Updates in Myeloproliferative Neoplasms

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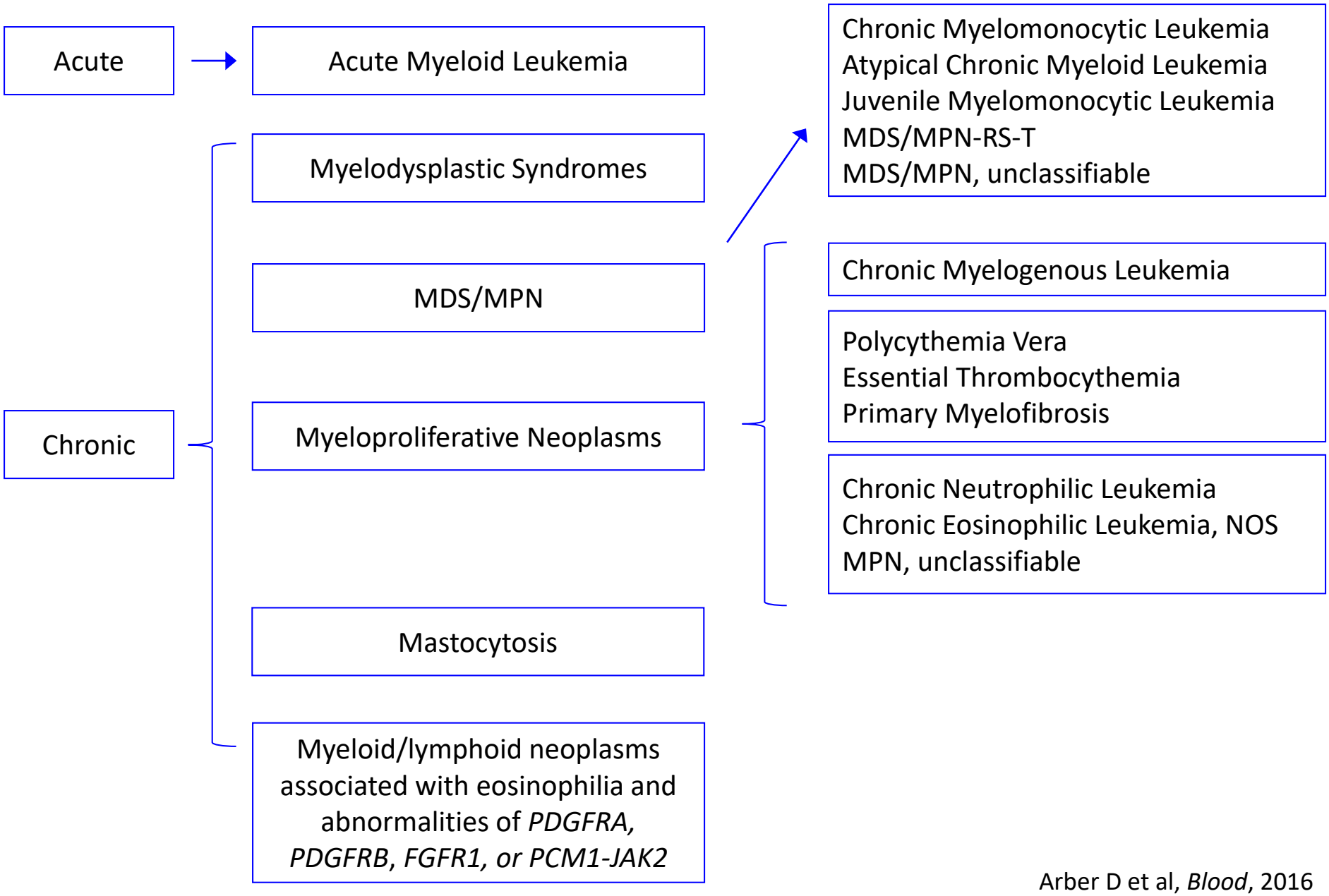
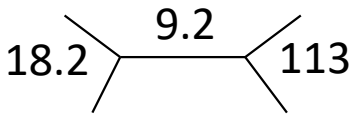
2016 WHO Classification Scheme for Myeloid Neoplasms



Arber D et al, *Blood*, 2016

Case Presentation

- A 72 year-old man complains of fatigue, occasional night sweats, early satiety, and poor appetite, with 10-15 pound weight loss over the past few months
- Splenomegaly ~14 cm below the left costal margin
- Leukocytosis with anemia and thrombocytopenia:



Seg 35, bands 20, metamyelocytes 10, myelocytes 8, promyelocytes 4, blasts 2, teardrop RBCs, nRBCs

- Bone marrow biopsy reveals a hypercellular marrow with megakaryocytic hyperplasia/atypia and 2+ fibrosis
- Cytogenetics normal, JAK2 negative, CALR type I (52 bp del) mutation positive
- NGS testing reveals a likely pathogenic ASXL1 mutation

What is this patient's expected survival?

A) 10+ years
B) ~6-7 years
C) ~2-3 years
D) < 1 yr

Case Presentation

- A 72 year-old man complains of fatigue, occasional night sweats, early satiety, and poor appetite, with 10-15 pound weight loss over the past few months
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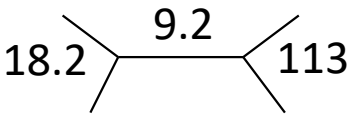


Seg 35, bands 20, metamyelocytes 10, myelocytes 8, promyelocytes 4, blasts 2, teardrop RBCs, nRBCs

- Bone marrow biopsy reveals a hypercellular marrow with megakaryocytic hyperplasia/atypia and 2+ fibrosis
- Cytogenetics normal
- JAK2 V617F negative
- *Is additional testing needed to confirm the diagnosis?*
- *Is there a role for additional genetic testing?*
- *What is this patient's overall prognosis? Risk of transformation to AML?*
- *Should treatment with ruxolitinib (JAK2 inhibitor) be considered?*
- *Are there alternative therapies that should be considered?*

2016 WHO Diagnostic Criteria for Primary Myelofibrosis

Table 7. WHO criteria for overt PMF

WHO overt PMF criteria
Major criteria
1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3*
2. Not meeting WHO criteria for ET, PV, *BCR-ABL1*$^{+}$ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of reactive myelofibrosis‡
Minor criteria
Presence of at least 1 of the following, confirmed in 2 consecutive determinations:
a. Anemia not attributed to a comorbid condition
b. Leukocytosis $\geq 11 \times 10^9$/L
c. Palpable splenomegaly
d. LDH increased to above upper normal limit of institutional reference range
e. Leukoerythroblastosis
Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

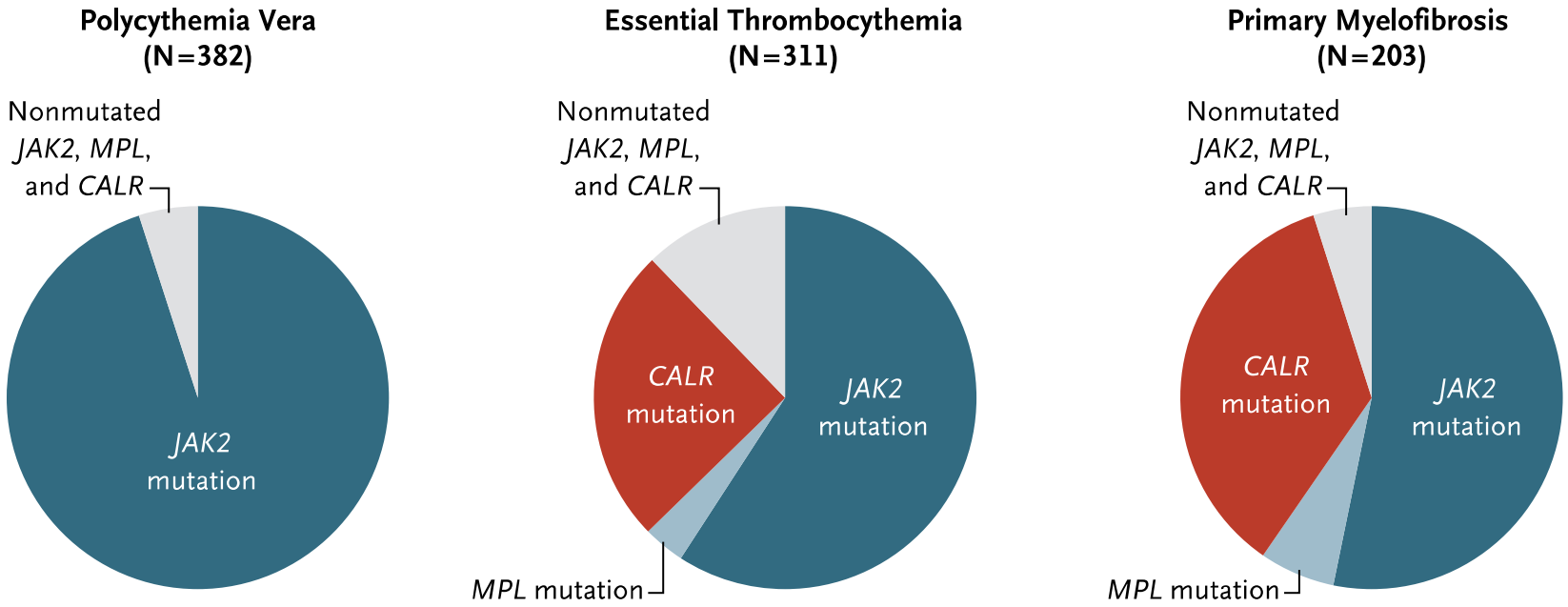
*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

‡BM fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Arber D et al, *Blood*, 2016

A **Distribution of *JAK2*, *MPL*, and *CALR* Mutations in Philadelphia Chromosome–Negative Myeloproliferative Neoplasms**



Klampfl et al, NEJM 2013

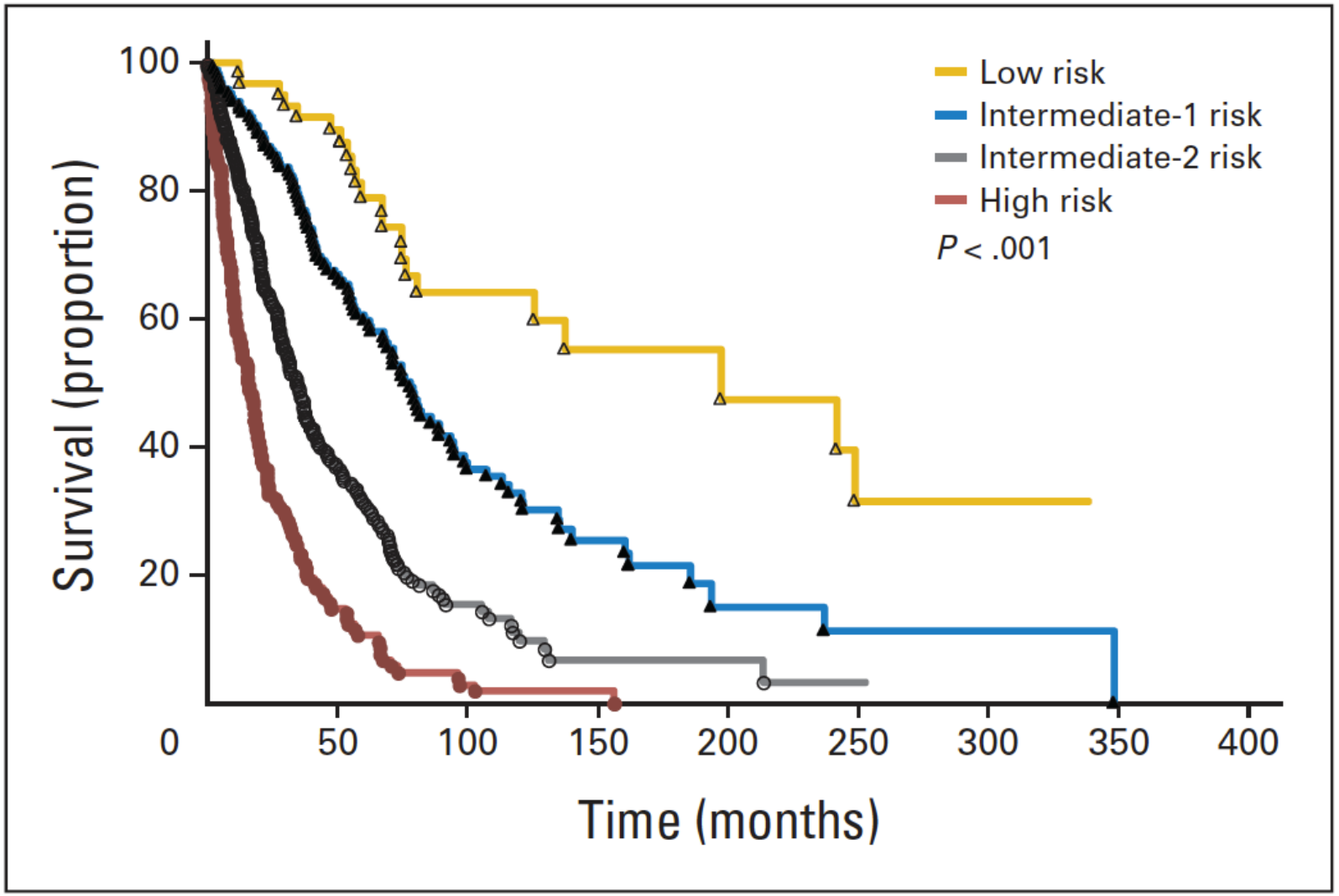
Primary Myelofibrosis: Prognosis

DIPSS:

- Age >65 years: 1 point
- Leukocyte count >25,000/microL: 1 point
- Hemoglobin <10 g/dL: 2 points
- Circulating blast cells ≥1 percent: 1 point
- Presence of constitutional symptoms: 1 point

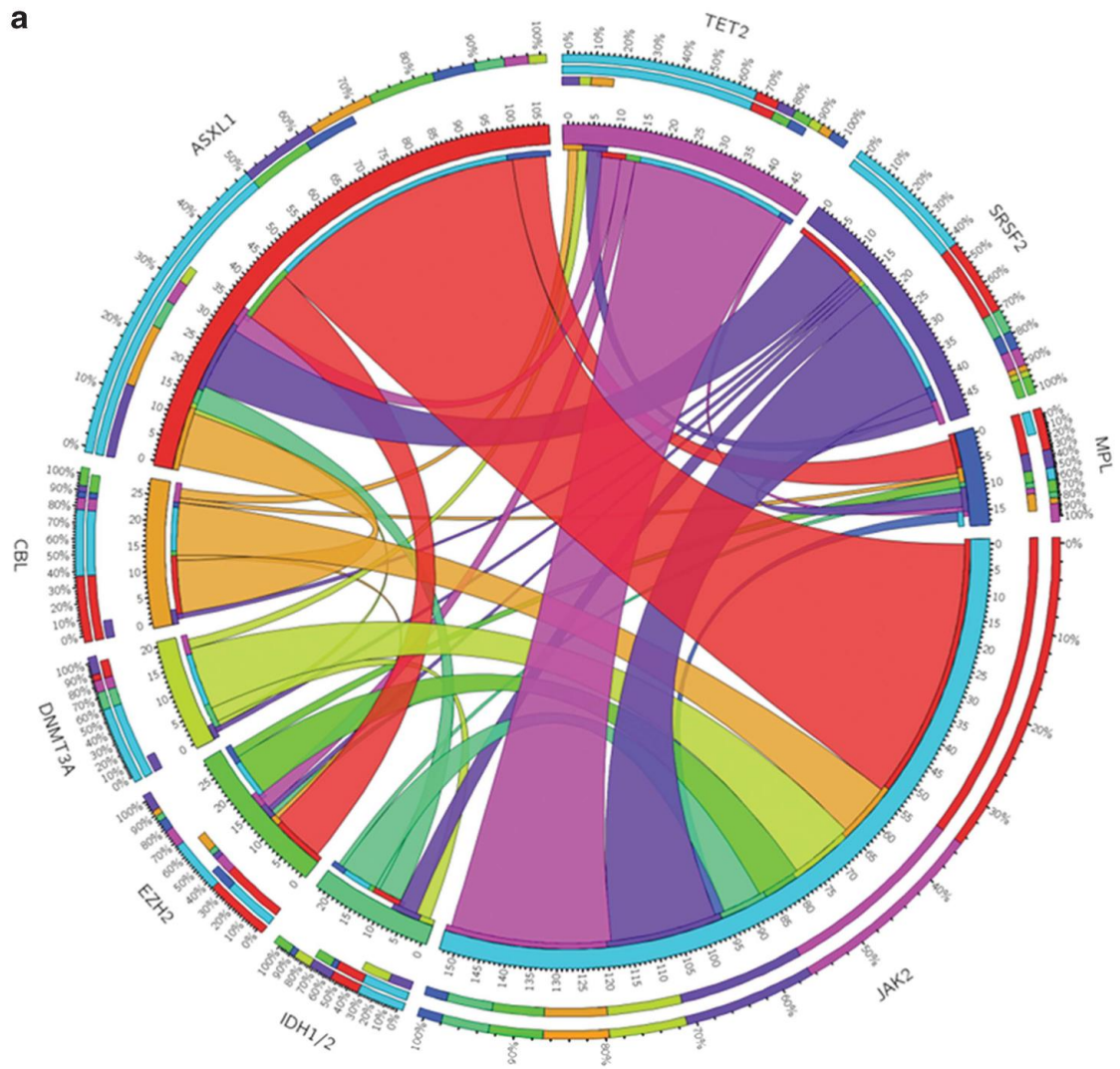
DIPSS category	Points	DIPSS-plus points	DIPSS-plus category
Low-risk	0	0	0
Intermediate-1	1-2	1	1
Intermediate-2	3-4	2	2-3
High-risk	5-6	3	4-6
Unfavorable karyotype		1	
Platelets < 100,000/microL		1	
RBC transfusion-dependence		1	

Primary Myelofibrosis: Prognosis (DIPSS-plus)



