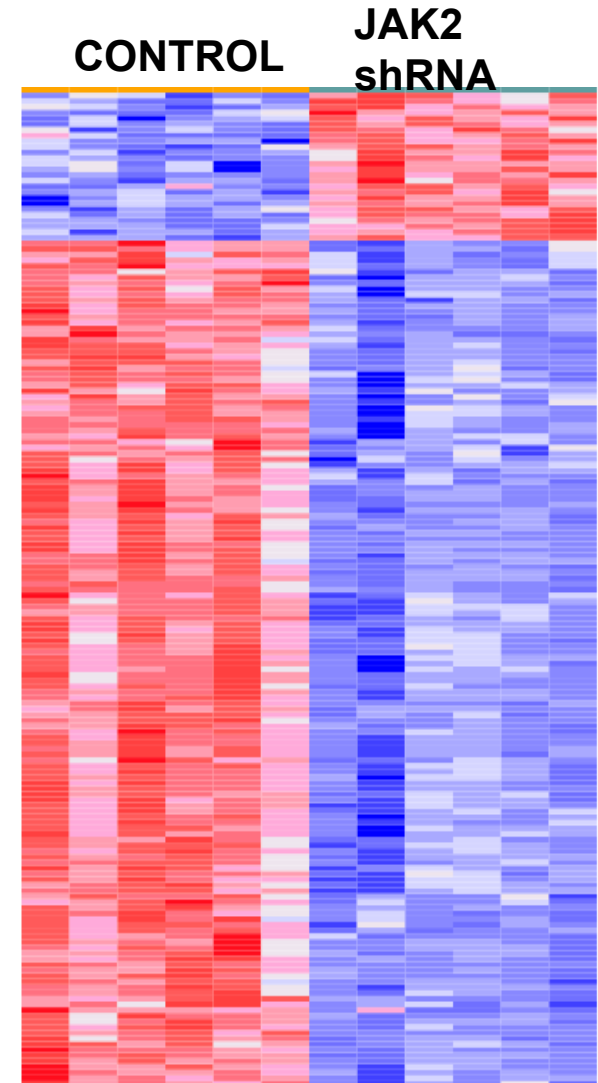


# Is there a JAK2 signature in heterozygous/WT MPN patients?

JAK2 shRNA in HEL cells to generate JAK2 signature

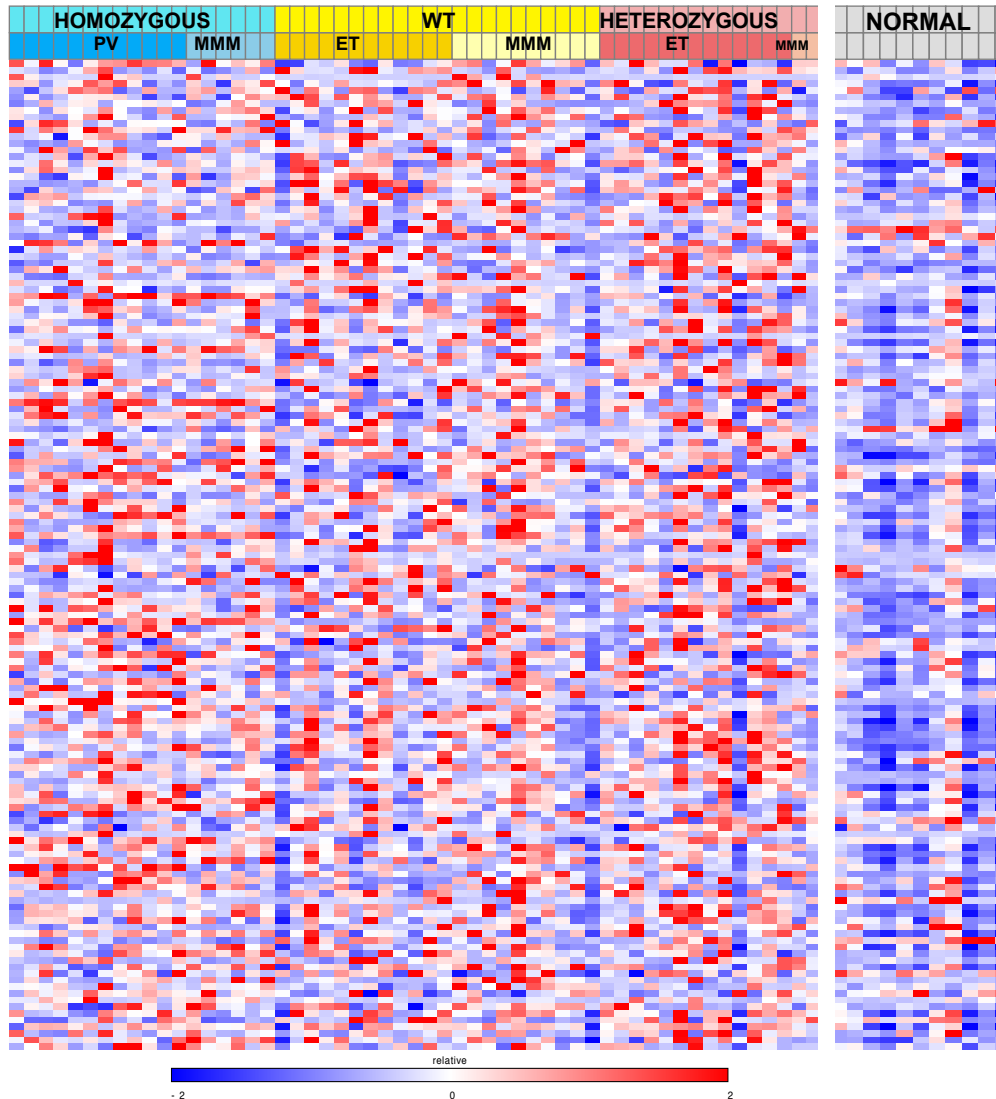


Similar data with JAK inhibitor





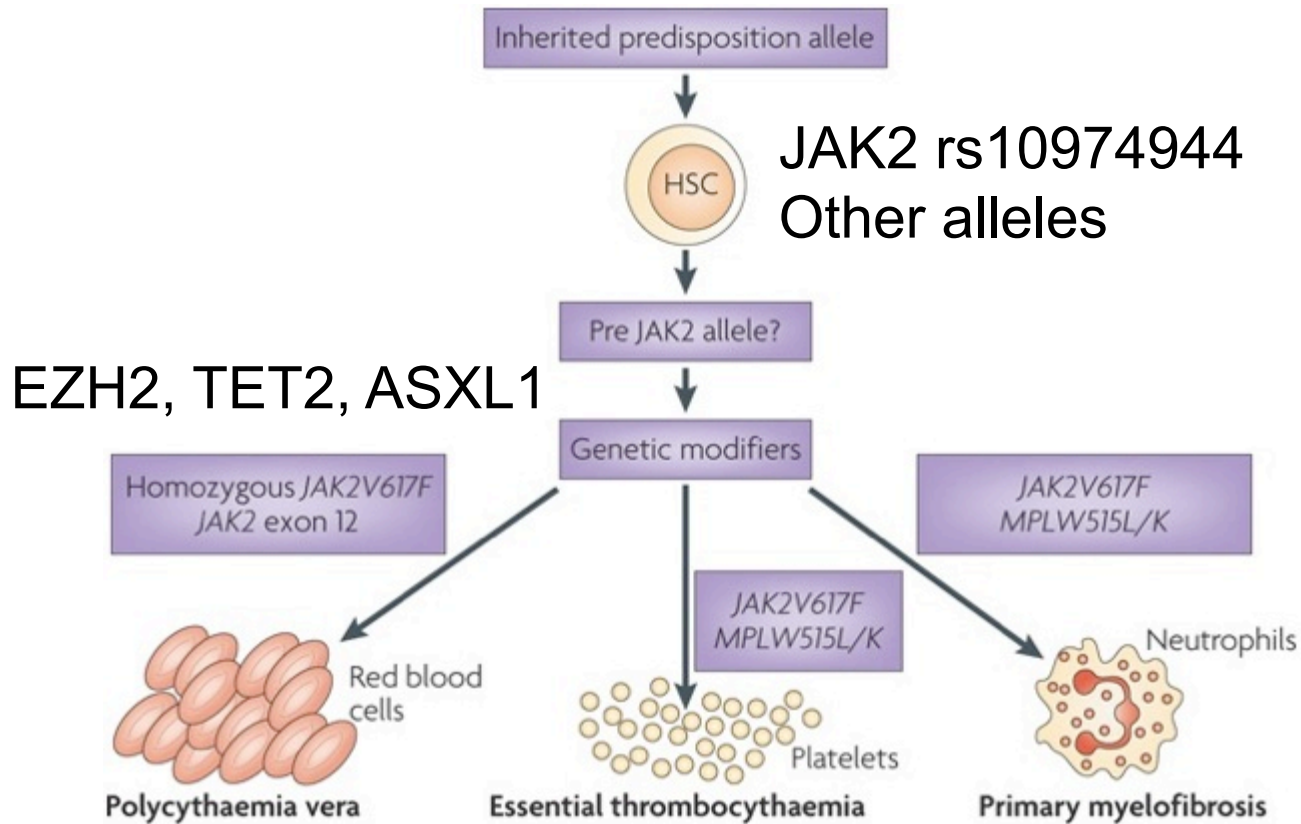
# JAK2 shRNA signature in MPN and Normal samples



Seen in all  
MPN patients,  
not in normals

Suggests JAK2  
is activated in  
all MPN  
patients  
regardless of  
specific  
mutation

# Model of MPN Pathogenesis



- Mutations which activate JAK2 are the most common lesion->best therapeutic target
- Possible other mutations affect response to JAK inhibitors

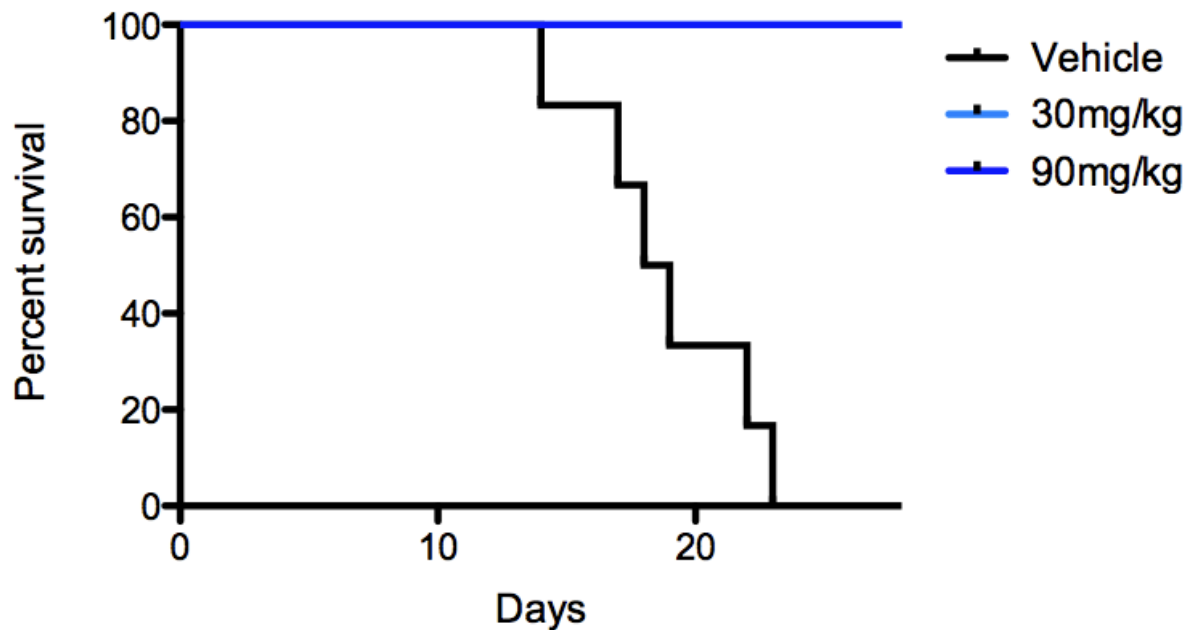


# Preclinical/Clinical Development of JAK inhibitors

- Agents in Current Clinical Development:
  - INCB18424: Potent dual JAK1/JAK2 inhibitor (approved)
  - TG101348/SAR302503: Most JAK2 selective, has FLT3 inhibitory activity (phase I completed)
  - CYT387: JAK1/JAK2 inhibitor (phase I completed)
  - SB1518: fairly JAK2 selective (phase I completed)
  - AZD1480: JAK2/JAK1 inhibitory activity (phase I)
  - LY2784544: phase I trial
  - Others in earlier phase development
- XL019: discontinued due to neurotoxicity->not clear if this is a JAK dependent effect or due to off-target effects
- Differences between these drugs may have a lot to do with pharmacokinetics and half-life, not just targets

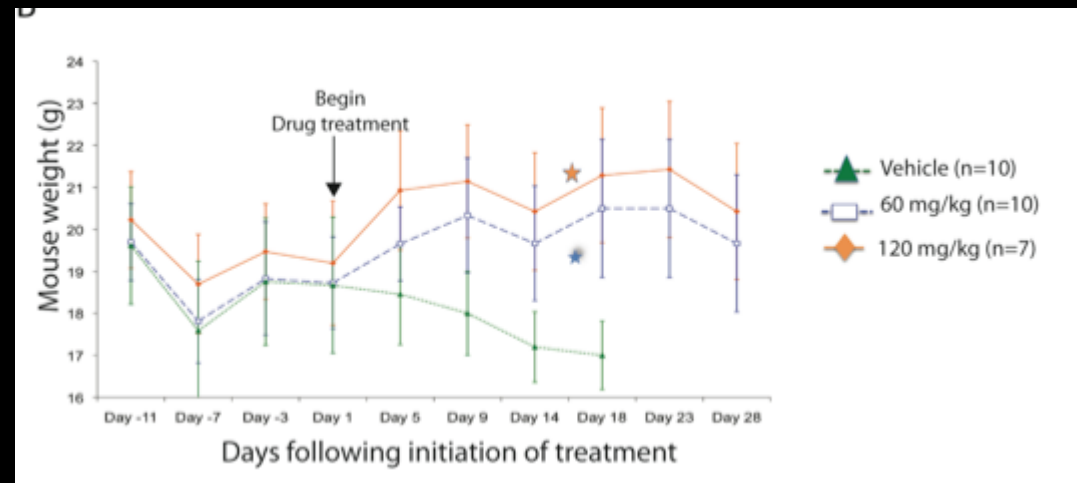
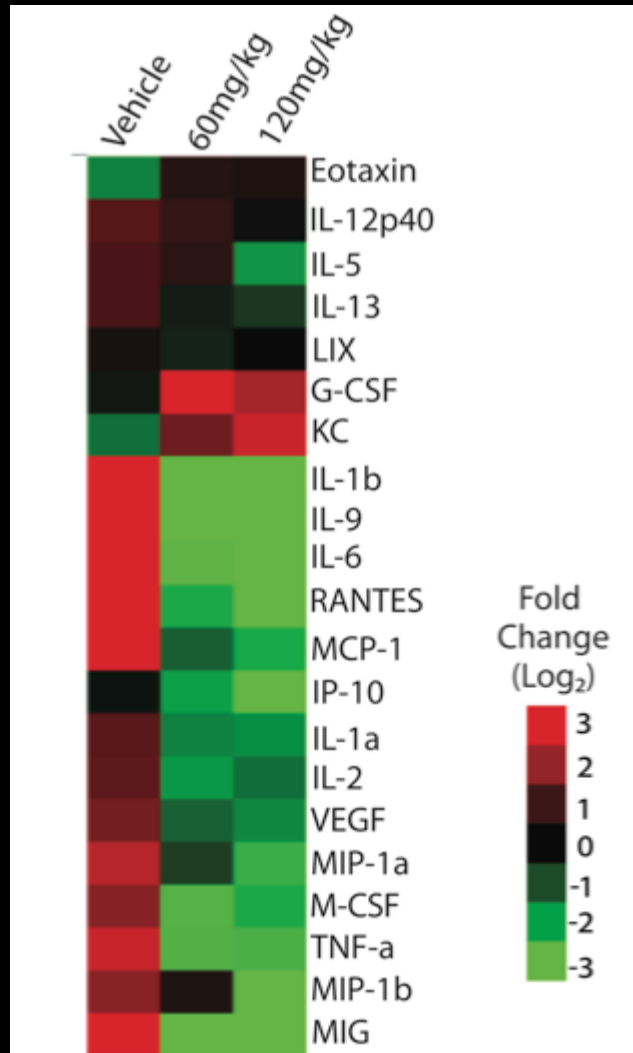
# INCB18424 treatment improves outcome in MPLW515L-mutant PMF mice\*

Survival Curve for INC18424



- Improved splenomegaly, thrombocytosis, leukocytosis, and myelofibrosis
- No reduction in mutant population in stem/progenitor or in differentiated cells