Tefferi et al, JCO 2011

Genetic complexity and prognosis in myelofibrosis

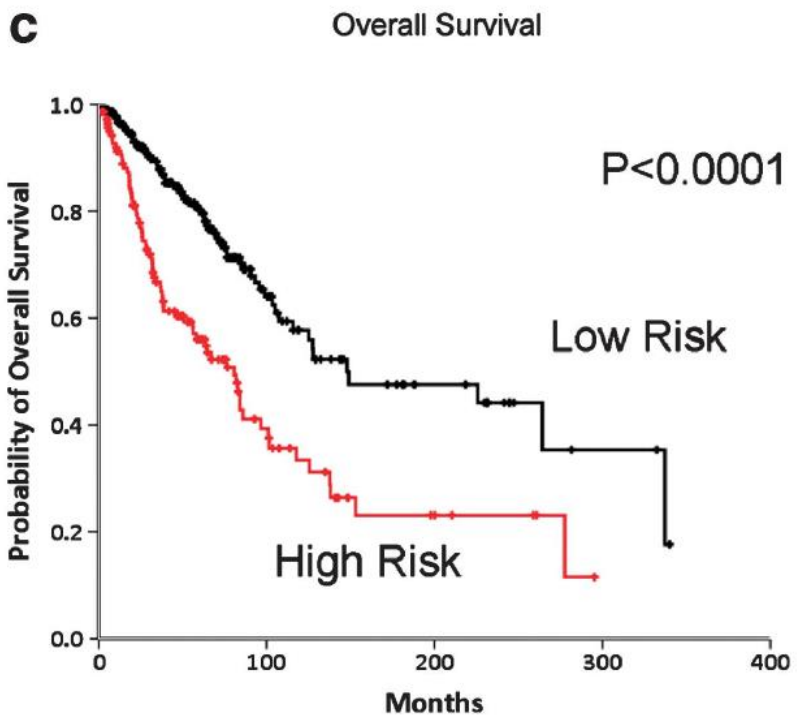


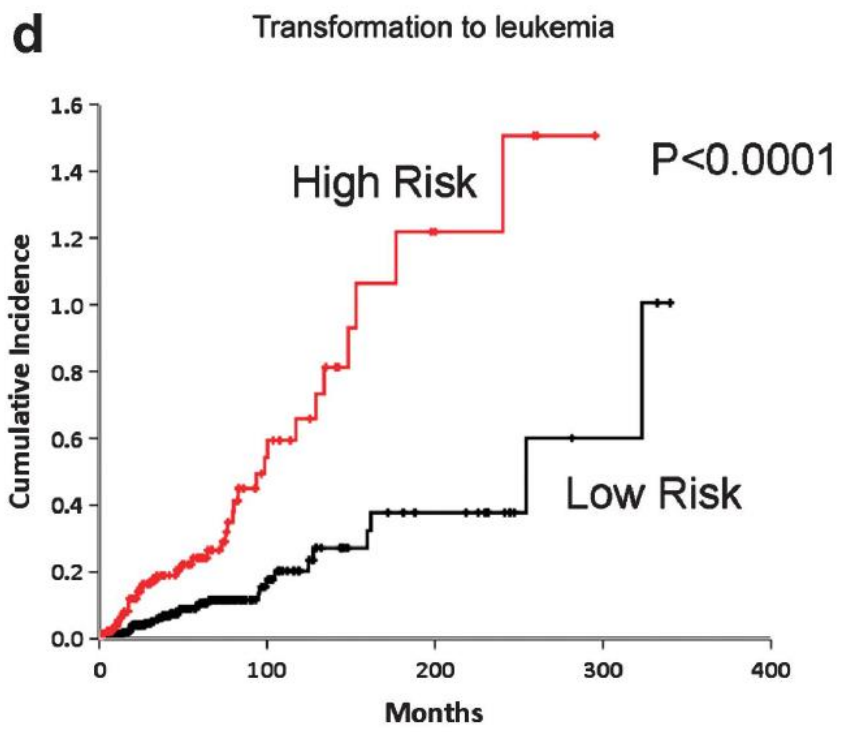
Gene	Overall Frequency (%)
JAK2	59.2
ASXL1	21.7
TET2	9.7
SRSF2	8.5
DNMT3A	5.7
MPL	5.2
EZH2	5.1
CBL	4.4
IDH1/2	2.6

Vannucchi et al, Leukemia 2013

Genetic complexity and prognosis in myelofibrosis

Mutationally high-risk patients*

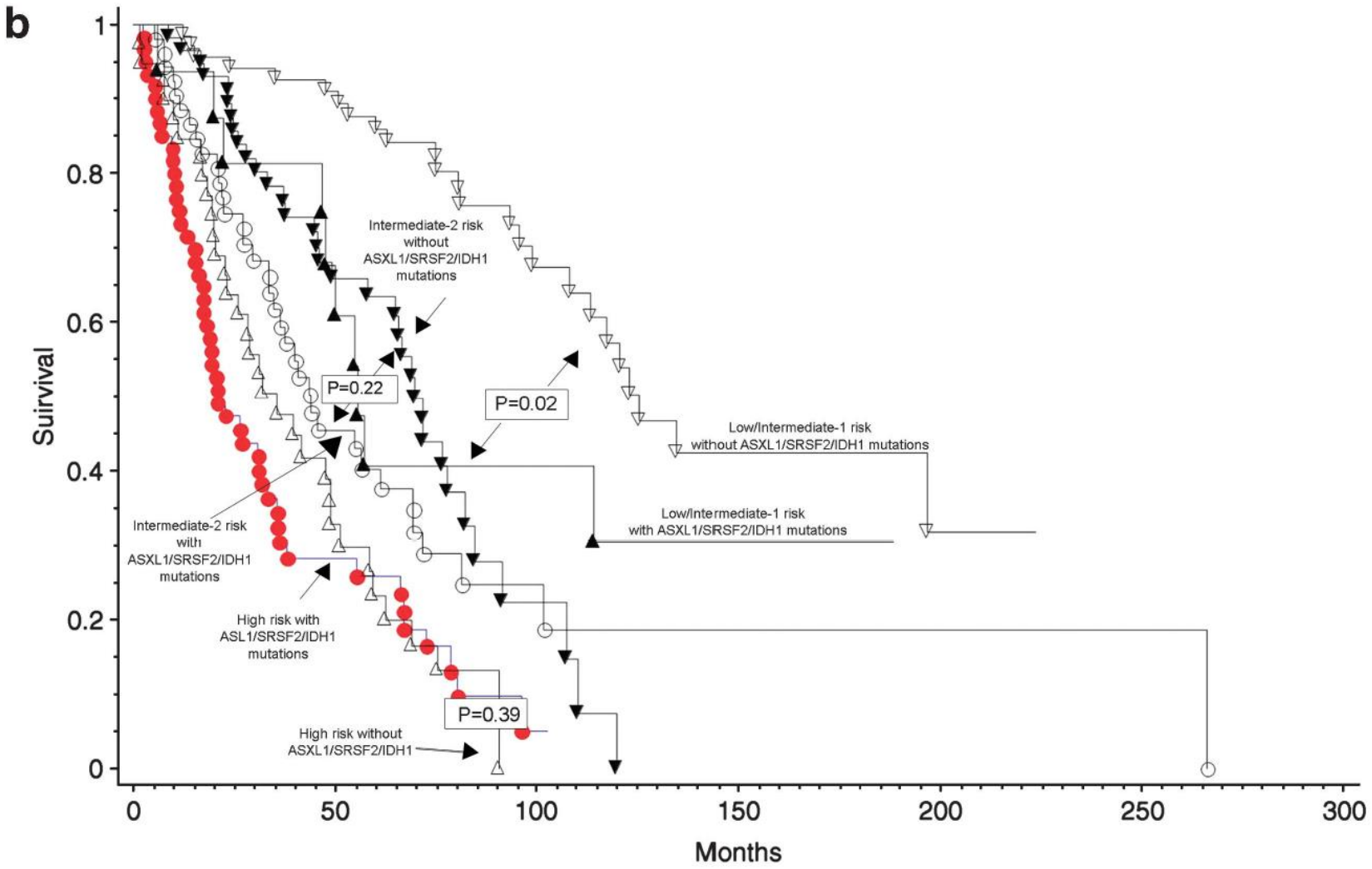




**Presence of mutation in EZH2, ASXL1, SRSF2, and/or IDH1/2*

Vannucchi et al, Leukemia 2013

Genetic complexity and prognosis in myelofibrosis

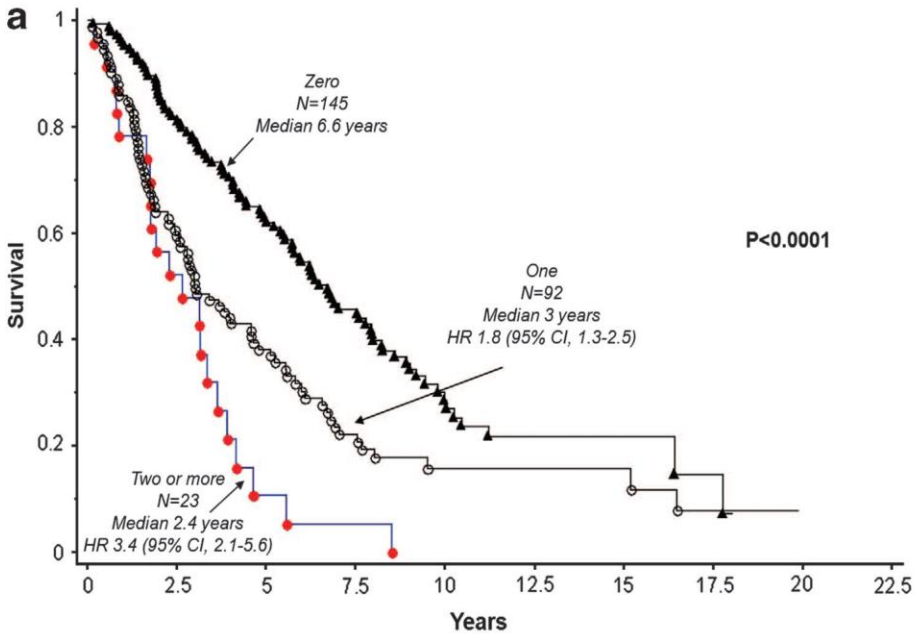


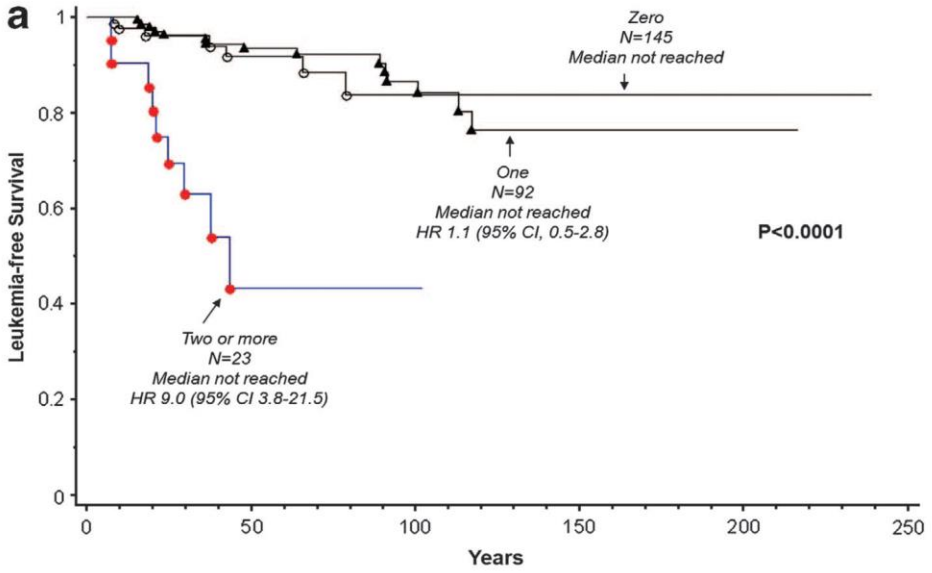
Vannucchi et al, Leukemia 2013

ORIGINAL ARTICLE

The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: an international study of 797 patients

P Guglielmelli[1,16], TL Lasho[2], G Rotunno[1], J Score[3], C Mannarelli[1], A Pancrazzi[1], F Biamonte[1], A Pardanani[2], K Zoi[4], A Reiter[5], A Duncombe[6], T Fanelli[1], D Pietra[7], E Rumi[7], C Finke[2], N Gangat[2], RP Ketterling[8], RA Knudson[8], CA Hanson[9], A Bosi[1], A Pereira[10], R Manfredini[11], F Cervantes[12], G Barosi[13], M Cazzola[14], NCP Cross[15], AM Vannucchi[1,16] and A Tefferi[2,16]





- *Presence of two or more mutations associated with worse LFS and OS (independent of DIPSS-plus)*

Guglielmelli et al, Leukemia 2014

JOURNAL OF CLINICAL ONCOLOGY

RAPID COMMUNICATION

MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis

Paola Guglielmelli, Terra L. Lasho, Giada Rotunno, Mythri Mudireddy, Carmela Mannarelli, Maura Nicolosi, Annalisa Pacilli, Animesh Pardanani, Elisa Rumi, Vittorio Rosti, Curtis A. Hanson, Francesco Mannelli, Rhett P. Ketterling, Naseema Gangat, Alessandro Rambaldi, Francesco Passamonti, Giovanni Barosi, Tiziano Barbui, Mario Cazzola, Alessandro M. Vannucchi, and Ayalew Tefferi

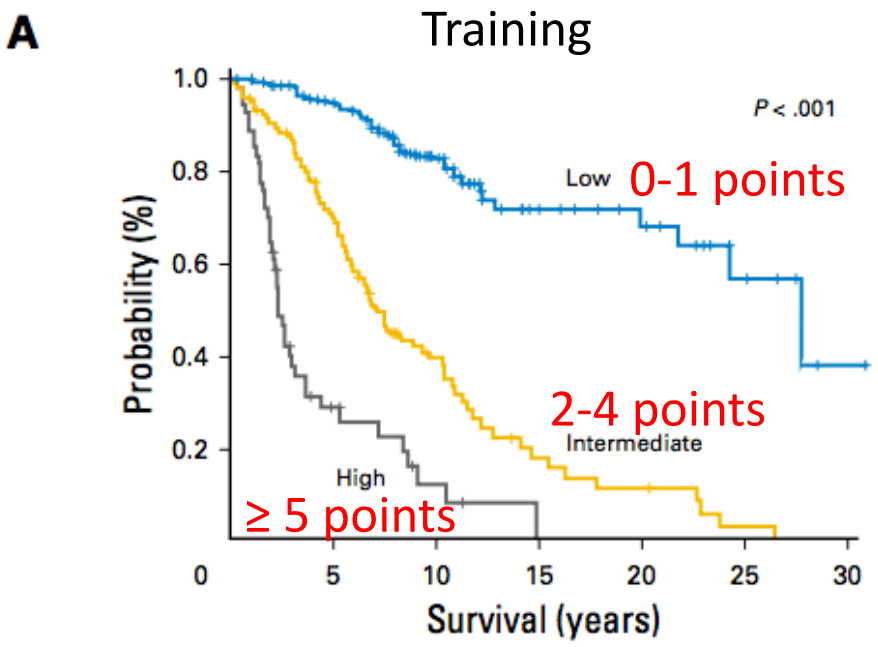
Published at jco.org on December 9, 2017.

Table 3. OS by MIPSS70 and MIPSS70-Plus Prognostic Scores in Training and Validation Cohorts

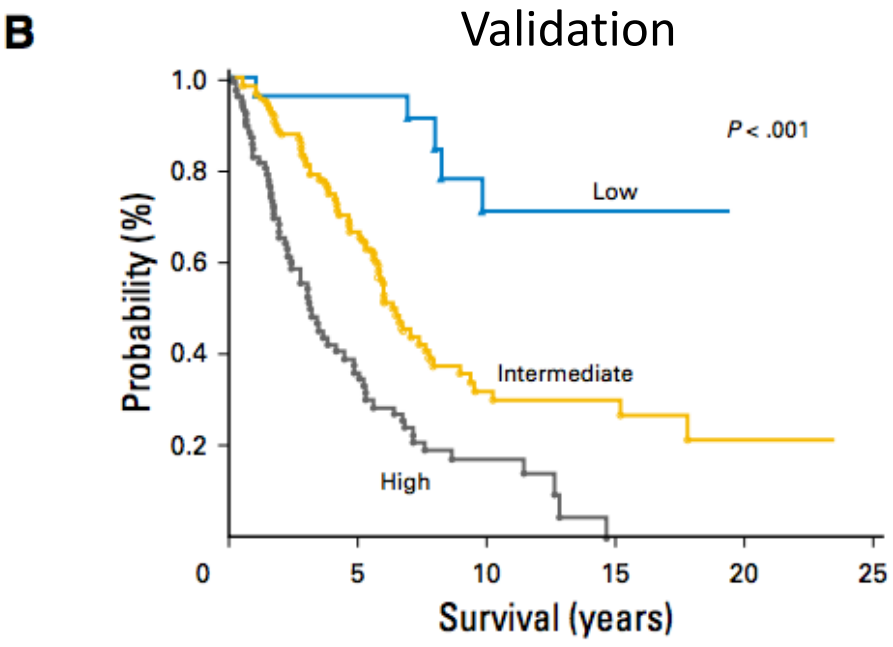
	Training Cohort				Validation Cohort			
Category (score range)	No. (%) of Patients	Median (range) OS (years)	HR (95% CI)	*P*	No. (%) of Patients	Median (range) OS (years)	HR (95% CI)	*P*
MIPSS70		Italian cohort				Mayo cohort		
Low (0-1)	238 (48.6)	27.7 (21.7-33.7)	1.00		27 (12.8)	Not reached	1.00	
Intermediate (2-4)	198 (40.4)	7.1 (6.2-8.1)	5.5 (3.8 to 8.0)	< .001	105 (49.8)	6.3 (0.1-23.5)	4.4 (1.8 to 11.1)	< .001
High (≥ 5)	54 (11.0)	2.3 (1.9-2.7)	16.0 (10.2 to 25.1)	< .001	79 (37.4)	3.1 (0.05-14.6)	9.9 (3.9 to 24.7)	< .001
MIPSS70-plus		Mayo cohort				Italian cohort		
Low (0-2)	86 (27.3)	20.0 (1.0-23.5)	1.00		25 (9.6)	Not reached	1.00	
Intermediate (3)	63 (20.0)	6.3 (0.6-30.9)	3.2 (1.9 to 5.2)	< .001	108 (41.4)	24.2 (12.3-36.1)	1.8 (0.9 to 5.1)	.303
High (4-6)	127 (40.3)	3.9 (0.05-17.1)	6.4 (4.1 to 10.0)	< .001	79 (30.3)	10.4 (7.1-13.6)	4.8 (1.7 to 13.8)	.004
Very high (≥ 7)	39 (12.4)	1.7 (0.14-7.7)	17.0 (9.8 to 29.2)	< .001	49 (18.7)	3.9 (0.7-7.1)	11.7 (4.1 to 33.7)	< .001

Abbreviations: HR, hazard ratio; OS, overall survival.

MIPSS70



At risk time					
Low	380	173	70	35	18
Intermediate	198	102	27	8	5
High	54	10	3	0	0

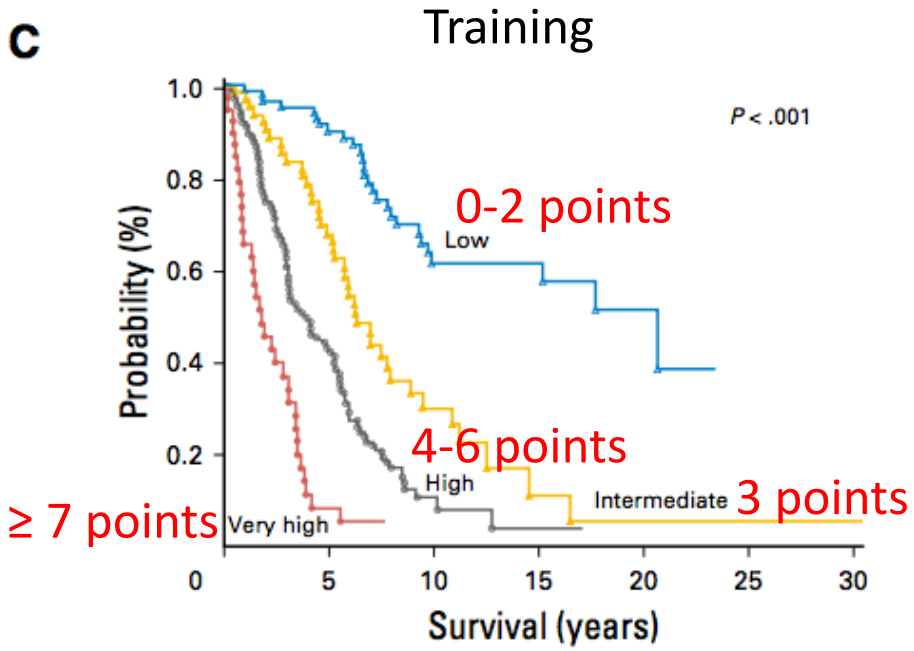


At risk time					
Low	27	21	9	5	0
Intermediate	105	54	17	9	2
High	79	23	5	0	0

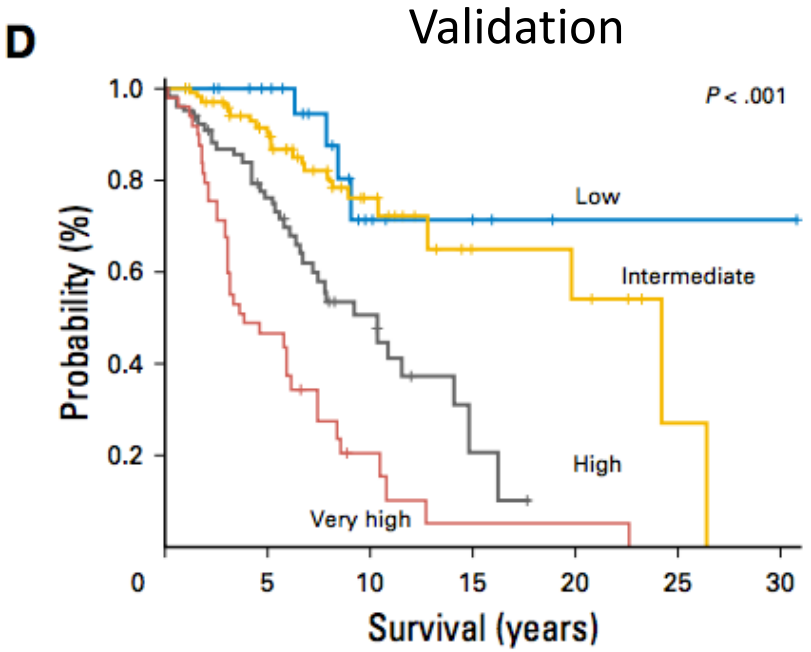
Anemia (Hgb < 10) = 1 point
Circulating blasts ≥ 2% = 1 point
Fibrosis grade ≥ 2 = 1 point
Constitutional symptoms = 1 point
Absence of CALR type-1 like mutation = 1 point
HMR category = 1 point

Leukocytosis (WBC > 25) = 2 points
Thrombocytopenia (plts < 100) = 2 points
≥ 2 HMR mutations = 2 points

MIPSS70-plus (incorporates cytogenetics)



At risk time					
Low	86	67	28	17	4
Intermediate	63	38	10	12	1
High	127	43	4	1	0
Very high	39	3	0	0	0



At risk time					
Low	25	20	6	3	1
Intermediate	108	74	24	7	0
High	79	50	18	2	0
Very high	49	18	4	1	0

Anemia (Hgb < 10) = 1 point
Circulating blasts ≥ 2% = 1 point
~~Fibrosis grade ≥ 2 = 1 point~~
Constitutional symptoms = 1 point
Absence of CALR type-1 like mutation = 2 points
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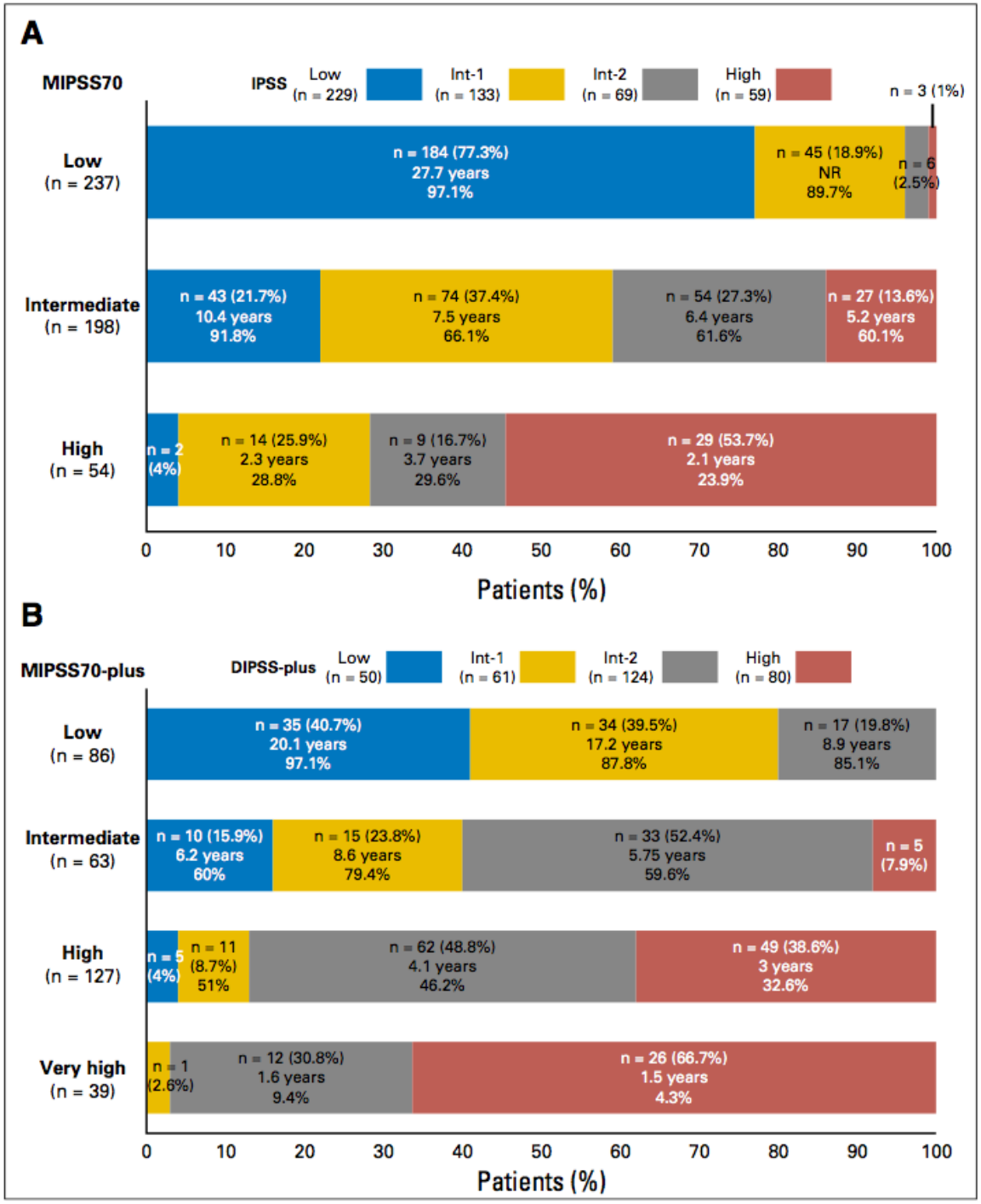

Fig 2. Categorization of patients according to (A) MIPSS70 and (B) MIPSS70-plus prognostic score versus the International Prognostic Scoring System (IPSS) and Dynamic IPSS-plus, respectively. Colored bars represent the IPSS/DIPSS-plus risk stratification (*x*-axis) in the context of the stratification based on the new scoring systems (represented by the rows). Shown is the number of patients for each IPSS/DIPSS-plus category within the new scoring system category, together with median overall survival (years) and 5-year survival (%). Survival data were omitted for groups with < 10 patients. Int, intermediate. DIPSS-plus, Dynamic International Prognostic Scoring System-plus; IPSS, International Prognostic Scoring System.

GENOMIC SEQUENCING

COMPREHENSIVE CANCER SET (VERSION 2)

INTERPRETATION

Targeted next-generation sequencing was performed on this sample of polycythemia vera 238.4, myelodysplastic syndrome 238.75 as part of the Genomics and Pathology Services Comprehensive Cancer Gene Set. A total of 23 variants predicted to alter protein coding were identified, including clinically actionable variant in *JAK2*.

SUMMARY OF SEQUENCING RESULTS:

- CLINICALLY ACTIONABLE *JAK2* p.V617F VARIANT DETECTED

A ***JAK2* p.V617F variant was identified**. Activating mutations in *JAK2*, including V617F, have been described in the majority of myeloproliferative neoplasms (Schnittger S et al.; Haematologica 94; 414-8; 2009 Mar). *JAK2* p.V617F mutation is seen in more than 95% of polycythemia vera (Levine RL, et al.; Cancer Cell 7; 387-97; 2005 Apr), (James C, et al.; Nature 434; 1144-8; 2005 Apr 28), (Kralovics R, et al.; N Engl J Med 352; 1779-90; 2005 Apr 28). One study showed that the higher *JAK2* p.V617F allele burden in polycythemia vera patients correlates with an increased risk of myelofibrosis transformation (Passamonti F, et al.; Leukemia 24; 1574-9; 2010 Sep). *JAK2* inhibitors are currently being tested in several clinical trials. Ruxolitinb showed significant clinical improvement in ET and PV patients (Verstovsek S, et al.; N Engl J Med 363; 1117-27; 2010 Sep 16), (Verstovsek S, et al.; N Engl J Med 366; 799-807; 2012 Mar 1) and has been recently approved by the FDA for the management of patients with intermediate- to high-risk myelofibrosis (Sonbol MB, et al.; Ther Adv Hematol 4; 15-35; 2013 Feb).

A non-synonymous variant p.D1127N was detected in *ASXL1* which has been previously reported in hematopoietic and lymphoid malignancies (COSMIC). This particular variant is seen in patients with inherited *GATA2* mutations (West RR, et al.; Haematologica 99; 276-81; 2014 Feb). Variants in ASXL1, in general, are found most often in association with myelodysplastic syndrome and chronic myelomonocytic leukemia (Thol F, et al.; J Clin Oncol 29;

2499-506; 2011 Jun 20). Prediction analysis predicts this variant to be neutral and benign. The clinical significance of this variant to the patient's tumor is not known.

Gene: ASXL1
Mutation: p.D1127N
Mutant Allele frequency: 48%

ASXL1 is a polycomb group chromatin-binding protein that directly regulates transcriptional gene expression. ASXL1 is involved in chromatin remodeling and epigenetic regulation of gene expression. Loss of function ASXL1 mutations have been identified across of spectrum of myeloid malignancies, most commonly in MDS, MPN and MDS/MPN. ASXL1 mutations have been detected in ~11% of de novo AML's, and 43% of CMML cases, and were associated with shorter overall survival (Chou, Blood. 2010;116(20):4086-4094). Specific targeted therapy for ASXL1 is not available (Katoh et al. Brit J Cancer (2013) doi: 10.1038/bjc.2013.281).

The D1127N mutation has been reported twice in the COSMIC database of tumor-specific mutations. However, this same variant has also been reported in the dbSNP database of predominantly "benign" sequence variants, so it is possible that this variant may not be pathologic. Protein prediction algorithms predict that the D1127N mutation will not have a major effect on protein function.

Average# of DNA sequence reads (ie, depth of coverage) for each of the 42 genes: 2095

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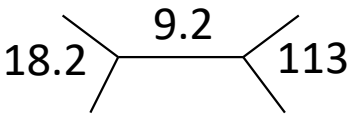

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- Cytogenetics normal
- JAK2 V617F negative
- *CALR type I (52 bp del) mutation positive*
- *NGS testing reveals a likely pathogenic ASXL1 mutation*

- *Does this additional information help guide treatment recommendations?*

Activation of JAK-STAT signaling in MPNs

A Gain-of-Function Mutation of *JAK2* in Myeloproliferative Disorders

Robert Kralovics, Ph.D., Francesco Passamonti, M.D., Andreas S. Buser, M.D., Soon-Siong Teo, B.S., Ralph Tiedt, Ph.D., Jakob R. Passweg, M.D., Andre Tichelli, M.D., Mario Cazzola, M.D., and Radek C. Skoda, M.D.

N Engl J Med. 2005 Apr 28;352(17):1779-90

A unique clonal *JAK2* mutation leading to constitutive signalling causes polycythaemia vera

Chloé James[1*], Valérie Ugo[1,2,3*], Jean-Pierre Le Couédic[1*], Judith Staerk[4], François Delhommeau[1,3], Catherine Lacout[1], Loïc Garçon[1], Hana Raslova[1], Roland Berger[5], Annelise Bennaceur-Griscelli[1,6], Jean Luc Villeval[1], Stefan N. Constantinescu[4], Nicole Casadevall[1,3] & William Vainchenker[1,7]

Nature. 2005 Apr 28;434(7037):1144-8

Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders

E Joanna Baxter, Linda M Scott*, Peter J Campbell*, Clare East, Nasios Fourouclas, Soheila Swanton, George S Vassiliou, Anthony J Bench, Elaine M Boyd, Natasha Curtin, Mike A Scott, Wendy N Erber, the Cancer Genome Project†, Anthony R Green*

Lancet. 2005 Mar 19-25;365(9464):1054-61

Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis

Ross L. Levine,[1,2,11] Martha Wadleigh,[2,11] Jan Cools,[6] Benjamin L. Ebert,[2,8] Gerlinde Wernig,[1] Brian J.P. Huntly,[1] Titus J. Boggon,[4] Iwona Wlodarska,[6] Jennifer J. Clark,[1] Sandra Moore,[1] Jennifer Adelsperger,[1] Sumin Koo,[1] Jeffrey C. Lee,[8] Stacey Gabriel,[8] Thomas Mercher,[1] Alan D'Andrea,[3] Stefan Fröhling,[1] Konstanze Döhner,[7] Peter Marynen,[6] Peter Vandenberghe,[6] Ruben A. Mesa,[9] Ayalew Tefferi,[9] James D. Griffin,[2] Michael J. Eck,[4] William R. Sellers,[2,8] Matthew Meyerson,[2,8] Todd R. Golub,[5,8,10] Stephanie J. Lee,[2,*] and D. Gary Gilliland[1,2,10,*]

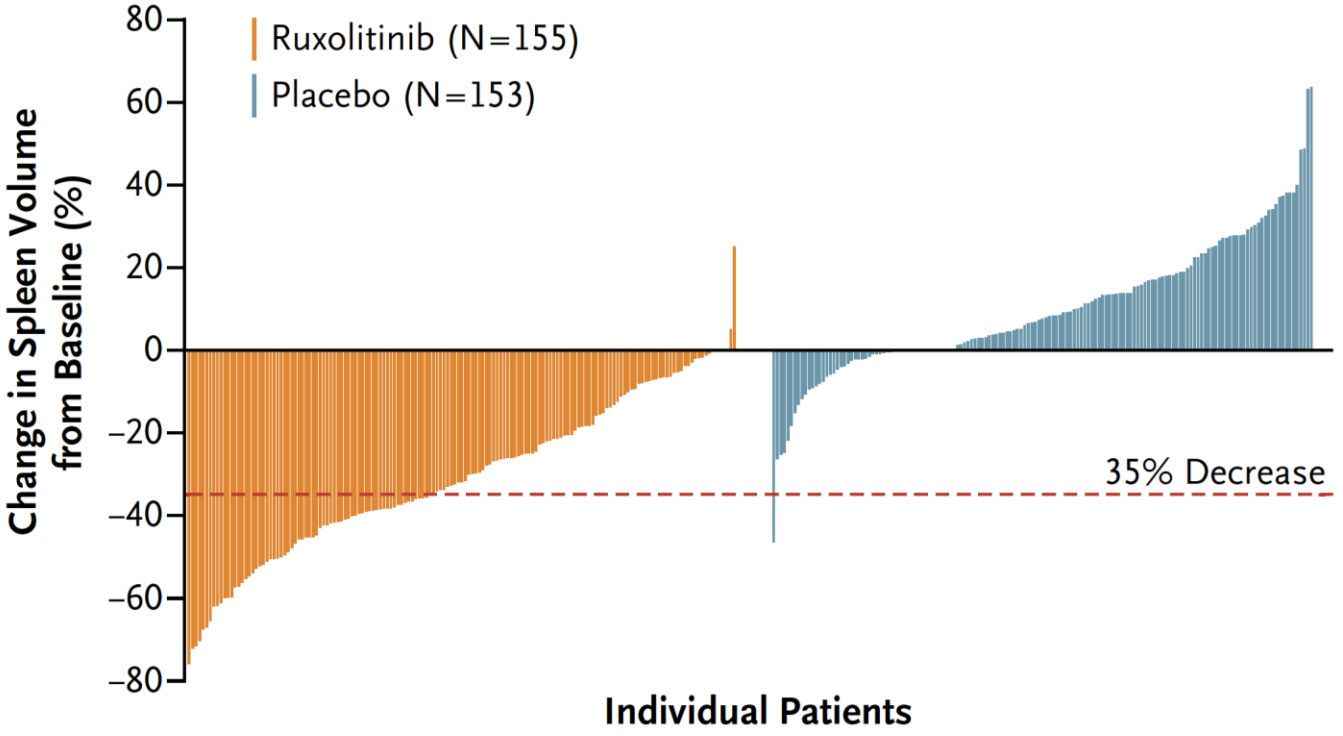
Cancer Cell. 2005 Apr;7(4):387-97

ORIGINAL ARTICLE

COMFORT-I

A Double-Blind, Placebo-Controlled Trial of Ruxolitinib for Myelofibrosis

Srdan Verstovsek, M.D., Ph.D., Ruben A. Mesa, M.D., Jason Gotlib, M.D., Richard S. Levy, M.D., Vikas Gupta, M.D., John F. DiPersio, M.D., Ph.D., John V. Catalano, M.D., Michael Deininger, M.D., Ph.D., Carole Miller, M.D., Richard T. Silver, M.D., Moshe Talpaz, M.D., Elliott F. Winton, M.D., Jimmie H. Harvey, Jr., M.D., Murat O. Arcasoy, M.D., Elizabeth Hexner, M.D., Roger M. Lyons, M.D., Ronald Paquette, M.D., Azra Raza, M.D., Kris Vaddi, Ph.D., Susan Erickson-Viitanen, Ph.D., Iphigenia L. Koumenis, M.S., William Sun, Ph.D., Victor Sandor, M.D., and Hagop M. Kantarjian, M.D.

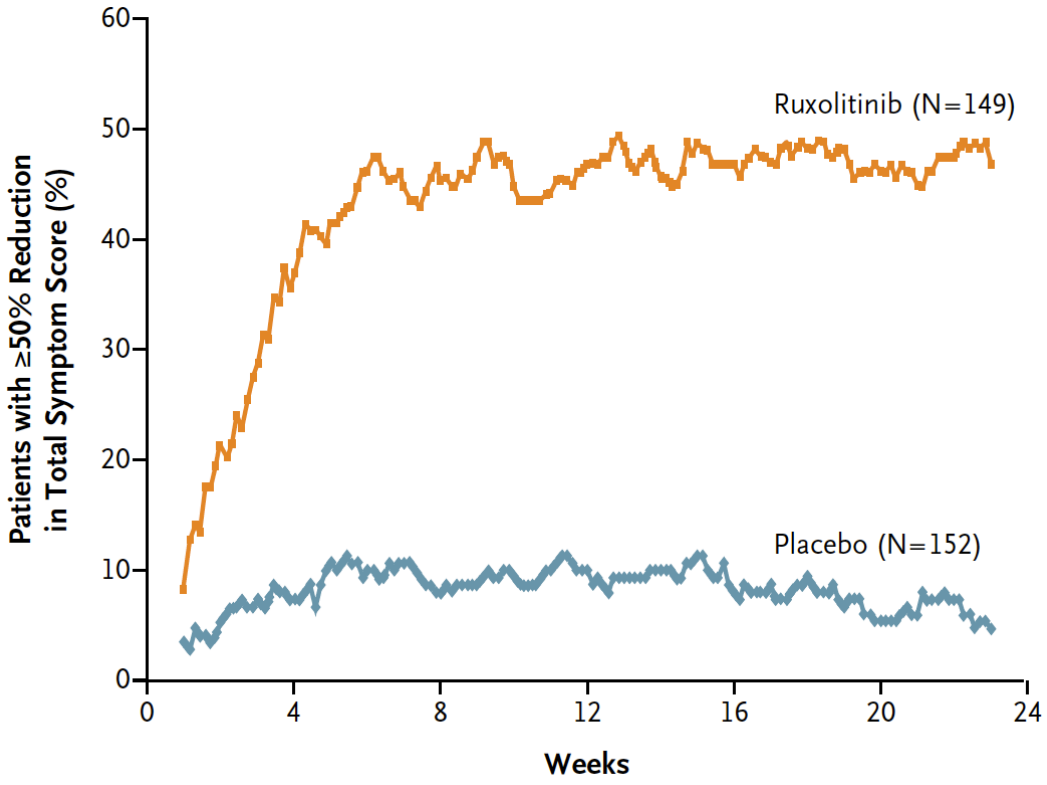


ORIGINAL ARTICLE

COMFORT-I

A Double-Blind, Placebo-Controlled Trial of Ruxolitinib for Myelofibrosis

Srdan Verstovsek, M.D., Ph.D., Ruben A. Mesa, M.D., Jason Gotlib, M.D., Richard S. Levy, M.D., Vikas Gupta, M.D., John F. DiPersio, M.D., Ph.D., John V. Catalano, M.D., Michael Deininger, M.D., Ph.D., Carole Miller, M.D., Richard T. Silver, M.D., Moshe Talpaz, M.D., Elliott F. Winton, M.D., Jimmie H. Harvey, Jr., M.D., Murat O. Arcasoy, M.D., Elizabeth Hexner, M.D., Roger M. Lyons, M.D., Ronald Paquette, M.D., Azra Raza, M.D., Kris Vaddi, Ph.D., Susan Erickson-Viitanen, Ph.D., Iphigenia L. Koumenis, M.S., William Sun, Ph.D., Victor Sandor, M.D., and Hagop M. Kantarjian, M.D.