# JAK Inhibitor Treatment Decreases Circulating Cytokine Levels and Improves Body Weight



# How effective are JAK inhibitors for treatment of MPN?

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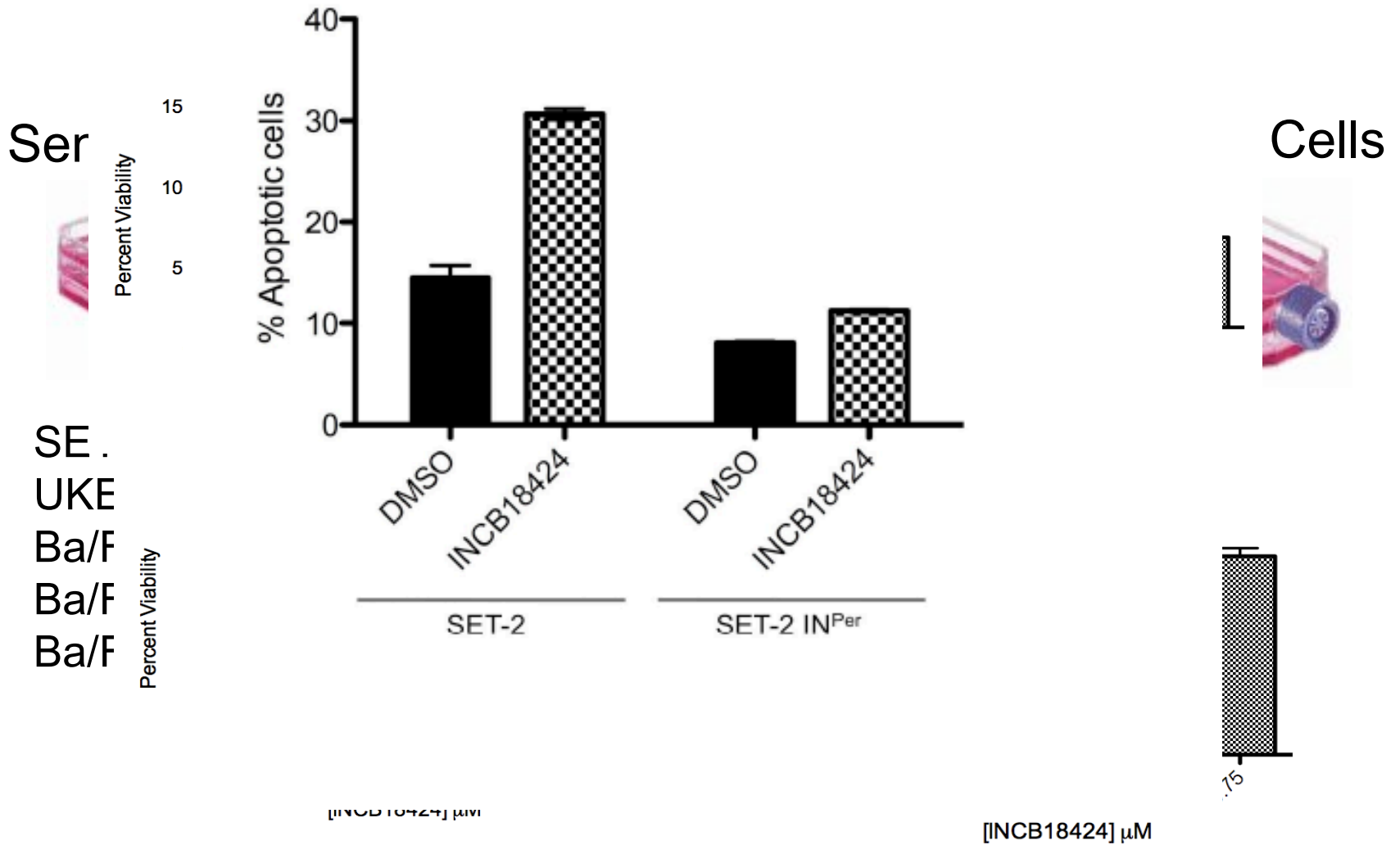
MPN mutant clone persists in the presence of chronic JAK2 kinase inhibition

- ▶ Phase II/III clinical trials with INCB18424 and other JAK inhibitors
  - ▶ Improvement in splenomegaly, constitutional symptoms, reduced progression to leukemia
  - ▶ No decrease in allele burden in the majority of MPN patients
- ▶ May be due, at least in part to presence of other (disease-initiating) alleles
- ▶ JAK2-driven preclinical models argue other factors contribute
  - ▶ JAK2V617F knockin model: Disease initiating cells are resistant to JAK2 inhibitor treatment (*Mullally et al. 2010*)
  - ▶ MPLW515L bone marrow transplant model: No decrease in allele burden
- ▶ We have not identified second-site mutations in patients treated with INCB18424

The lack of an initial response argues for inherent insensitivity to JAK inhibitors: **persistence**

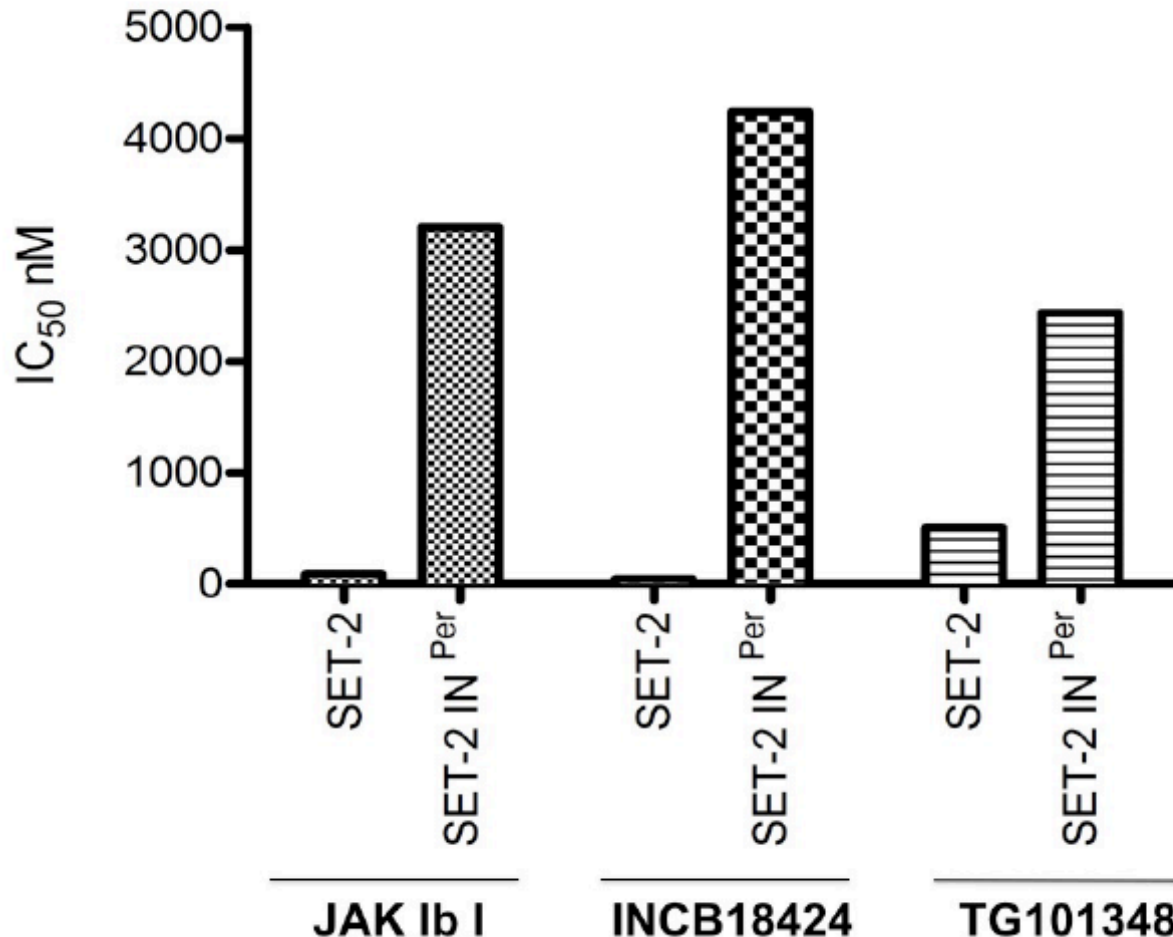


# Generation of JAK Kinase Inhibitor Persistent Cell Lines



In each case can generate persistent cells in absence of second-site resistance mutations

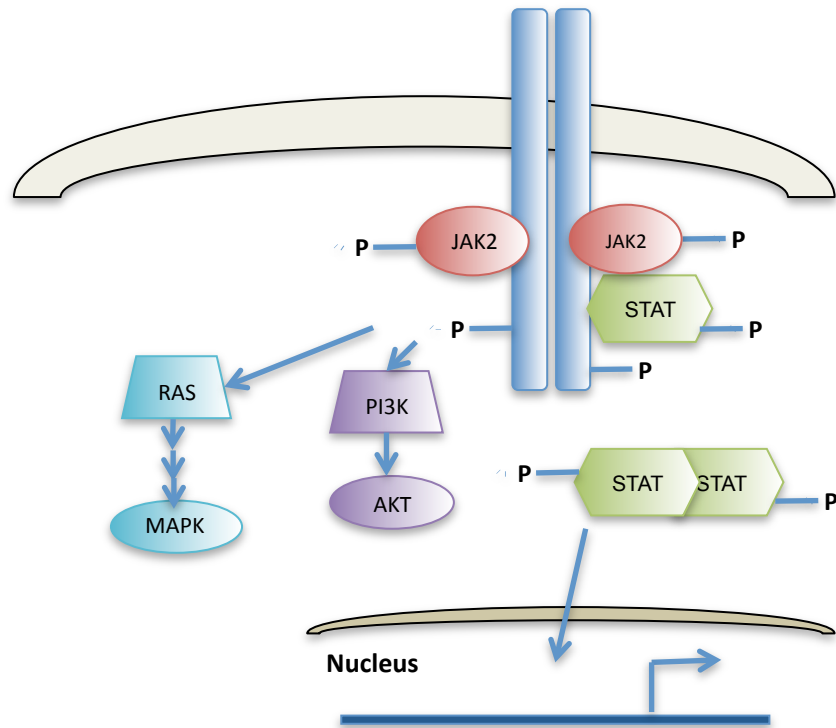
# JAK Inhibitor Persistent Cells are insensitive to different JAK kinase inhibitors



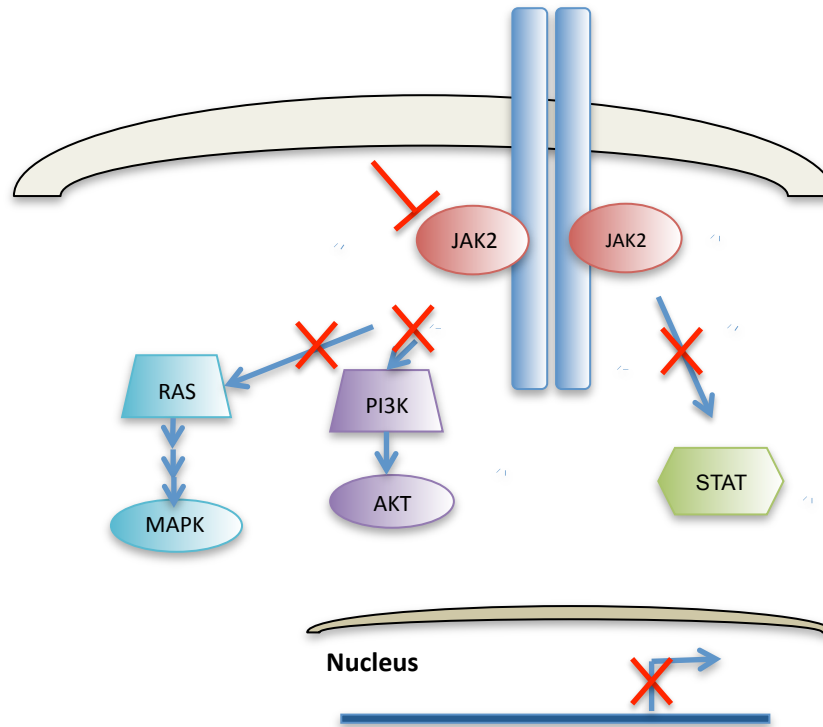
INCB18424  
persistent cells  
do not respond  
to TG101348 or  
JAK Inhibitor I



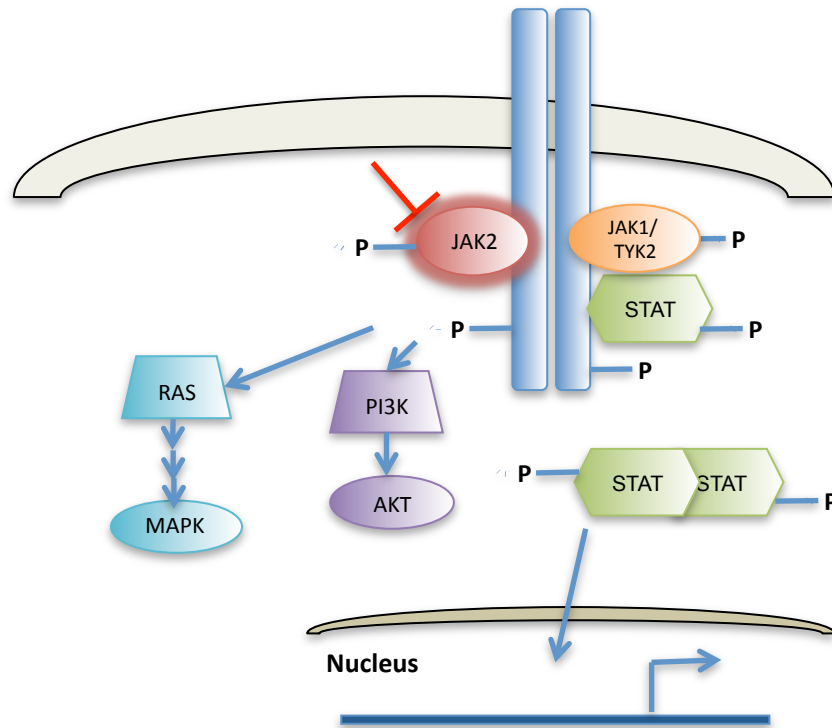
# JAK-STAT signaling in naïve MPN cells



# JAK inhibitors block homodimeric JAK2 activation and downstream signaling in naïve cells

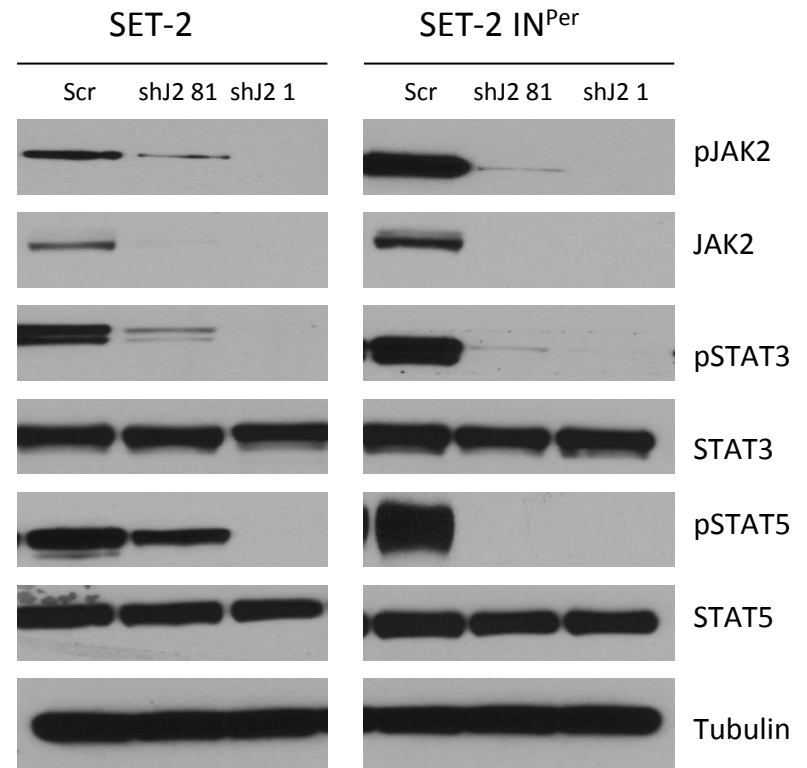
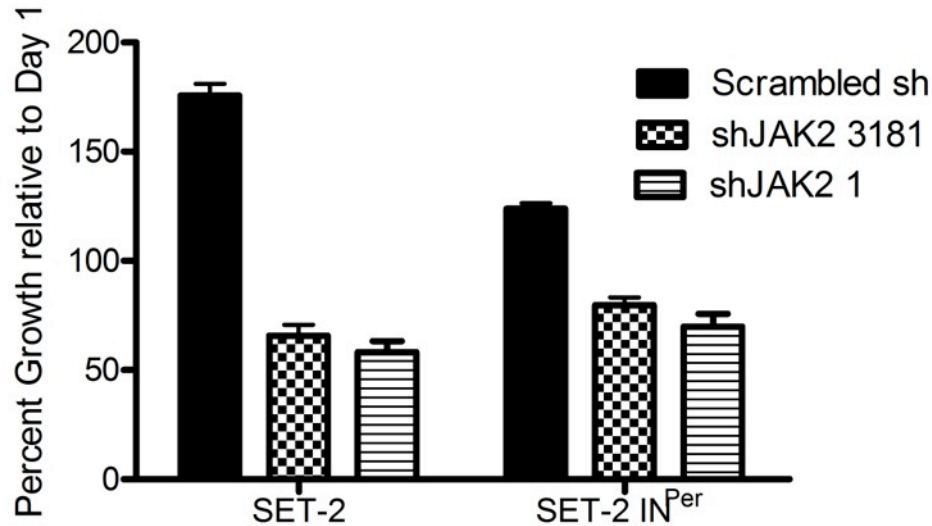