ORIGINAL ARTICLE

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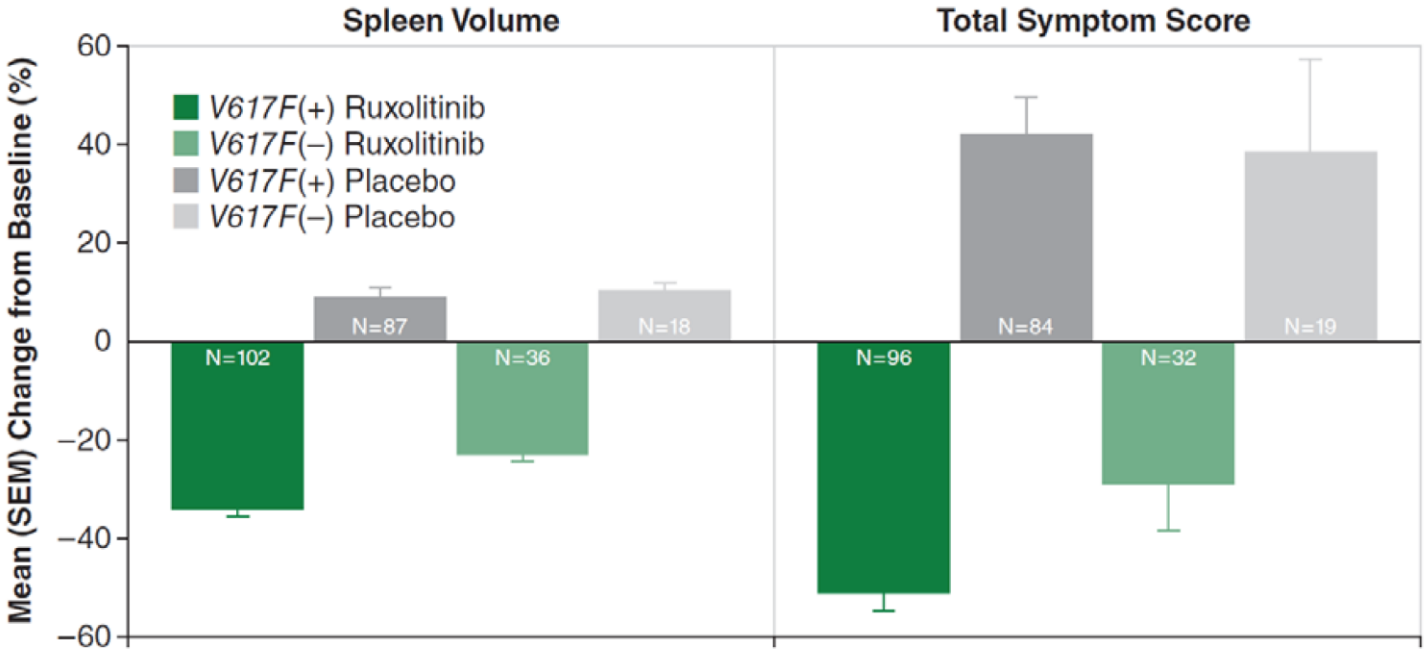
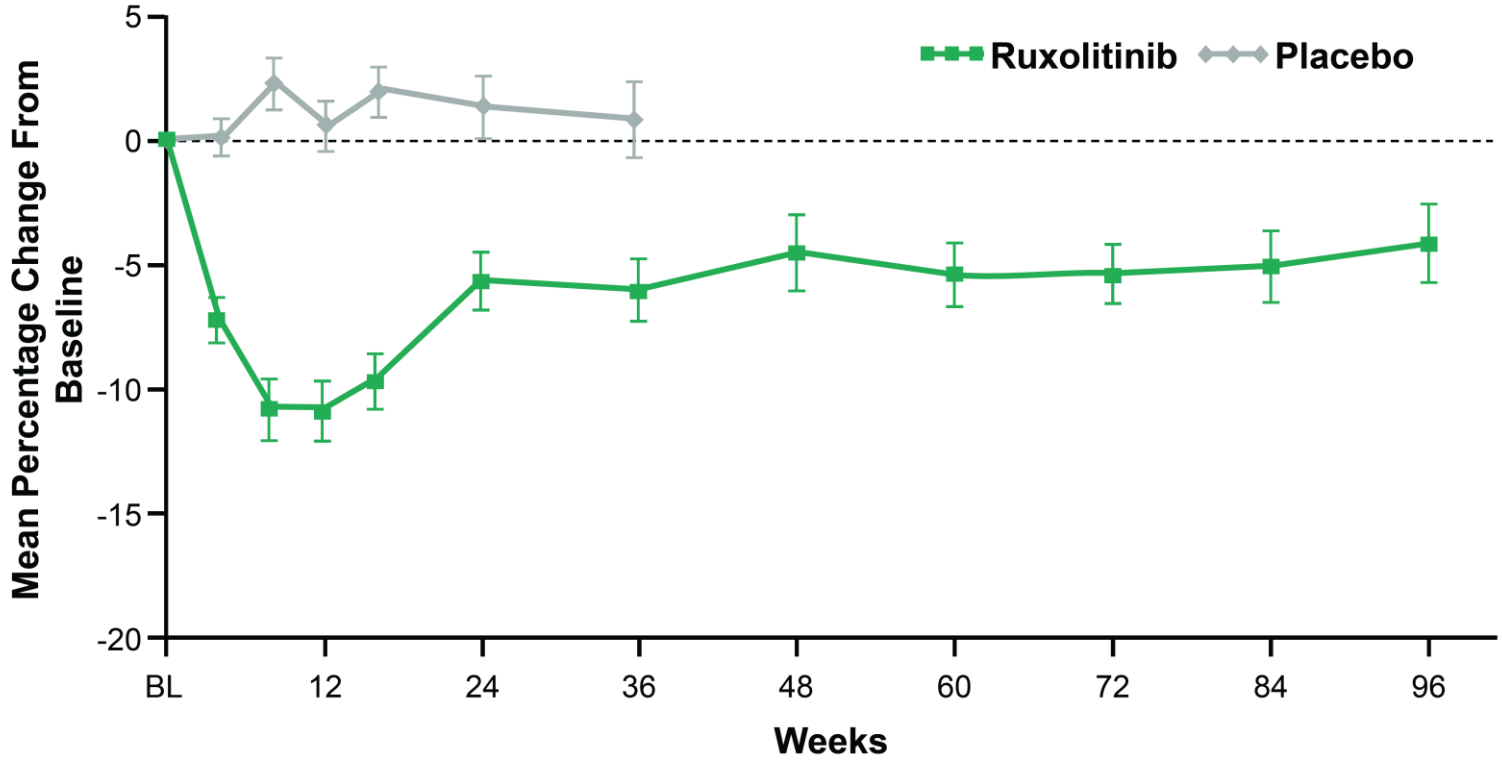


Mean Hemoglobin Levels Over Time

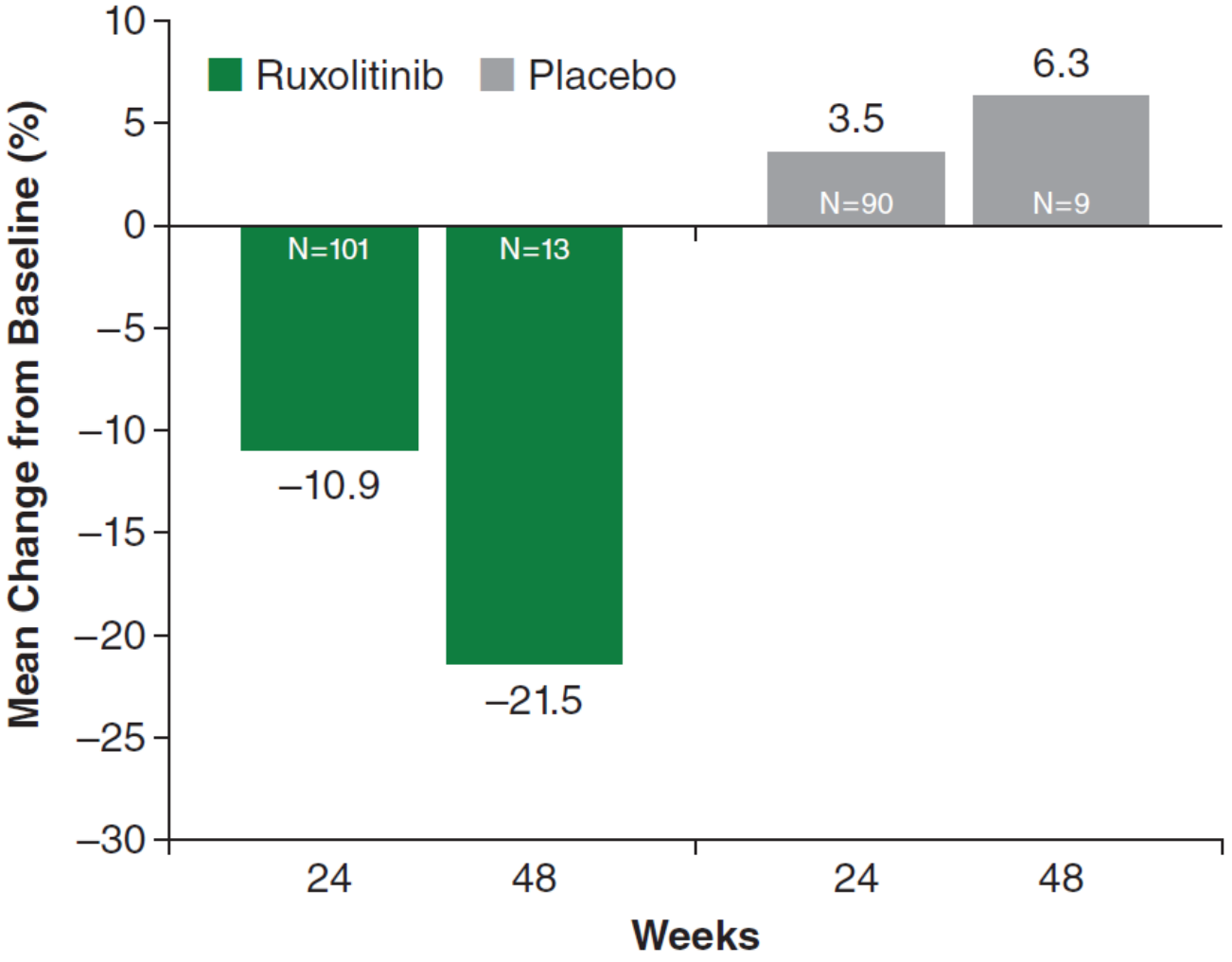
- Mean hemoglobin nadirs after 8–12 weeks of therapy and recovers to a new steady state which remains stable with longer-term therapy



Median hemoglobin at baseline: Ruxolitinib, 105 g/L; Placebo, 105 g/L.

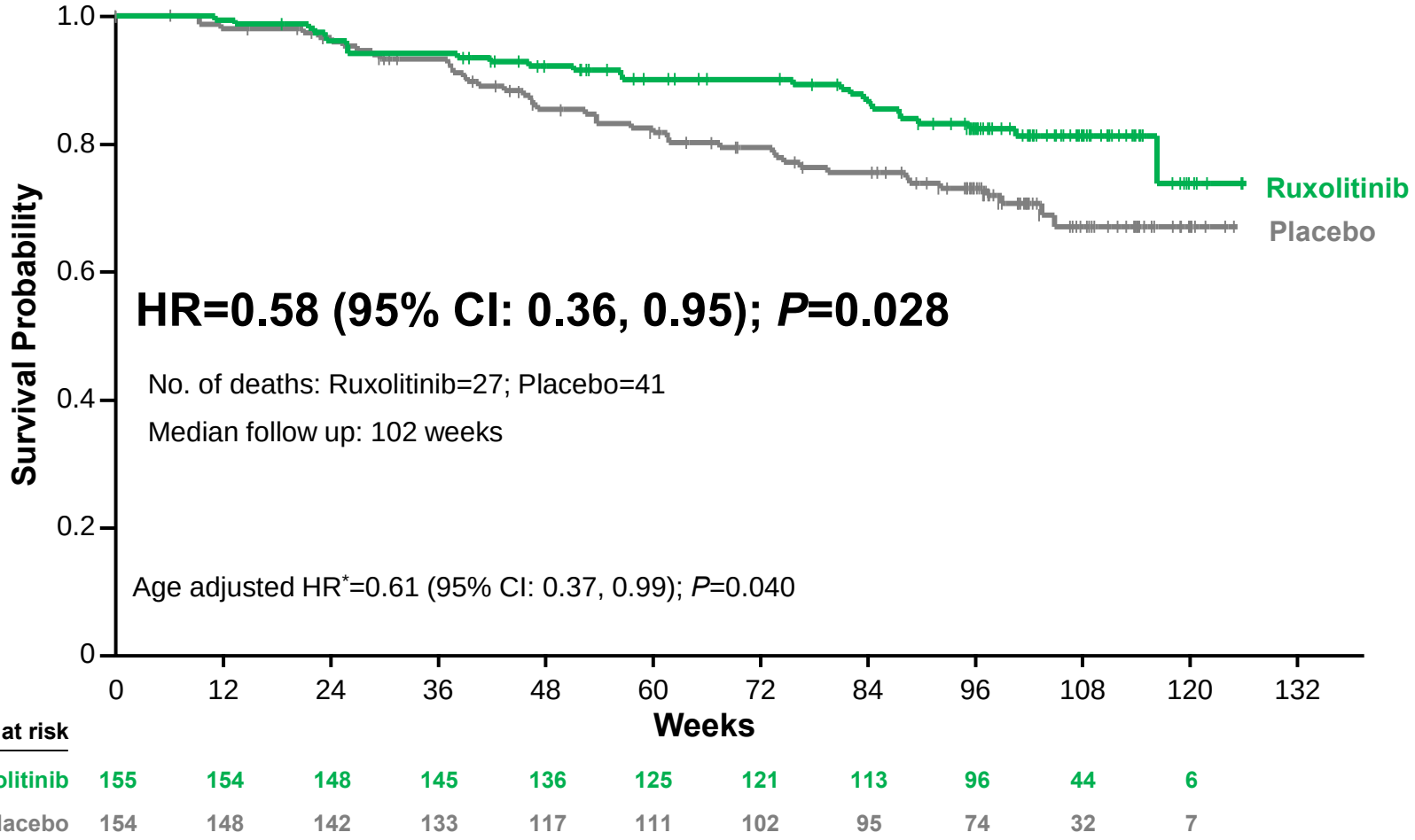
Verstovsek et al, ASH 2012

Mean Percent Change from Baseline in Percent *JAK2V617F* at Weeks 24 and 48



Verstovsek et al, NEJM 2012

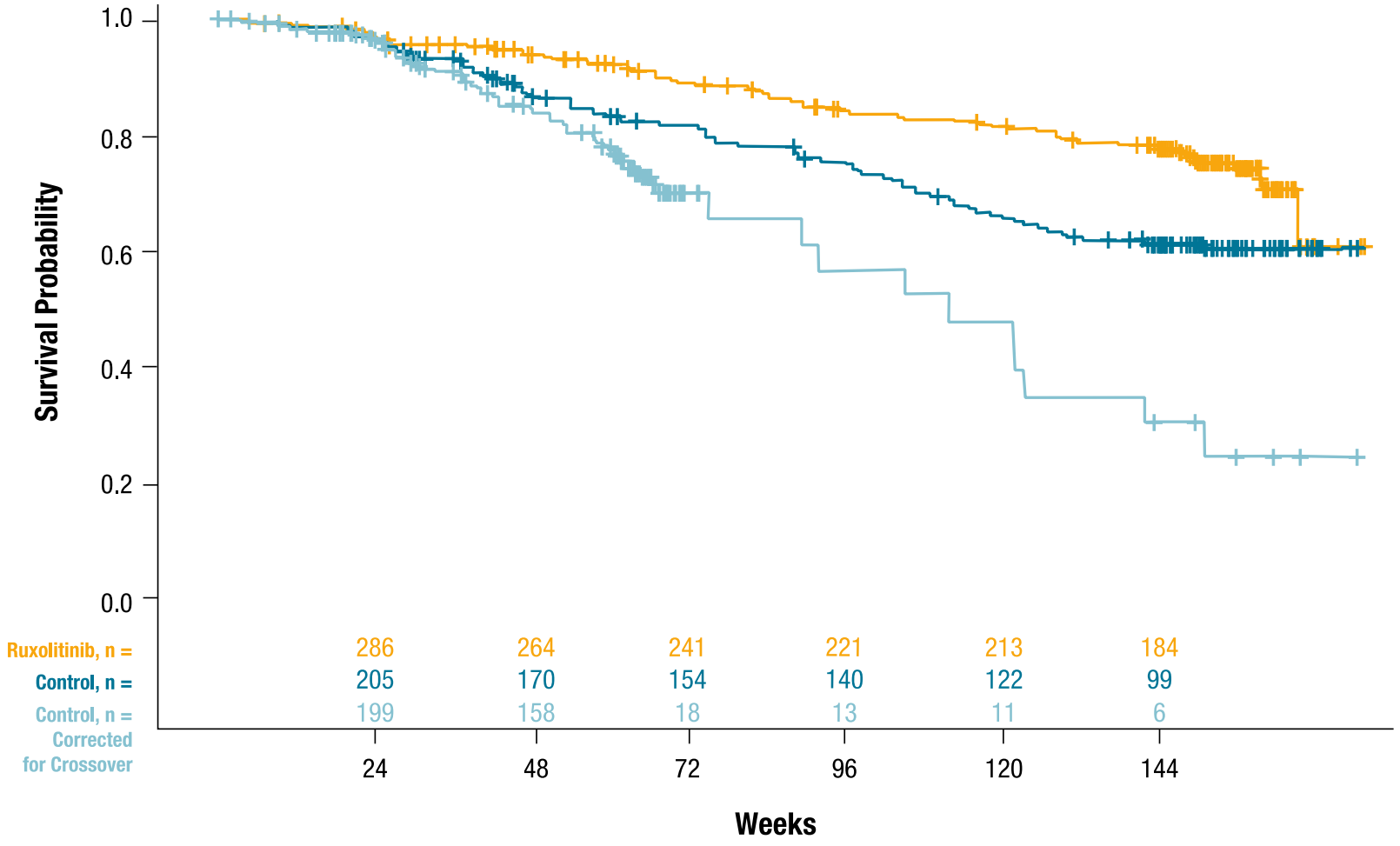
Overall Survival: ITT Population



Note: For this unplanned analysis, *P*-values are descriptive and nominally significant.
*Age was the only baseline characteristic that differed significantly between treatment groups as reported in Verstovsek S, et al. *N Engl J Med* 2012;366:799-807 (median age: ruxolitinib, 66 years; placebo, 70 years; *P*<0.05).