# JAK inhibitors cannot inhibit heterodimeric JAK2 activation and downstream signaling in persistent cells



- Do persistent cells remain JAK2 dependent?
- Can persistence be targeted with agents which degrade JAK2 or block JAK2 transactivation?

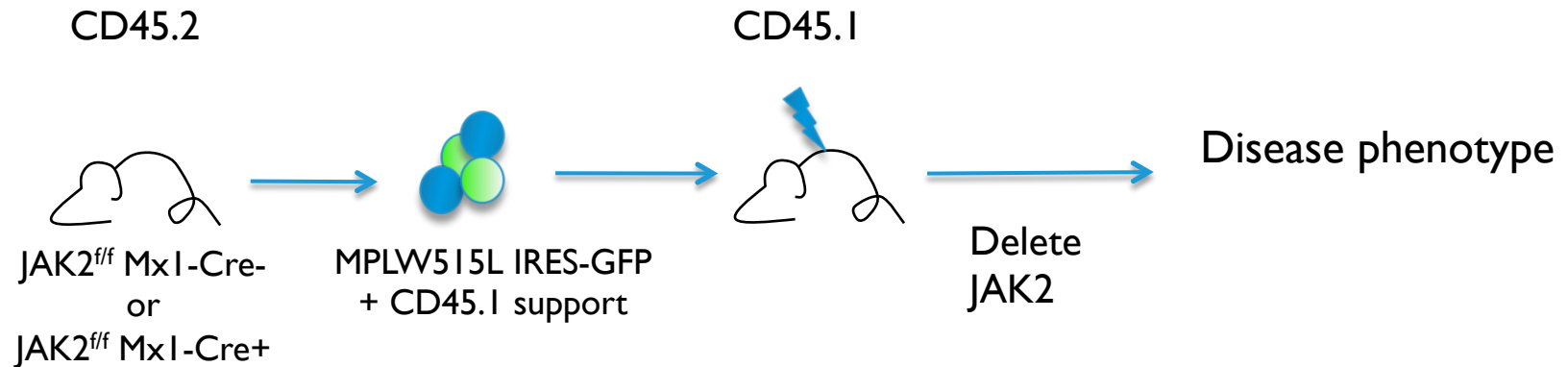
# Persistent Cells are JAK2 dependent



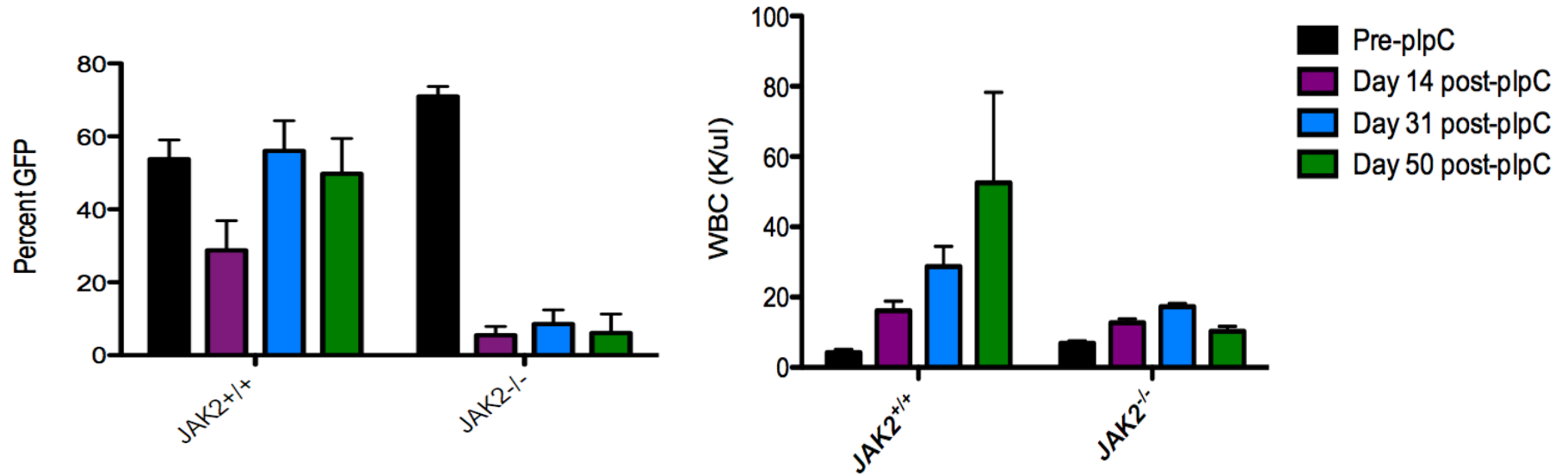
- Loss of JAK2 inhibits growth and signaling in persistent cells

# Are MPN Cells JAK2 Dependent? Testing the Genetic Requirement for JAK2 in PMF *in vivo*

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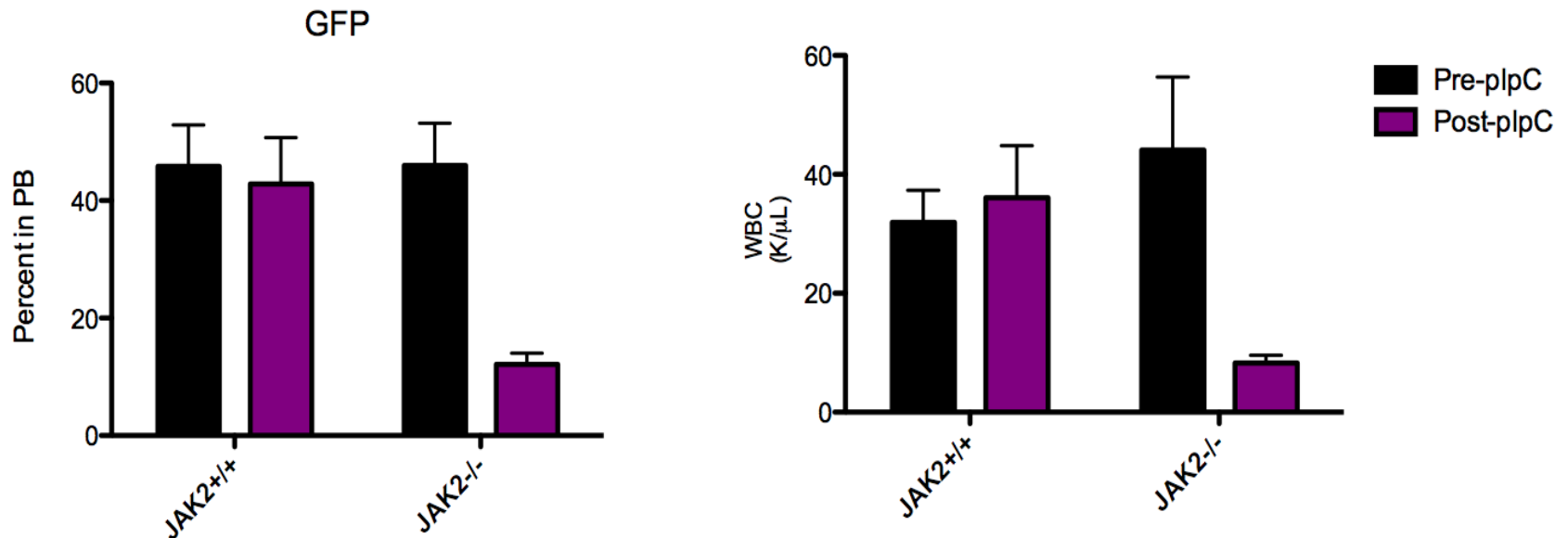


# JAK2 Deletion at Disease Initiation Reveals a JAK2 Requirement *in vivo* in PMF



# JAK2 Deletion After Disease Establishment Results in Potent Reduction in Disease Burden

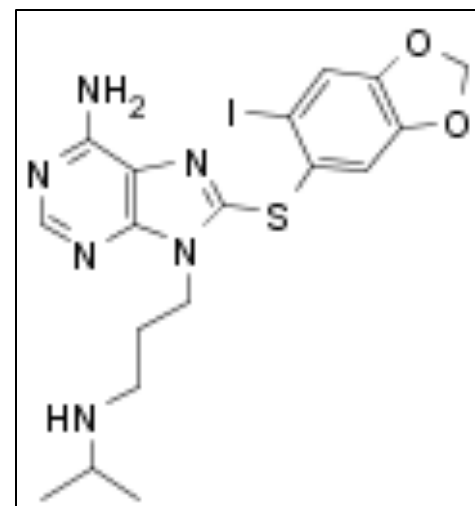
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- Dramatic reduction in GFP not seen with maximal JAK kinase inhibition in same model
- These studies suggest a requirement for JAK2 *in vivo* in PMF cells – can this be leveraged pharmacologically?

# Can we improve our ability to target JAK2\*

- It is presumed the hematopoietic toxicities are due to inhibition of JAK2 in normal cells->has this been clearly delineated in vivo?
- Can we develop better therapies which improve the therapeutic window and target the malignant cell?
  - additional therapies
  - alternate dosing strategies for JAK2 inhibitors
- Collaborated with Gabriela Chiosis and Jay Bradner to test ability of additional compounds to inhibit JAK2 dependent proliferation