Verstovsek et al, ASH 2012

Figure 3. Kaplan-Meier Plot of Overall Survival



Ruxolitinib vs control (ITT): HR = 0.65; 95% CI, 0.46-0.90; *P* = .01.
Ruxolitinib vs control (RPSFT-corrected for crossover) HR = 0.29; 95% CI, 0.13-0.63; *P* = .01.

Vannucchi et al, ASH 2013

JAK inhibitors approved/in development for MPNs

JAK inhibitor	JAK2 IC50	JAK selectivity	Non-JAK targets	Clinical trials
Ruxolitinib FDA approved	4.5 nM	JAK1 0.6x JAK3 72x TYK2 4x		MF PV ET
Momelotinib Phase III	18 nM	JAK1 0.6x JAK3 8.6x TYK2 Unk	JNK1 CDK2	MF PV ET
Pacritinib Phase III	22 nM	JAK1 58x JAK3 24x TYK2 Unk	FLT3	MF
Fedratinib Phase III	3 nM	JAK1 35x JAK3 332x TYK2 135x	FLT3 RET	MF PV ET
NS-018 Phase I/II	< 1 nM	JAK1 33x JAK3 39x TYK2 22x	SRC, FLT3, ABL	MF

Anemia benefit

Minimal thrombocytopenia

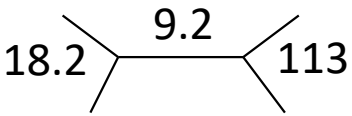
Wernicke's encephalopathy

Table 2. Clinical Trials (Nontransplant) of Ruxolitinib-Based Combinations in MF

Clinicaltrials.gov Identifier	Partner Drug	Major Inclusion Criteria	Phase	Comments
NCT02718300	INCB050465 (PI3K delta inhibitor)	PMF or post-PV/ET MF; spleen >10 cm below LCM or 5–10 cm with MF symptoms	II	
NCT01433445	Panobinostat (HDAC inhibitor)	PMF or post-PV/ET MF; spleen ≥5 cm below LCM, platelets >100 x 10^9/L, <10% blasts	I/II	RP2D, 15 mg bid of rux and 25 mg 3 times a week of panobinostat[70]
NCT01693601	Panobinostat (HDAC inhibitor)	PMF or post-PV/ET MF in CP or AP; intermediate-2– or high-risk; platelets ≥75 x 10^9/L, ANC ≥0.75 x 10^9/L	I/II	
NCT01732445	Danazol	PMF or post-PV/ET MF, intermediate- or high-risk; Hgb <10 g/dL or TD; platelets ≥50 x 10^9/L, ANC ≥1 x 10^9/L	II (pilot)	Closed early[61]
NCT02370706	PIM447 (PIM kinase inhibitor) and/or LEE011 (CDK4/6 inhibitor)	PMF or post-PV/ET MF, JAK2 V617F–positive; splenomegaly ≥5 cm by MRI; platelets ≥100 x 10^9/L, ANC ≥1.5 x 10^9/L; Hgb ≥9 g/dL	I	Dose escalation and expansion parts have different inclusion criteria: only patients with insufficient spleen response to ≥6 mo of rux allowed in escalation phase
NCT01787487	Azacitidine (HMA)	PMF or post-PV/ET MF, intermediate- or high-risk if newly diagnosed; platelets ≥50 x 10^9/L, ANC ≥1 x 10^9/L	II	Completed accrual to MF arm[74]; currently accruing patients with MDS/MPN
NCT02493530	TGR-1202 (PI3K delta inhibitor)	PMF or post-PV/ET MF, intermediate- or high-risk, with grade ≥1 marrow fibrosis; patients with PV meeting rux indications	I	Escalation stage 1 enrolls only patients with insufficient response to ≥8 wk of rux; stage 2 is for JAK inhibitor–naïve patients; PI3K or mTOR inhibitors not allowed in prior 6 mo
NCT02436135	Idelalisib (PI3K delta inhibitor)	PMF or post-PV/ET MF, intermediate- or high-risk, with disease relapse or progression on rux	I	Patients must have been on a stable dose of rux for ≥4 wk
NCT02267278	Pracinostat (HDAC inhibitor)	PMF or post-PV/ET MF, intermediate- or high-risk if newly diagnosed, spleen ≥5 cm below LCM ; platelets ≥50 x 10^9/L, ANC ≥1 x 10^9/L	II	Prior rux allowed only if duration <3 mo; no prior HDAC inhibitor allowed
NCT01375140	Lenalidomide (IMiD)	PMF or post-PV/ET MF, intermediate- or high-risk if newly diagnosed; platelets ≥100 x 10^9/L, ANC ≥1 x 10^9/L	II	Simultaneous administration of rux and lenalidomide is difficult due to excessive myelosuppression[60]
NCT01644110	Pomalidomide (IMiD)	PMF or post-PV/ET MF, splenomegaly >11 cm (total diameter) and/or leuko-erythroblastosis; Hgb <10 g/dL or TD; platelets ≥100 x 10^9/L, ANC ≥0.5 x 10^9/L	I/II	Although promising in a phase II study as a treatment for anemia of MF,[106] pomalidomide was not superior to placebo in a phase III study in MF[107]
NCT02593760	Vismodegib (Hedgehog inhibitor)	PMF or post-PV/ET MF, intermediate- or high-risk; spleen >5 cm below LCM; ANC >1 x 10^9/L, platelets ≥100 x 10^9/L, <10% peripheral blasts	I/II	Placebo-controlled trial; prior JAK or hedgehog inhibitor not allowed
NCT02742324	Pegylated interferon-α-2a	PMF or post-PV/ET MF, intermediate- or high-risk, with need for active therapy; ANC ≥ 1.5 x 10^9/L, platelets ≥150 x 10^9/L, ≤10% peripheral blasts	I/II	Interferon-α alone has been disappointing in MF,[108,109] although it may retard the progression of early PMF[110]; prior interferon-α or JAK2 inhibitor not allowed
NCT01787552	Sonidegib (Hedgehog inhibitor)	PMF or post-PV/ET MF, symptomatic, spleen ≥5 cm below LCM, intermediate- or high-risk; platelets ≥75 x 10^9/L	I/II	RP2D, 20 mg bid of rux and 400 mg/d of sonidegib[83]; prior JAK or smoothened inhibitors not allowed
NCT02076191	Decitabine (HMA)	MPN in AP or post-MPN AML	I/II	
NCT02257138	Decitabine (HMA)	Phase I portion: R/R AML Phase II portion: post-MPN AML or MDS/MPN with >20% blasts	I/II	

Case Presentation

- A 72 year-old man complains of fatigue, occasional night sweats, early satiety, and poor appetite, with 10-15 pound weight loss over the past few months
- Splenomegaly ~14 cm below the left costal margin
- Leukocytosis with anemia and thrombocytopenia:

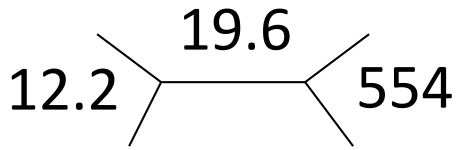


Seg 35, bands 20, metamyelocytes 10, myelocytes 8, promyelocytes 4, blasts 2, teardrop RBCs, nRBCs

- Bone marrow biopsy reveals a hypercellular marrow with megakaryocytic hyperplasia/atypia and 2+ fibrosis
- Cytogenetics normal
- JAK2 V617F negative
- *Is additional testing needed to confirm the diagnosis?*
- *Is there a role for additional genetic testing?*
- *What is this patient's overall prognosis? Risk of transformation to AML?*
- *Should treatment with ruxolitinib (JAK2 inhibitor) be considered?*
- *Are there alternative therapies that should be considered?*

Clinical Vignette

- A 67 year-old man presents for routine evaluation
- CBC reveals erythrocytosis with mild leukocytosis and thrombocytosis:



- He generally feels well but c/o pruritus that occurs after hot showers
- PEX unremarkable, no splenomegaly
- Epo level < 1.0 (2.6-18.5)
- JAK2 V617F: positive (68.2%)
- Patient declines bone marrow biopsy
- *Does the patient meet diagnostic criteria for polycythemia vera (PV)?*
- *Should additional testing be done? Role for genomic profiling?*
- *What is this patient's overall prognosis? Risk of transformation to AML?*
- *Should he be treated with ASA, phlebotomy, and/or hydroxyurea?*
- *Should treatment with ruxolitinib (JAK2 inhibitor) be considered?*

Overview of polycythemia vera (PV)

- Chronic myeloproliferative neoplasm (MPN)
- Characterized by erythrocytosis often accompanied by thrombocytosis and/or leukocytosis
- Driven by genetic abnormalities involving the JAK-STAT signaling pathway
- Associated with increased risk of thrombotic complications
- Propensity for transformation to myelofibrosis (MF) and/or secondary acute myeloid leukemia (sAML)

Genetic basis of PV

Table 1. Frequency of *JAK2* and *MPL* mutations in the classic myeloproliferative neoplasms.

Mutation	Myeloproliferative neoplasms and mutation frequency		
	Polycythemia vera	*Essential thrombocythemia*	*Primary myelofibrosis*
JAK2 V617F	>95%	~50–60%	~50–60%
JAK2 exon 12	~1%	Not present	Not present
MPL W515L/K	Not present	~1%	~5%

Frequencies for the representative mutations refer to somatically acquired myeloproliferative neoplasms. Rare cohorts of hereditary essential thrombocythemia have been reported with mutations in the *MPL* gene at codon 505 or other codons, and several essential thrombocythemia families have been reported with mutations in the *TPO* gene.

Oh, Gotlib, Expert Review of Hematology 2010

How does one mutation (*JAK2* V617F) "cause" three different diseases?

JAK2 V617F allele burden segregates with MPN phenotype

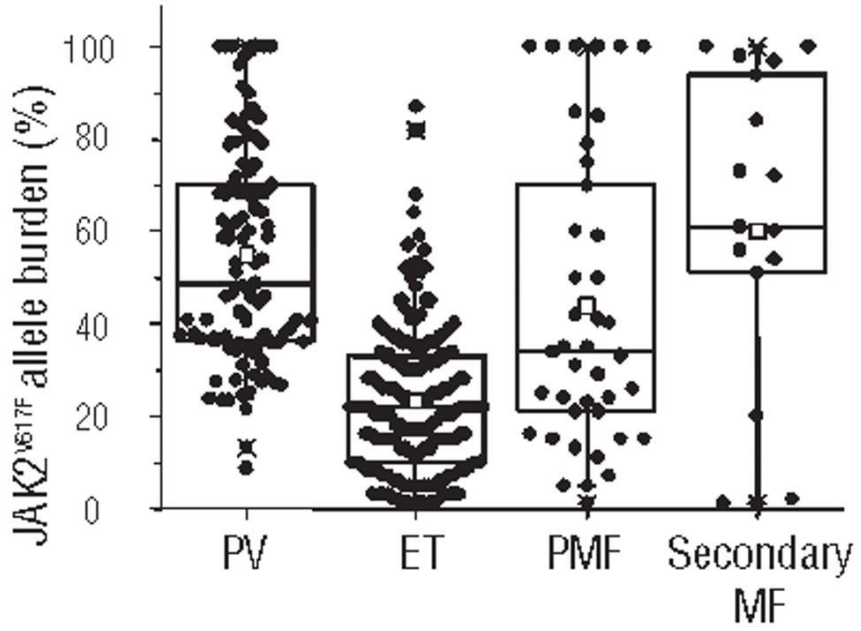
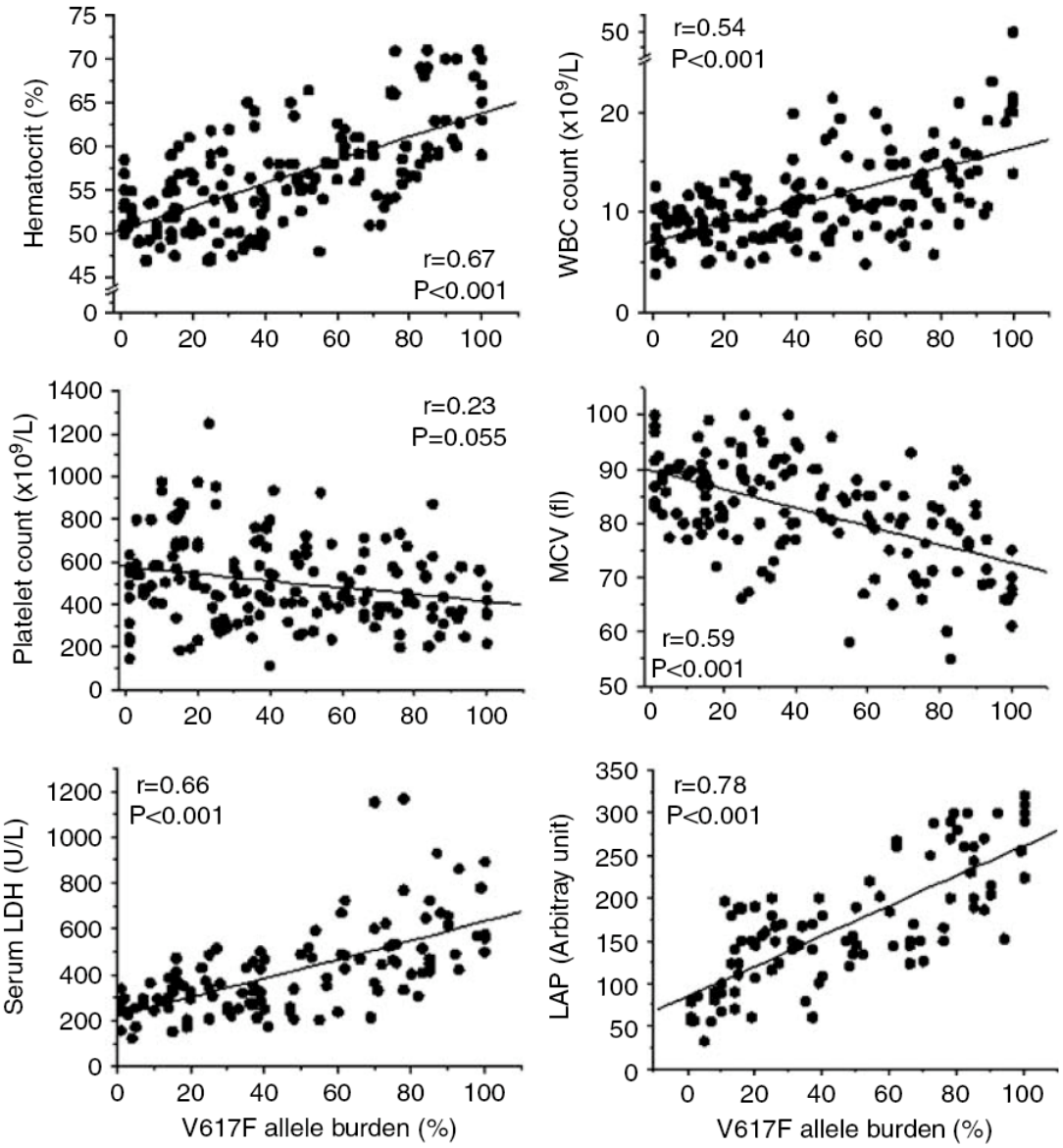


Figure 1. *JAK2*^{V617F} allele burden in the 165 patients with the *JAK2* mutation included in the study. For comparison, results from patients with PV (n=135)[44], primary myelofibrosis (PMF) (n=55) or secondary forms of myelofibrosis (Post-MF) (n=20) are presented. The level found in ET patients was significantly lower than the levels in all other MPD categories ($p<0.001$). Boxes represent the interquartile range that contains 50% of the subjects, the small square inside the mean value, and the horizontal line inside marks the median; the bars show the upper and lower range of values.

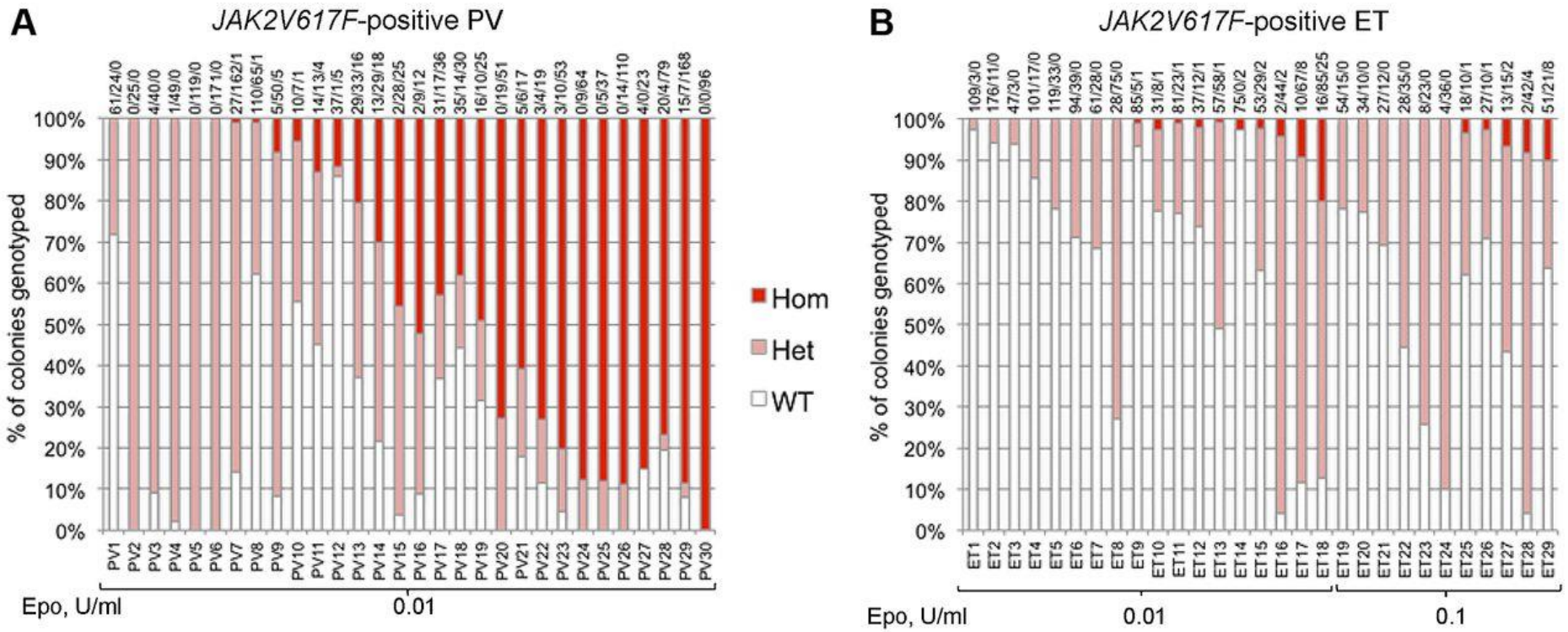
Antonioli et al, Haematologica 2008

JAK2 V617F allele burden segregates with MPN phenotype



Vannuchi AM et al, Leukemia Sep 2007

JAK2 V617F heterozygous and homozygous clones may co-exist in the same patient



Godfrey et al, Blood 2012

Diagnosing PV: WHO Criteria (2008)

Major Criteria

1. Hg >18.5 g/dL in men and >16.5 g/dL in women, or other evidence of increased red cell volume

2. Presence of *JAK2* V617F mutation or other functionally similar mutation, such as *JAK2* exon 12 mutation

For PV Diagnosis

- ❑ Both Major and One Minor Criteria *or*
- ❑ Major Criteria 1 + Any Two Minor Criteria

Minor Criteria

- Bone marrow biopsy showing hypercellularity (for age) with trilineage myeloproliferation
- Serum erythropoietin level below reference range of normal
- Endogenous erythroid colony formation in vivo

Vardiman JW et al. *Blood*. 2009;114:937-951.