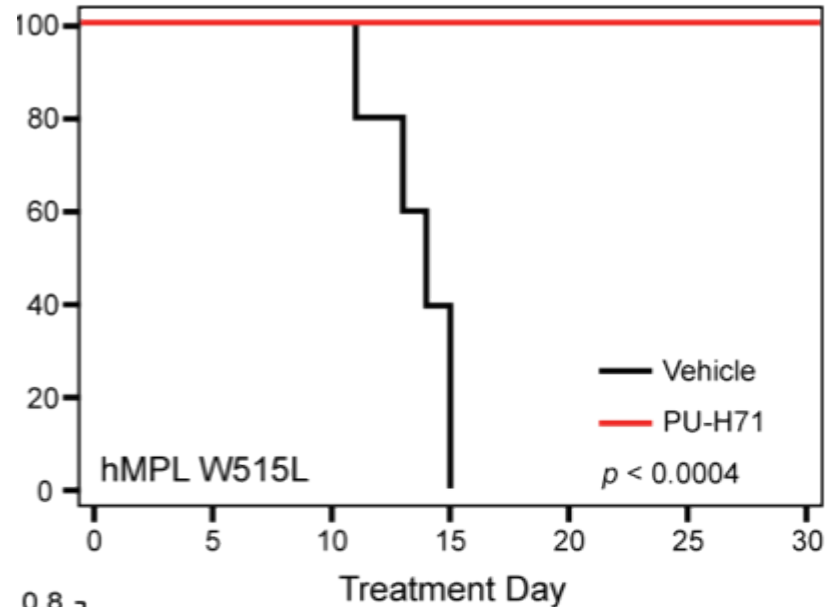


PU-H71

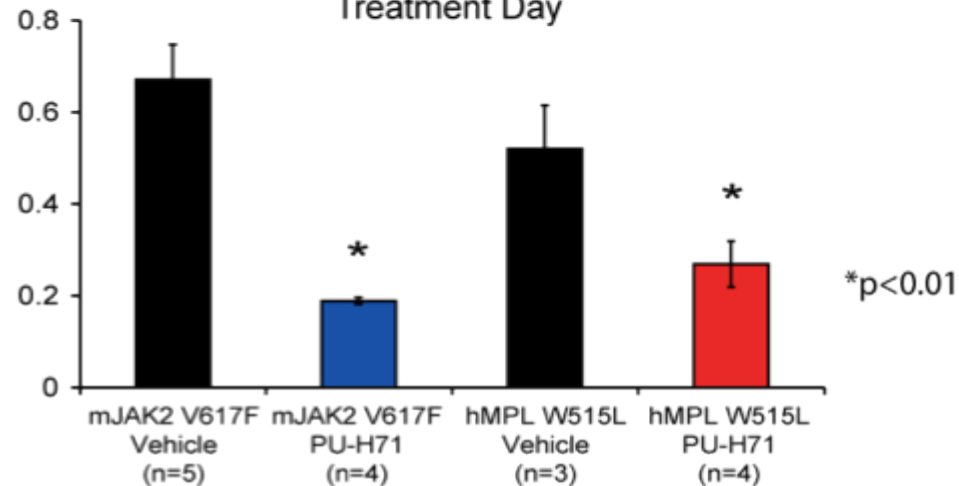


# PU-H71 demonstrates efficacy in vivo in JAK2V617F and MPLW515L transplant models

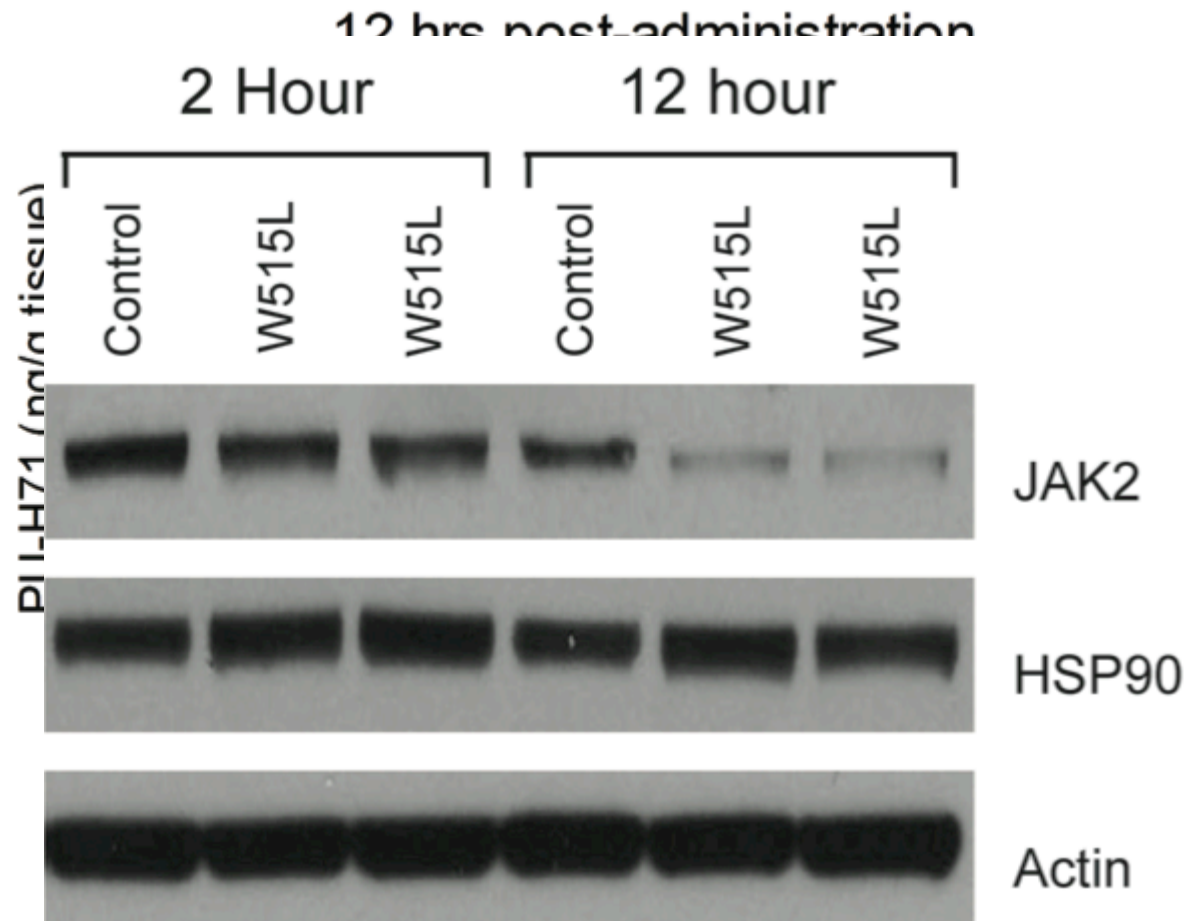
**Survival**



**Spleen Weight**

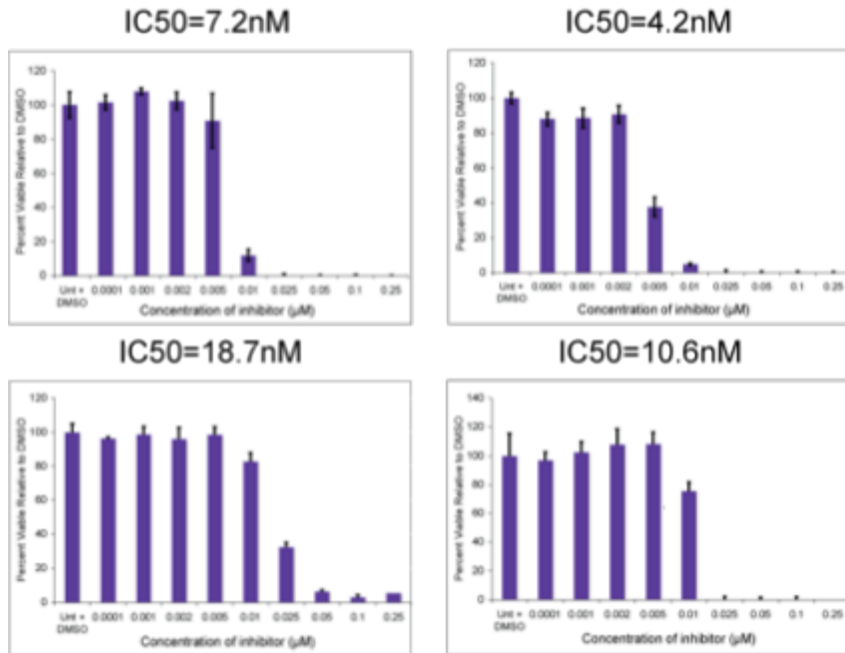


# PU-H71 Depletes JAK2 in leukemic, but not normal hematopoietic cells



**PK/PD studies show PU-H71 is selectively taken up and maintained in tumor, but not normal cells – basis for therapeutic index**

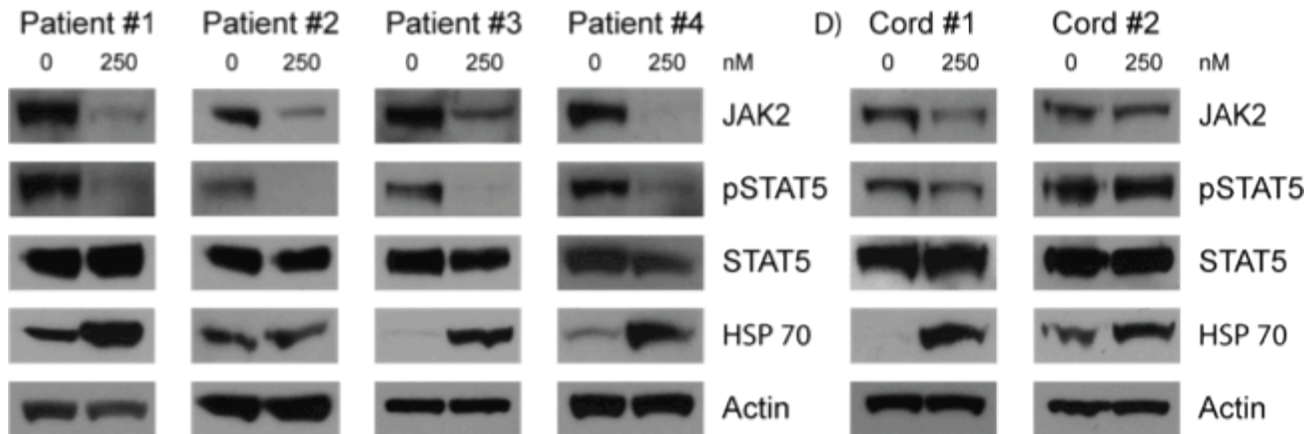
# PU-H71 Degrades JAK2/Inhibits JAK-STAT signaling in 1° MPN Samples



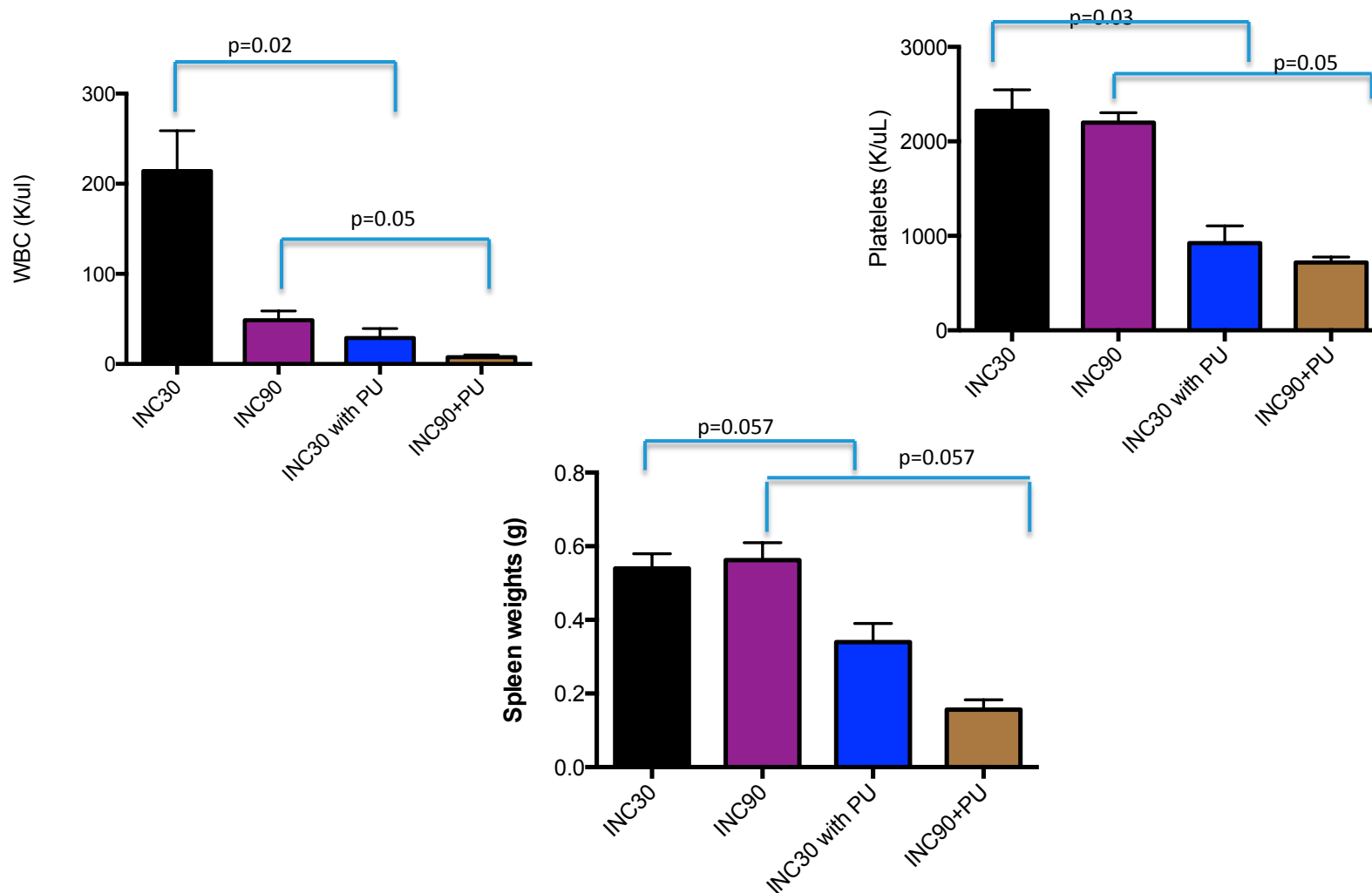
MPD Samples

Cord Blood Samples

Clinical studies of HSP90 inhibitors are underway (AUY922, PUH71 at MSKCC)