Diagnosing PV: WHO Criteria (2016)

Table 4. WHO criteria for PV

WHO PV criteria
Major criteria
1. Hemoglobin >16.5 g/dL in men
Hemoglobin >16.0 g/dL in women
or,
Hematocrit >49% in men
Hematocrit >48% in women
or,
increased red cell mass (RCM)*
2. BM biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
3. Presence of *JAK2*V617F or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion†

*More than 25% above mean normal predicted value.

†Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5 g/dL in men (hematocrit, 55.5%) or >16.5 g/dL in women (hematocrit, 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV MF).

Arber et al, *Blood* 2016

PV Symptom Burden

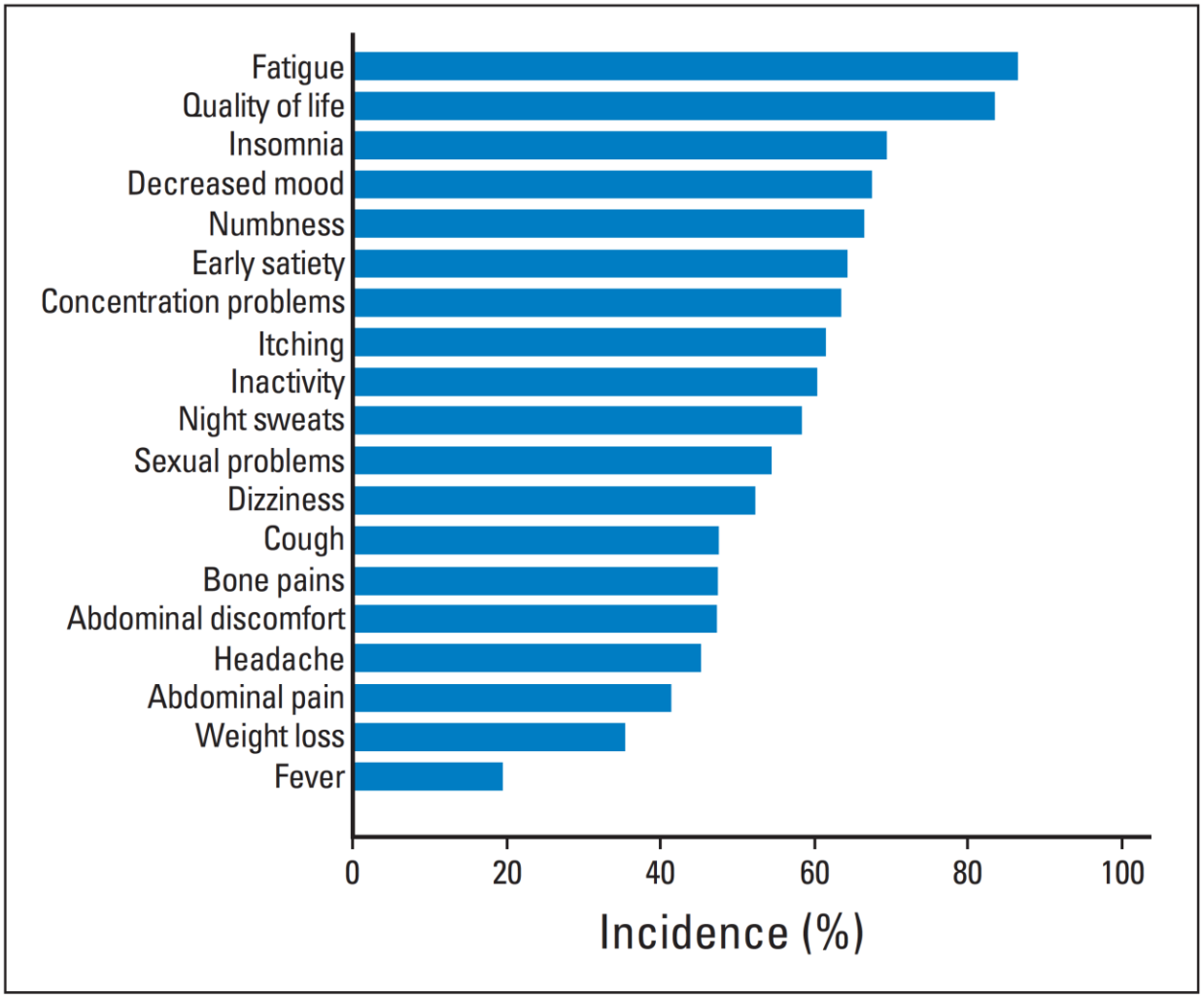
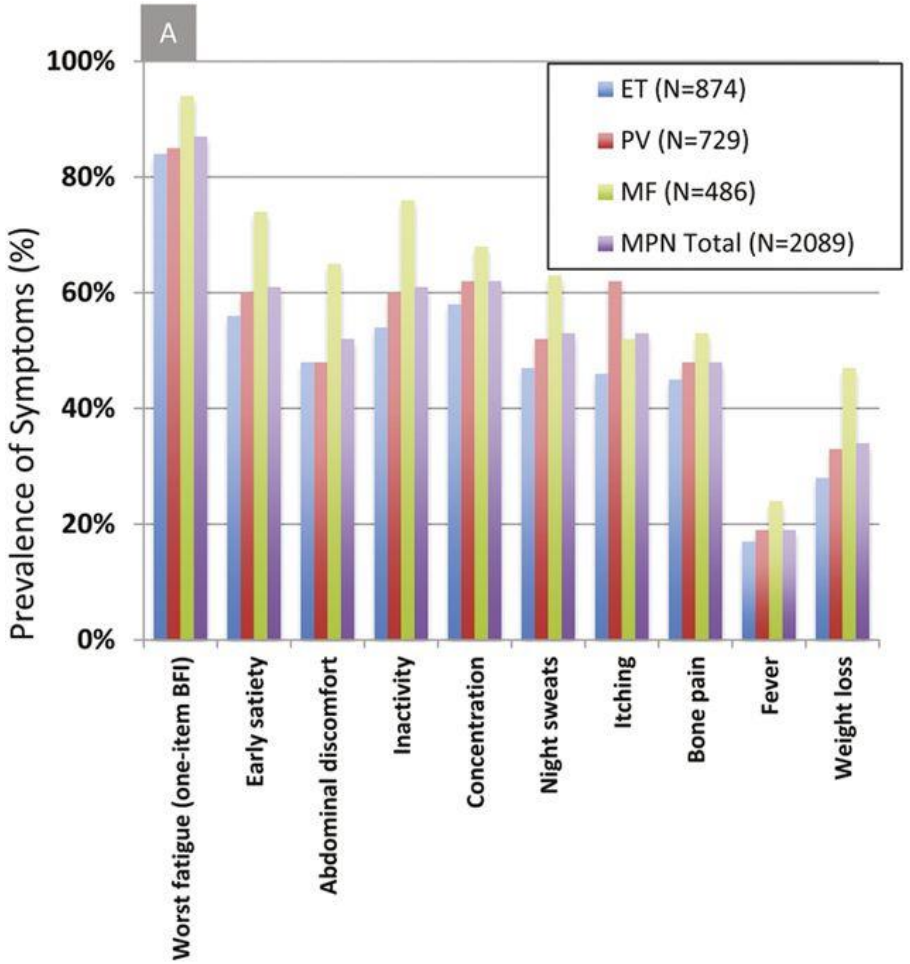
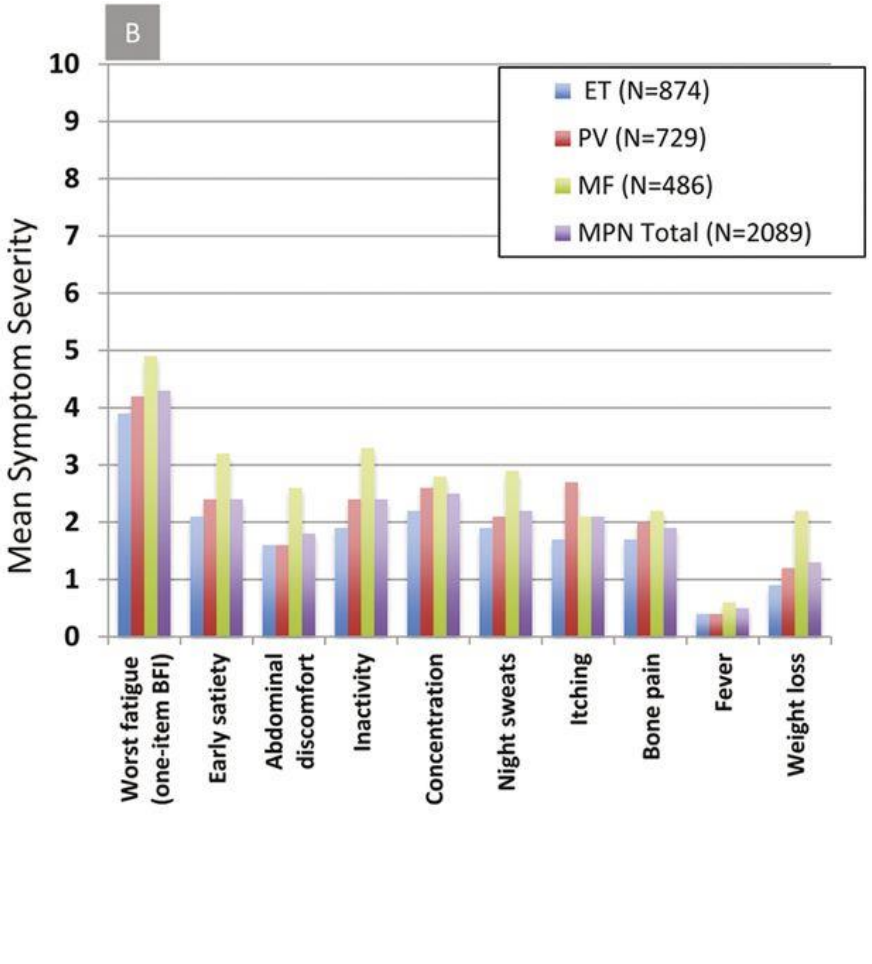


Fig 2. Patient-reported symptoms in polycythemia vera. Incidence of each symptom is estimated based on data from Scherber et al,[35a] Emmanuel et al,[35b] Johansson et al,[35c] and Abelsson et al.[35d]

Stein et al. JCO 2015

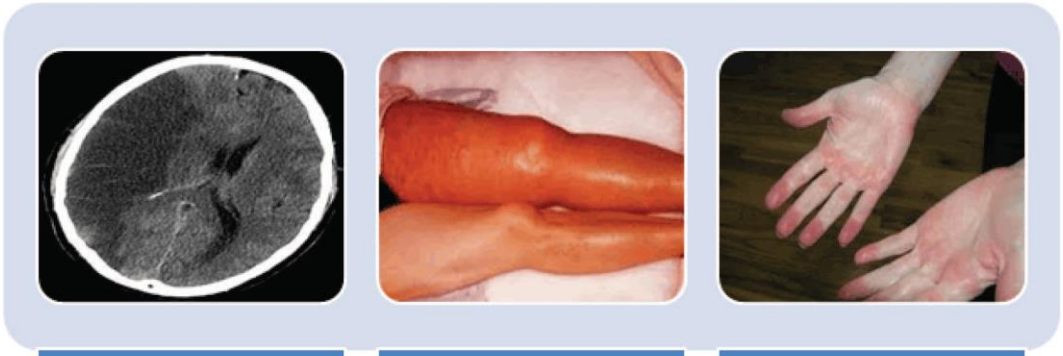
Symptom Burden Across MPNs





Geyer and Mesa, Blood 2014;124:3529-3537

Thrombotic Complications in PV



Arterial thrombosis	Venous thrombosis	Microcirculatory disturbancies
• Myocardial infarction • Unstable angina • Ischemic stroke • Transient ischemic attack • Acute peripheral and visceral thromboembolism	• Deep venous thrombosis (legs and arms) • Pulmonary embolism • Unusual sites venous thrombosis (visceral vein thrombosis and cerebral sinus and venous thrombosis. • Superficial venous thrombosis	• Erythromelalgia • Seizures • Migraine • Vertigo • Tinnitus • Scintillating scotomas • Amaurosis fugax

Falanda and Marchetti, Am Soc Hematol Educ Program 2012

<u>Risk Factors for thrombosis:</u>

- Age > 60 years
- Previous history of thrombosis
- Leukocytosis
- Higher *JAK2* V617F allele burden

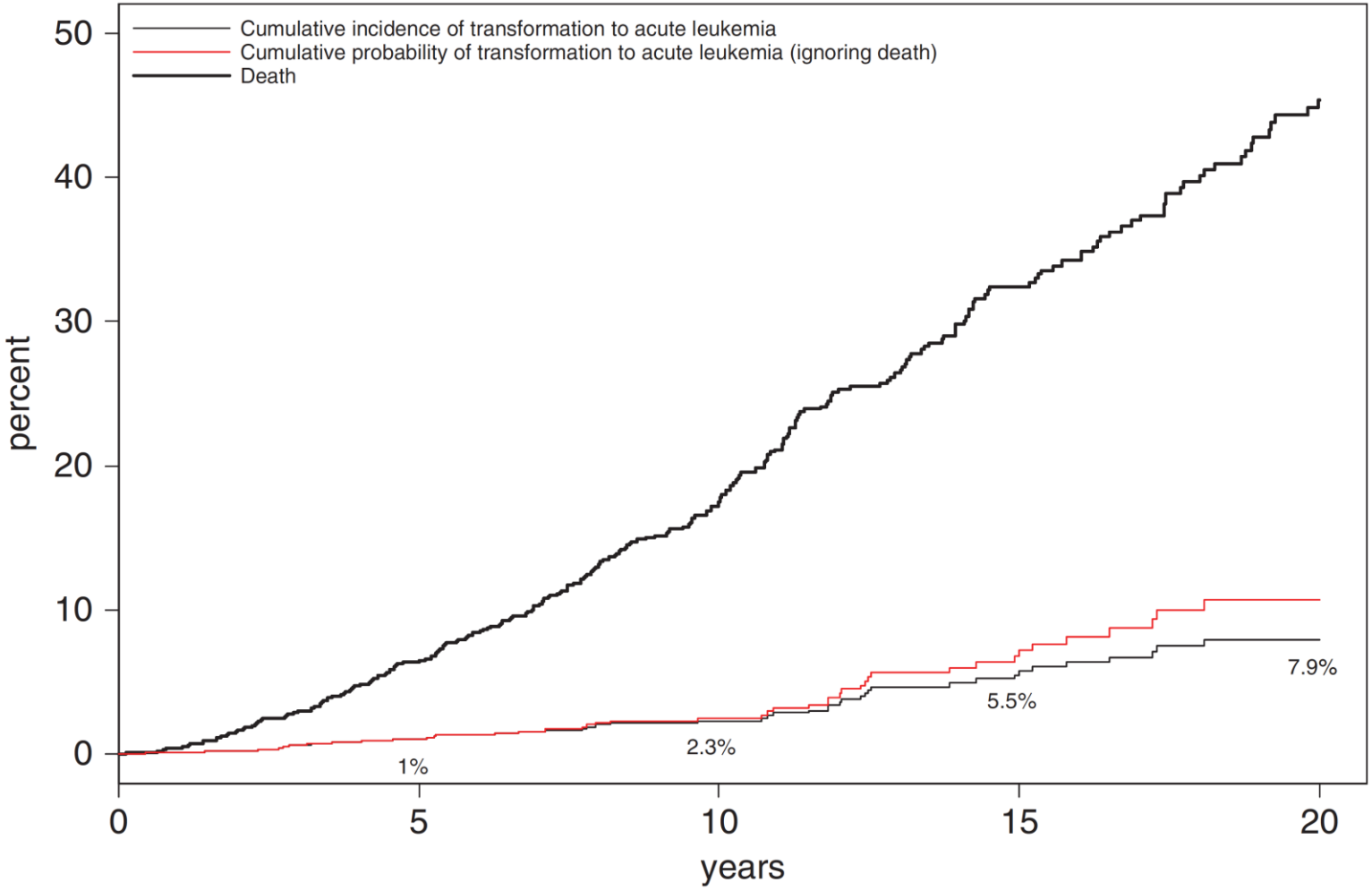
Polycythemia Vera: The Natural History of 1213 Patients Followed for 20 Years

Gruppo Italiano Studio Policitemia*

1 November 1995 • *Annals of Internal Medicine*

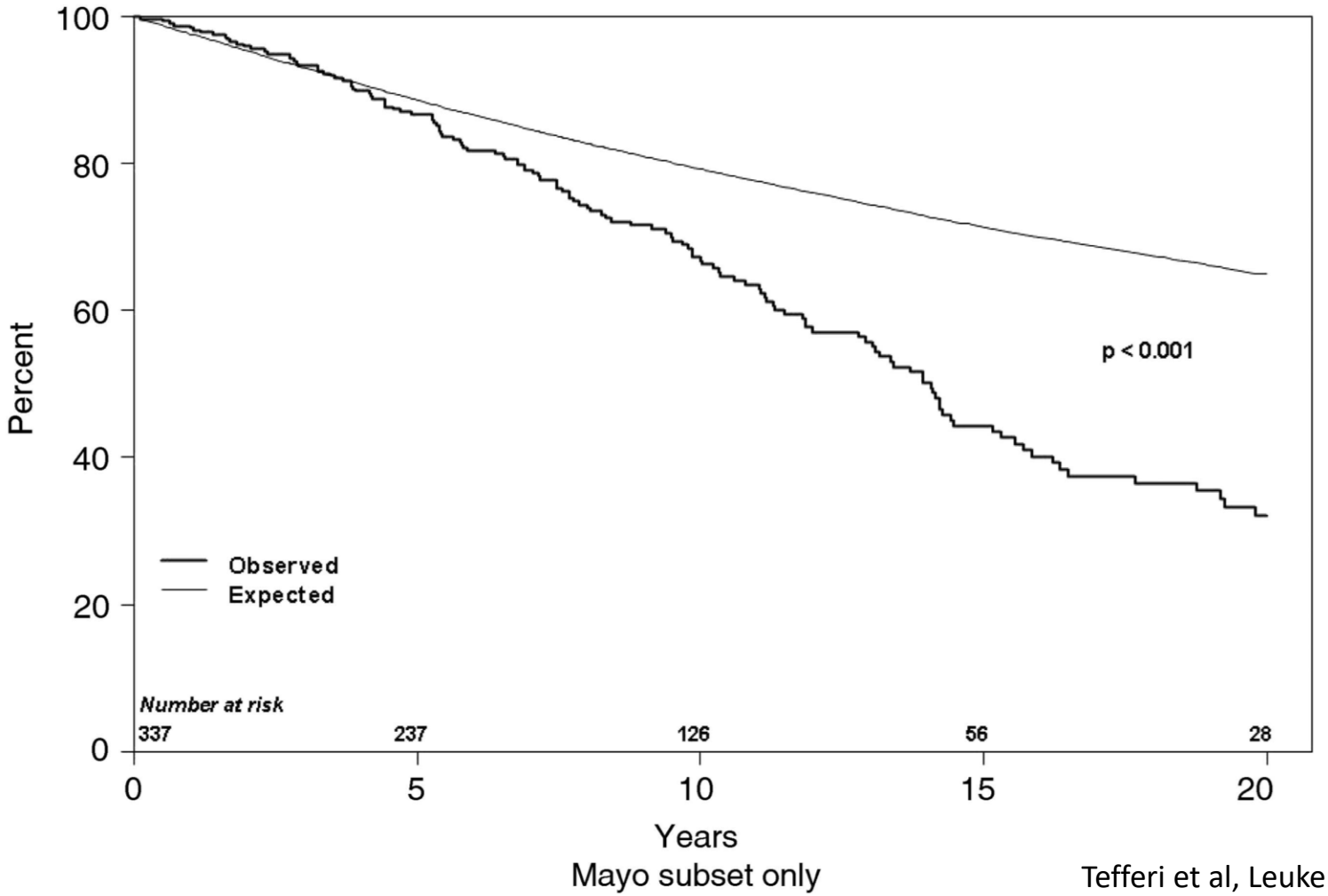
- Thrombotic events in 41% of the patients (arterial >> venous)
- 20% with thrombosis as presenting symptom
- 19% with thrombosis during follow-up period (3.4%/year)

PV Prognosis: Disease Transformation



Tefferi et al, Leukemia 2013

PV Prognosis: Overall Survival



Tefferi et al, Leukemia 2013

Causes of Death in PV

	All patients, n = *1545*
Median follow-up years (range)	6.9 (0–39)
Deaths, *n* (%)	347 (23%)
Causes of death	
Acute leukemia	36
Second malignancies	36
Thrombotic complications	32
Heart failure	13
Non-leukemic progressive disease	12
Infection	7
Respiratory failure	7
Bleeding	5
End-stage liver disease	3
Cardiopulmonary arrest	3
Other causes with incidences of 2 or less	10
Unknown	183 (53%)

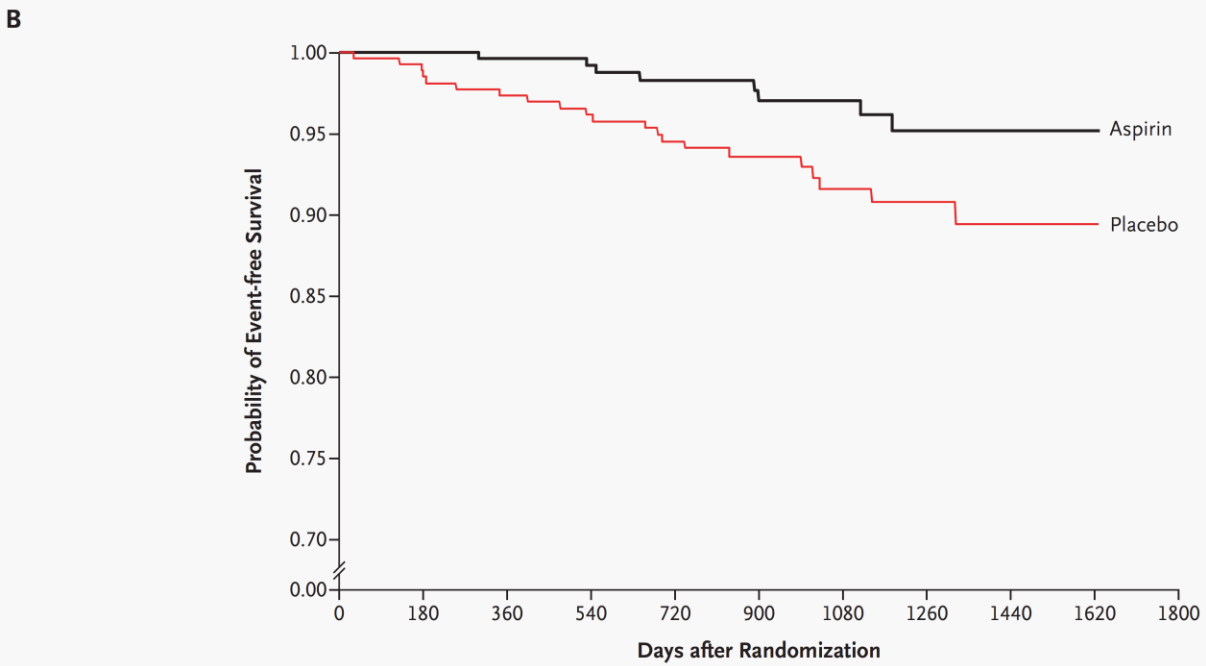
Tefferi et al, Leukemia 2013

Overview of Treatment for PV

- Low dose aspirin recommended for all patients (unless contraindicated)
- Phlebotomy: Goal Hct < 45
- Cytoreductive therapy (usually hydroxyurea)
  - Indicated for patients at high risk for thrombosis
    - Age > 60 or prior h/o thrombosis
- Alpha-interferon (younger high-risk patients)
- Ruxolitinib (JAK2 inhibitor) – for PV patients refractory/intolerant to hydroxyurea

Efficacy and Safety of Low-Dose Aspirin in Polycythemia Vera

Raffaele Landolfi, M.D., Roberto Marchioli, M.D., Jack Kutti, M.D., Heinz Gisslinger, M.D., Gianni Tognoni, M.D., Carlo Patrono, M.D., and Tiziano Barbui, M.D., for the European Collaboration on Low-Dose Aspirin in Polycythemia Vera Investigators*



- Aspirin reduced the combined risk of non-fatal MI, non-fatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes (relative risk, 0.40)

Landolfi R et al. *N Engl J Med*. 2004;350:114-124.

What is the Goal Hematocrit in PV?

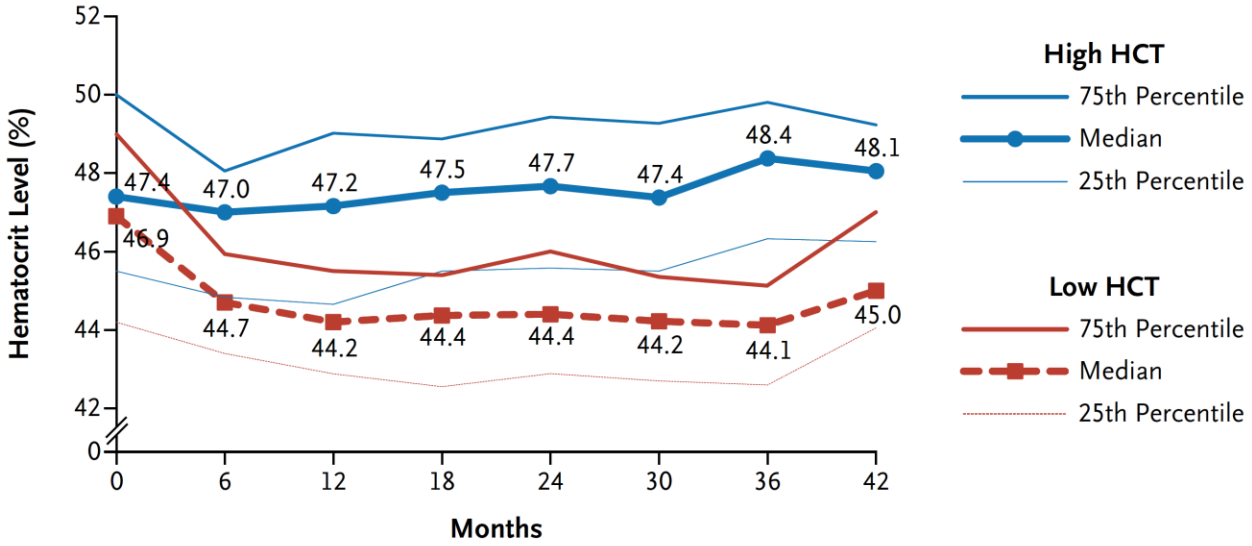
Cardiovascular Events and Intensity of Treatment in Polycythemia Vera

Roberto Marchioli, M.D., Guido Finazzi, M.D., Giorgina Specchia, M.D., Rossella Cacciola, M.D., Ph.D., Riccardo Cavazzina, Sc.D., Daniela Cilloni, M.D., Ph.D., Valerio De Stefano, M.D., Elena Elli, M.D., Alessandra Iurlo, M.D., Ph.D., Roberto Latagliata, M.D., Francesca Lunghi, M.D., Monia Lunghi, M.D., Rosa Maria Marfisi, M.S., Pellegrino Musto, M.D., Arianna Masciulli, M.D., Ph.D., Caterina Musolino, M.D., Ph.D., Nicola Cascavilla, M.D., Giovanni Quarta, M.D., Maria Luigia Randi, M.D., Davide Rapezzi, M.D., Marco Ruggeri, M.D., Elisa Rumi, M.D., Anna Rita Scortechini, M.D., Simone Santini, M.D., Marco Scarano, Sc.D., Sergio Siragusa, M.D., Antonio Spadea, M.D., Ph.D., Alessia Tieghi, M.D., Emanuele Angelucci, M.D., Giuseppe Visani, M.D., Alessandro Maria Vannucchi, M.D., and Tiziano Barbui, M.D., for the CYTO-PV Collaborative Group*

N ENGL J MED 368;1 NEJM.ORG JANUARY 3, 2013

- 365 PV patients randomized to low HCT (target < 45%) vs high HCT (target 45-50%) groups
- HCT control via phlebotomy and/or cytoreductive therapy
- Low-dose ASA recommended for all patients unless contraindicated
- Primary composite endpoint – time until death from CV causes or major thrombotic events

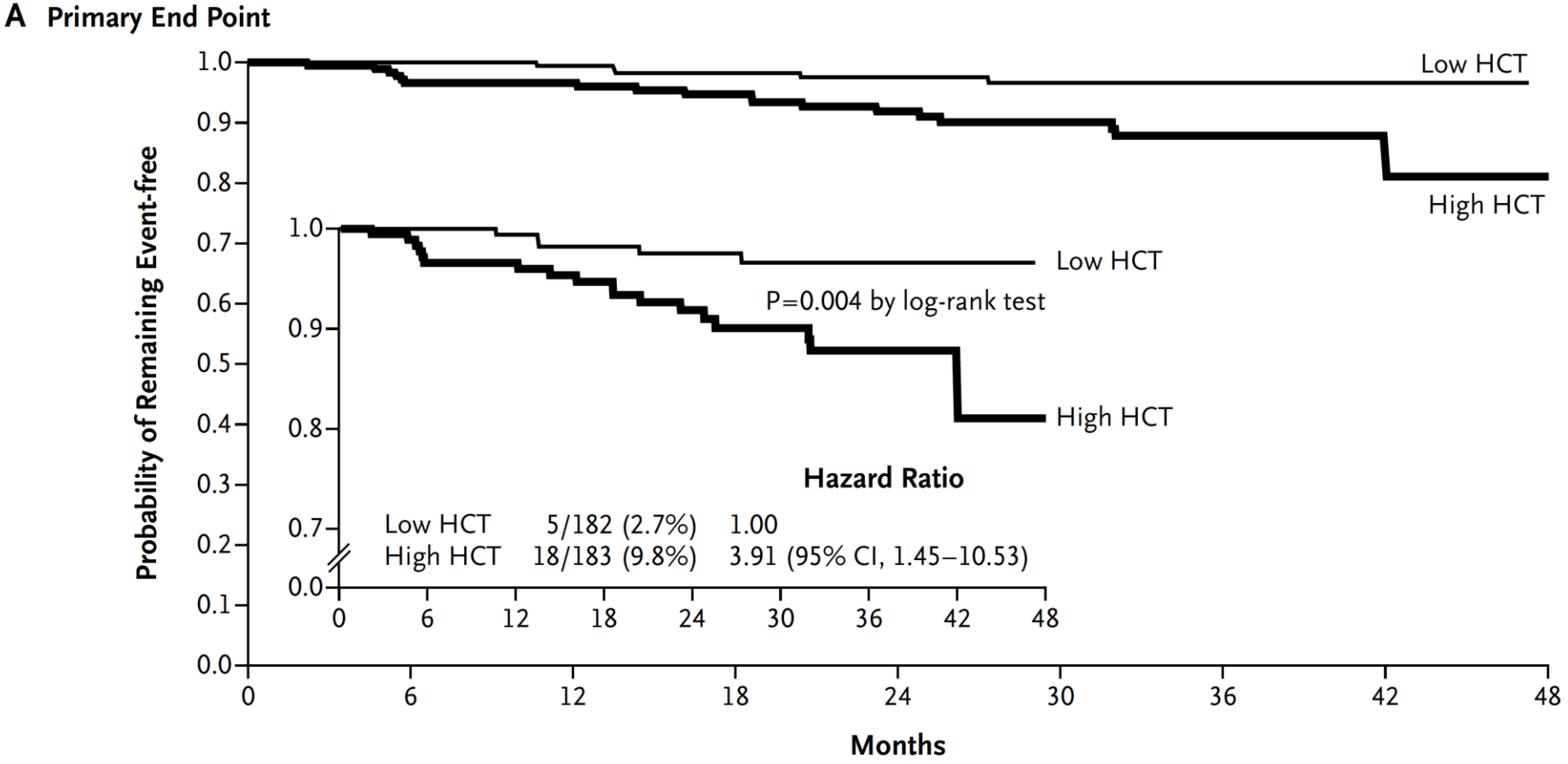
A Hematocrit



No. of Patients

Low HCT	182	179	171	157	135	103	64	26
High HCT	183	178	166	145	127	97	63	22

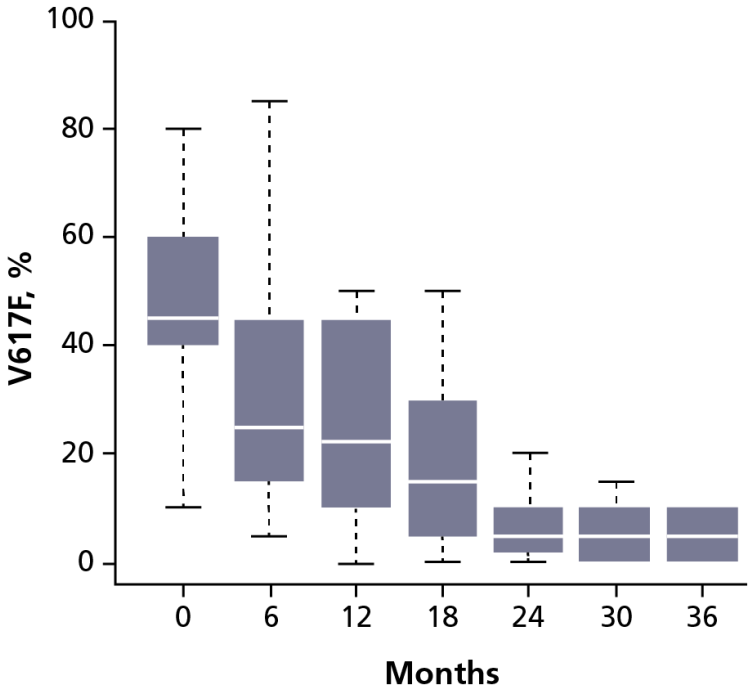
What is the Goal Hematocrit in PV?



No. at Risk

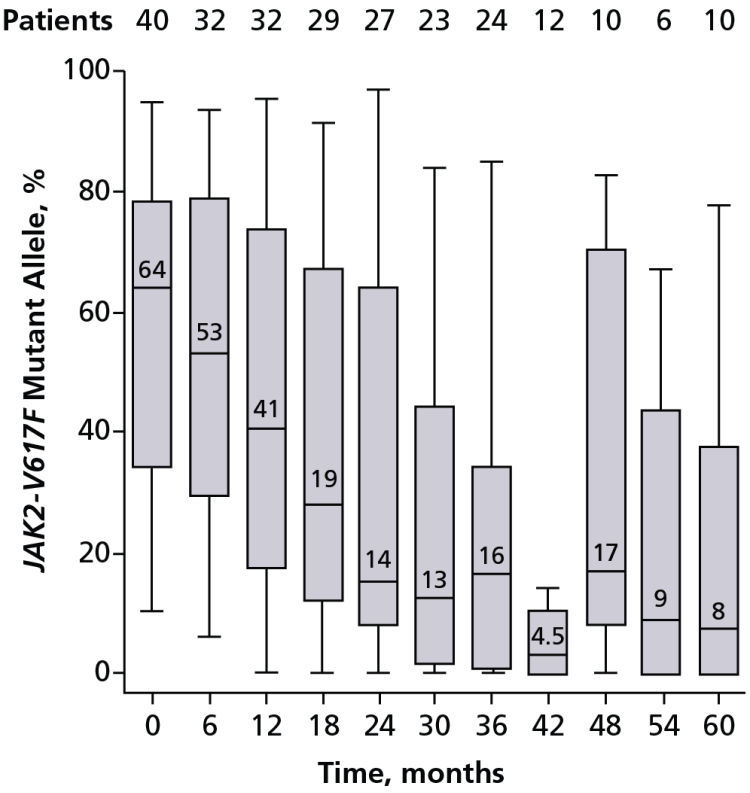
Low HCT	182 (0)	177 (1)	168 (2)	154 (1)	129 (1)	95 (0)	62 (0)	18 (0)	0
High HCT	183 (6)	168 (0)	160 (3)	143 (4)	110 (2)	92 (2)	54 (1)	12 (0)	1

PEG-IFN in PV



- Complete hematologic response at 12 mo: 94.6%
- Complete molecular response: 7/29 (24%)
- AEs in 89% (grade 1, 2); decreasing over time
- Treatment discontinuation: 35% (24% for toxicity)

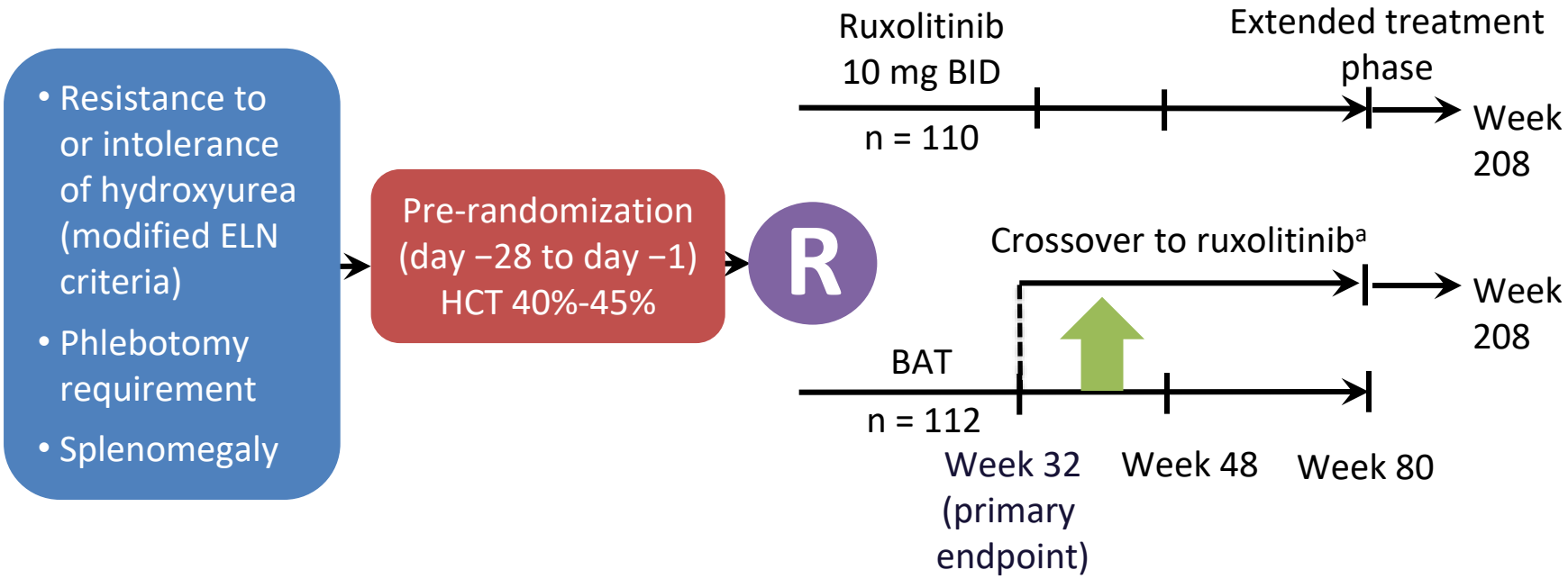
Kiladjian et al, *Blood* 2008



- Complete hematologic response: 76%
- Complete molecular response: 18%
- Drug-related treatment discontinuation: 20%