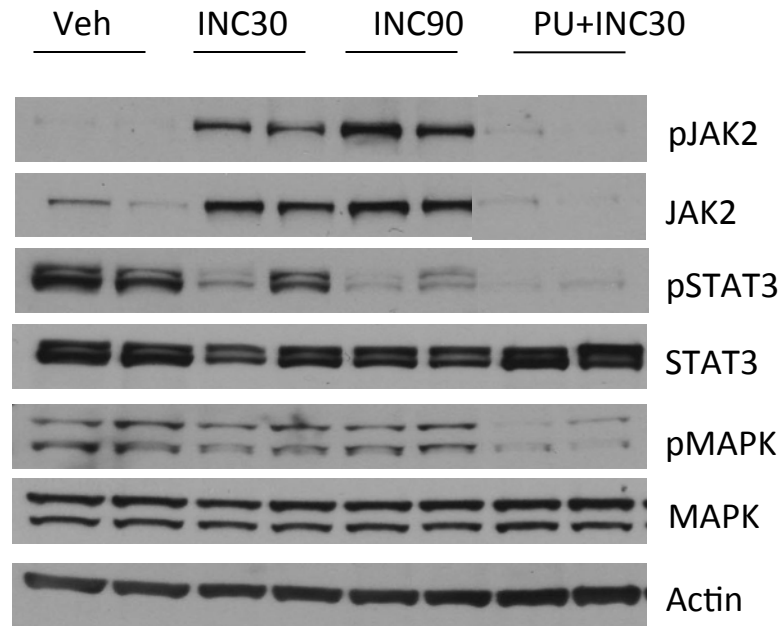


# Combination Treatment with JAK and HSP90 Inhibitor Shows Improved Efficacy Compared to Either Therapy Alone



# Combination Therapy with JAK/HSP90 Inhibition Hits the Target Better than Either Therapy Alone

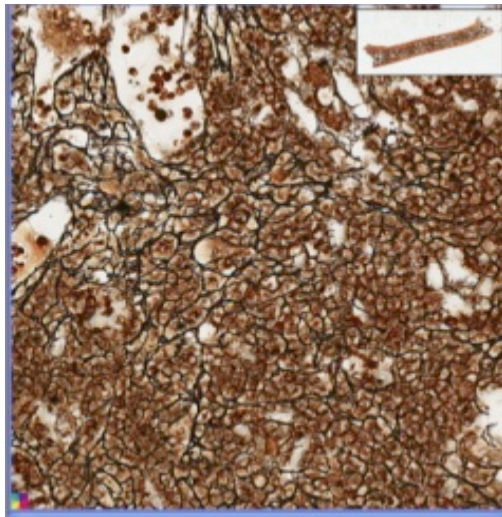
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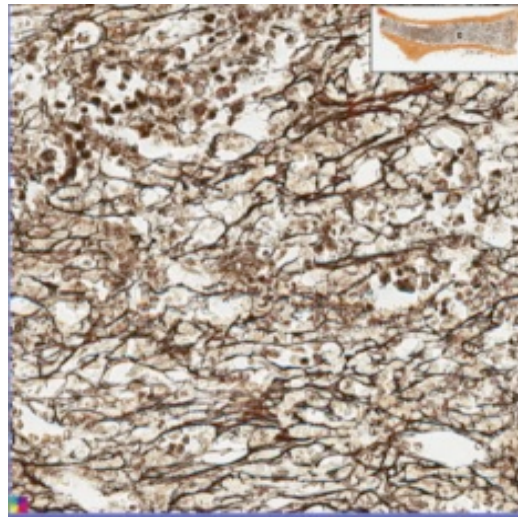
# Reduced Reticulin Fibrosis with Combination Therapy

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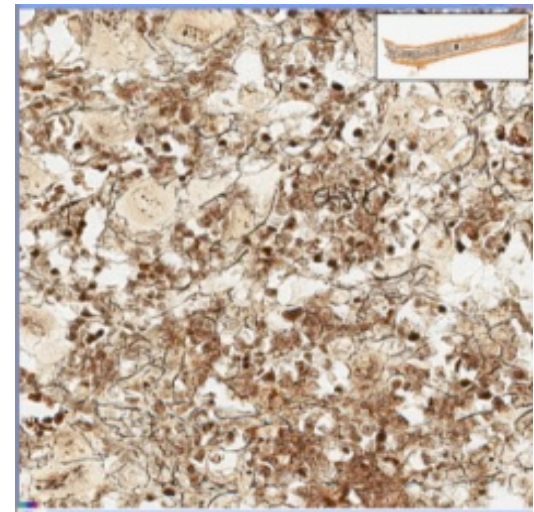
Vehicle



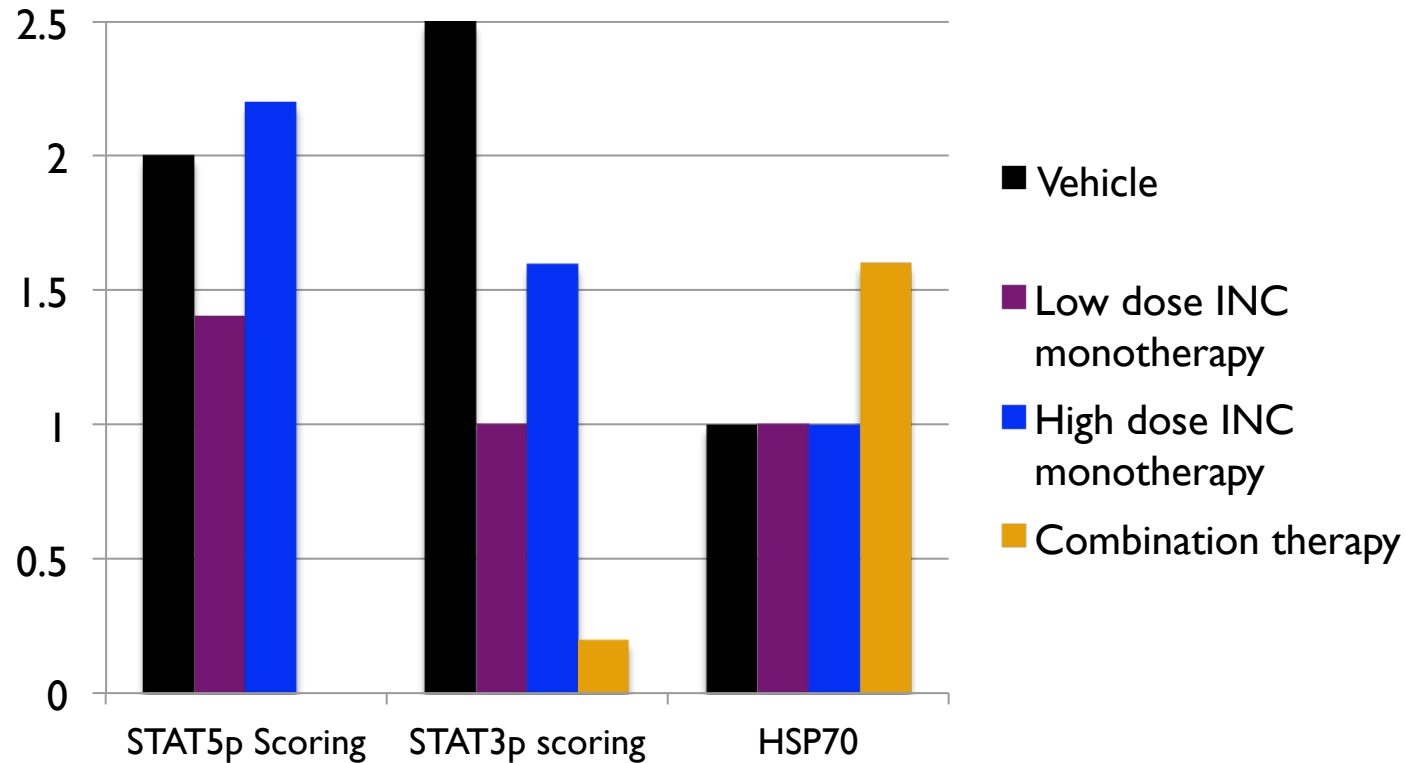
INC90



INC30 with PU



# IHC Improvement in Mice Treated With Combination Therapy



# Summary

- Mutations which activate JAK-STAT Signaling are seen in almost all MPN patients->but there are additional genetic lesions seen in MPN patients which contribute to MPN/MDS/AML stem cell survival
- Additional novel therapeutic approaches targeted at JAK2 and at other oncogenic signaling pathways might offer benefit alone or in conjunction with JAK2 inhibitors
- Genetic studies of myeloid malignancies will likely identify novel mutations with pathogenetic and therapeutic relevance



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  - Levine et al. Cancer Cell 2005
  - Levine et al. Blood 2005
  - Levine et al. Blood 2006
  - Pikman et al. Plos Medicine 2006
  - Scott et al. NEJM 2007
  - Kawamata et al. Experimental Hematol 2007
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