Quintás-Cardama et al, *Blood* 2013

Phase 3 RESPONSE Study



Primary endpoint: phlebotomy independence and spleen volume reduction at week 32

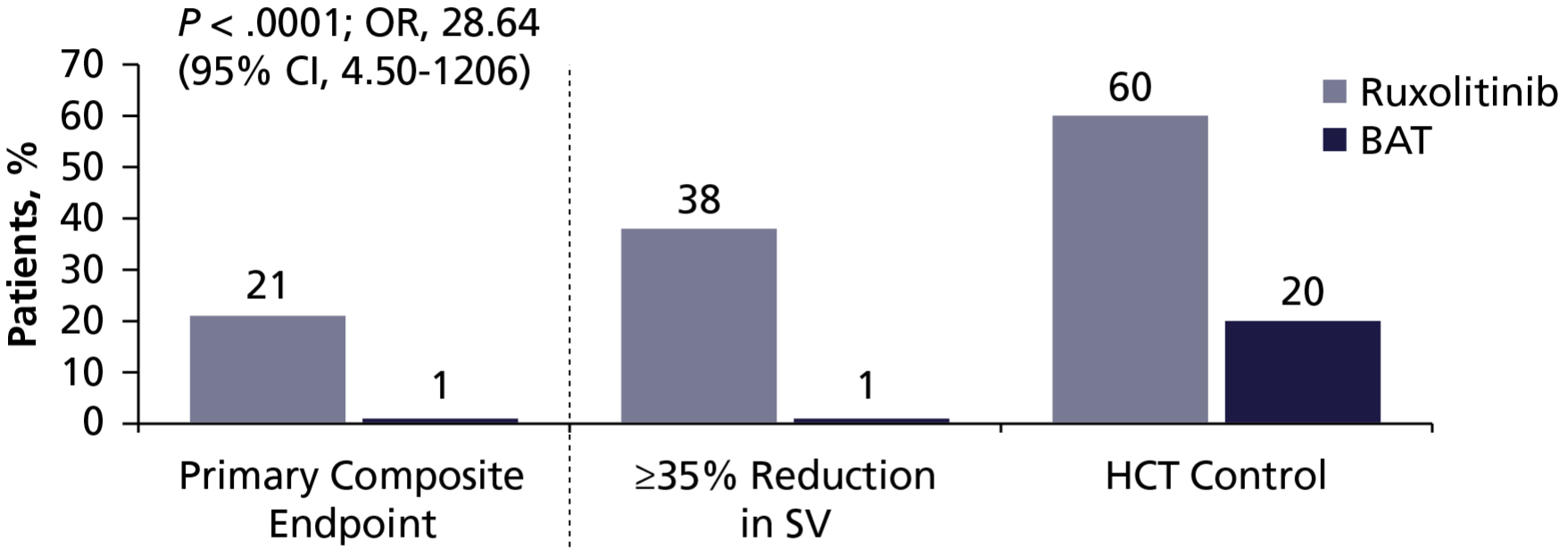
Investigator-selected BAT: hydroxyurea, IFN/PEG-IFN, anagrelide, pipobroman, IMIDs, or observation

[a] At wk 32 if patients on BAT failed to meet the primary endpoint or later in case of progression (phlebotomy requirement and/or splenomegaly progression).

Verstovsek S et al. NEJM 2015

RESPONSE: Primary Response at Week 32

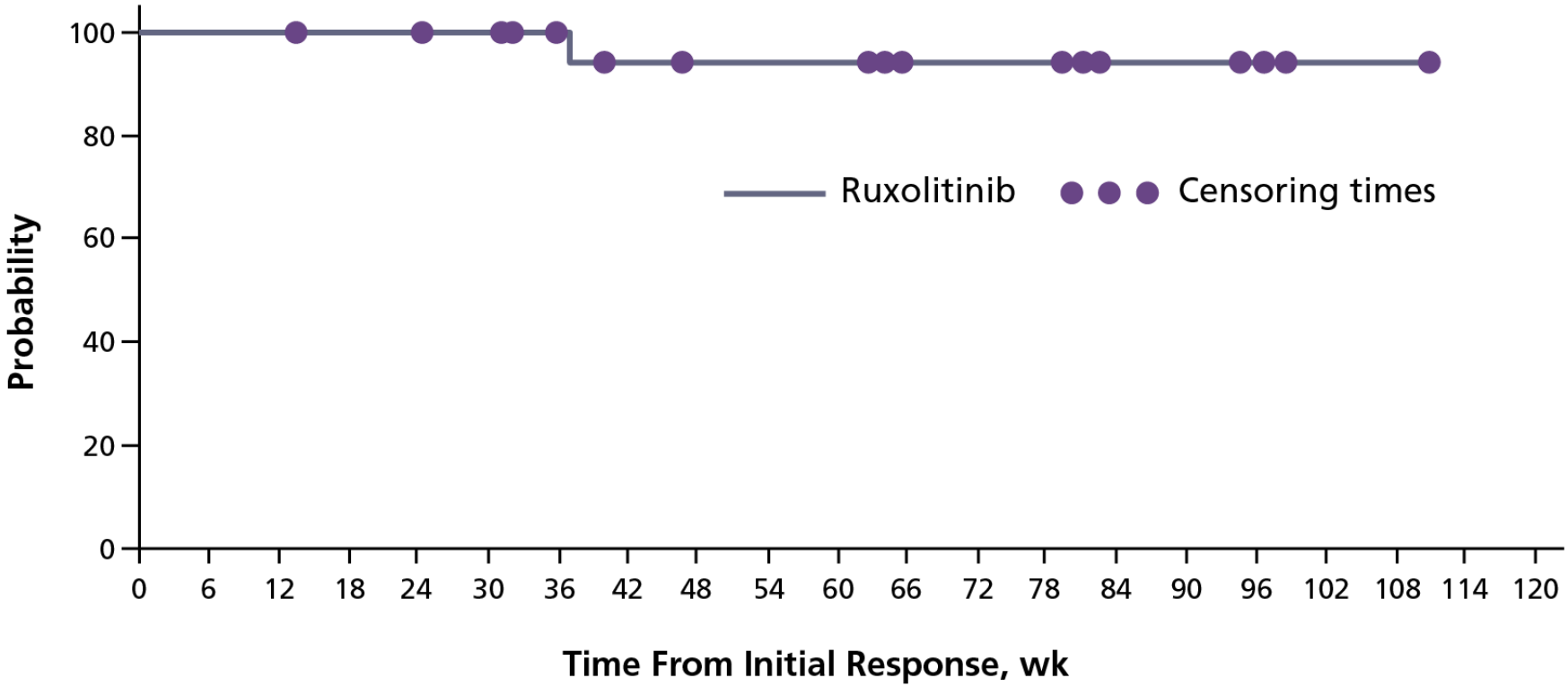
- 77% of patients randomized to ruxolitinib met at least one component of the primary endpoint
- 91% of patients who met the primary endpoint had a confirmed response at wk 48



SV: spleen volume.

Verstovsek S et al. NEJM 2015

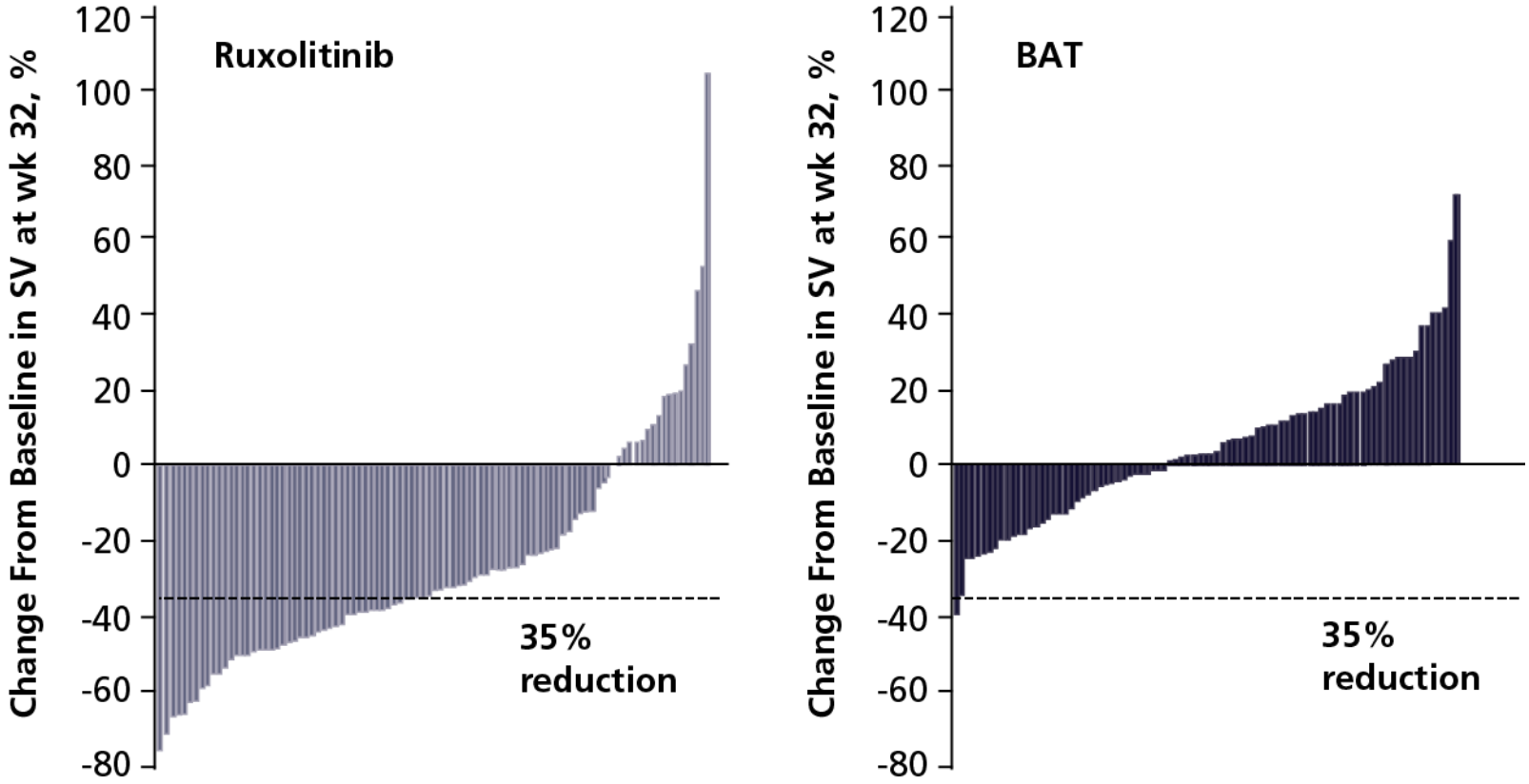
RESPONSE: Duration of Primary Response



At risk	23	23	23	22	22	21	18	15	14	14	14	10	10	10	6	6	4	1	1	0	0
Events	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1

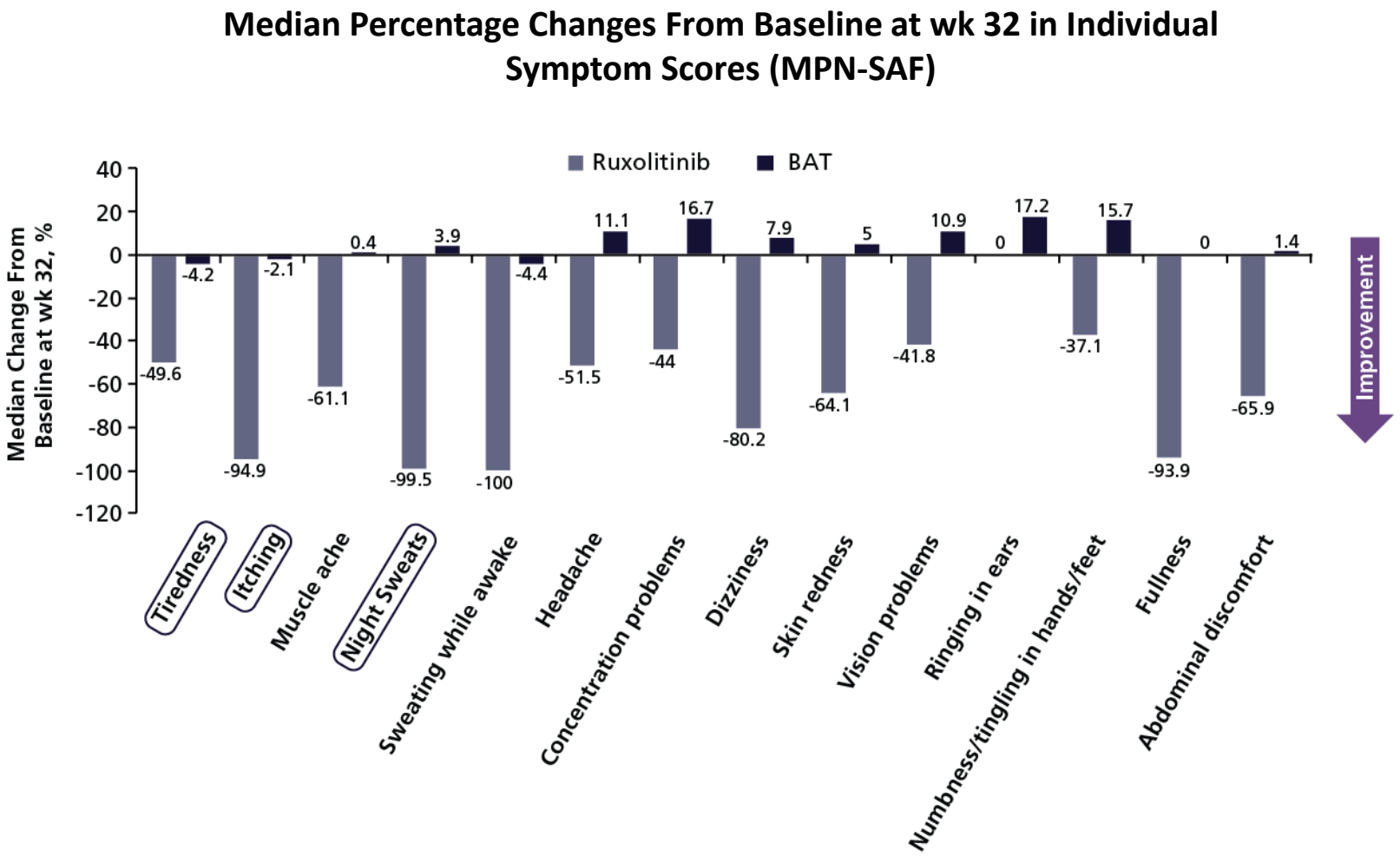
Verstovsek S et al. NEJM 2015

RESPONSE: Percentage Change in Spleen Volume at Week 32



Verstovsek S et al. NEJM 2015

RESPONSE: Improvement in Individual Symptoms



Patients with assessments at baseline and wk 32, with baseline value >0.

Verstovsek S et al. NEJM 2015

RESPONSE: Thromboembolic Events Up to Week 32

Patients, n (%)	Ruxolitinib (n = 110)		BAT (n = 111)	
	All Grade	Grade 3/4	All Grade	Grade 3/4
All thromboembolic events	1 (0.9)	1 (0.9)	6 (5.4)[a]	2 (1.8)[a]
Portal vein thrombosis	1 (0.9)	1 (0.9)	0	0
Myocardial infarction	0	0	1 (0.9)	1 (0.9)
Deep vein thrombosis	0	0	2 (1.8)	1 (0.9)
Pulmonary embolism	0	0	1 (0.9)	1 (0.9)
Splenic infarction	0	0	1 (0.9)	0
Thrombophlebitis	0	0	1 (0.9)	0
Thrombosis	0	0	1 (0.9)	0

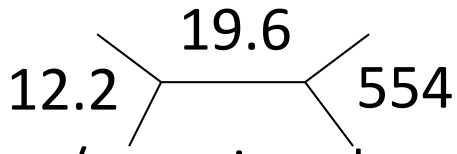
- A higher proportion of patients in the ruxolitinib arm had a history of prior thromboembolic events at baseline than in the BAT arm (35.5% vs 29.5%)
- There was one additional event in the ruxolitinib group over the course of randomized treatment (median exposure 81 wk)

[a] 1 patient in the BAT group had both myocardial infarction and pulmonary embolism.

Verstovsek S et al. NEJM 2015

Clinical Vignette

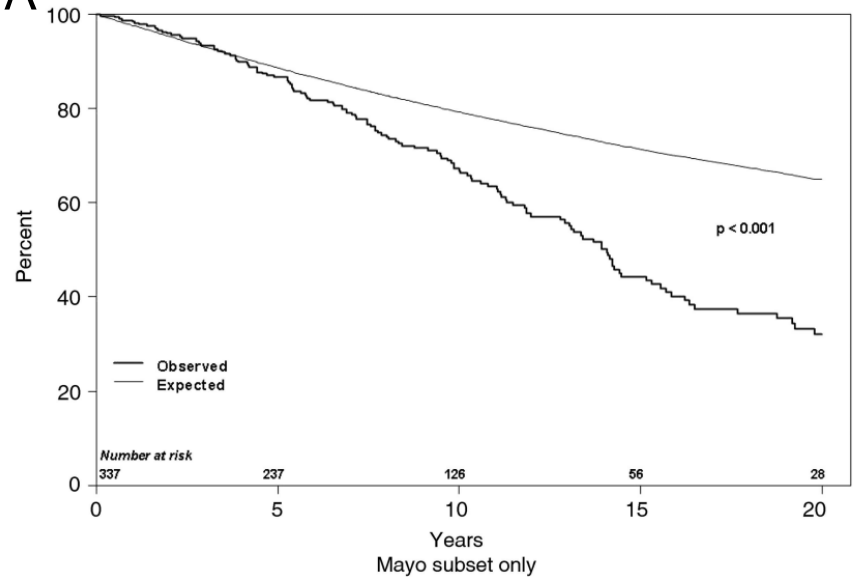
- A 67 year-old man presents for routine evaluation
- CBC reveals erythrocytosis with mild leukocytosis and thrombocytosis:



- He generally feels well but c/o pruritus that occurs after hot showers
- PEX unremarkable, no splenomegaly
- Epo level < 1.0 (2.6-18.5)
- JAK2 V617F: positive (68.2%)
- Patient declines bone marrow biopsy
- *Does the patient meet diagnostic criteria for polycythemia vera (PV)?*
- *Should additional testing be done? Role for genomic profiling?*
- *What is this patient's overall prognosis? Risk of transformation to AML?*
- *Should he be treated with ASA, phlebotomy, and/or hydroxyurea?*
- *Should treatment with ruxolitinib (JAK2 inhibitor) be considered?*

Age-Related Differences in Disease Phenotype in PV

A



B

PV clinical features	Age ≤45 (n = 120)	Age ≥ 65 (n = 84)	P-value
% Female	76	55	0.0023
Palpable splenomegaly	60 (51%)	22 (27%)	0.0012
WBC (x 10^9/L), median	9.2	13.4	0.004
JAK2 V617F allele burden (%)	51	66	0.015
Splanchnic vein thrombosis	16 (13%)	2 (2%)	0.0056
MF transformation	18 (15%)	8 (10%)	0.29
AML transformation	2 (2%)	5 (6%)	0.13

Figure 1. Reduced survival and age-associated clinical characteristics in polycythemia vera. (A) Survival in 337 Mayo Clinic patients with PV (44% followed to death; media survival 14.1 years) compared with expected survival based on individuals of the same age and gender from the US total population, adapted from Tefferi et al.[6] (B) Retrospective analysis of a cohort of 120 younger (age ≤ 45) and 84 older (age ≥ 65) patients with PV, adapted from Stein et al.[7]

Age-Related Differences in Disease Phenotype in PV

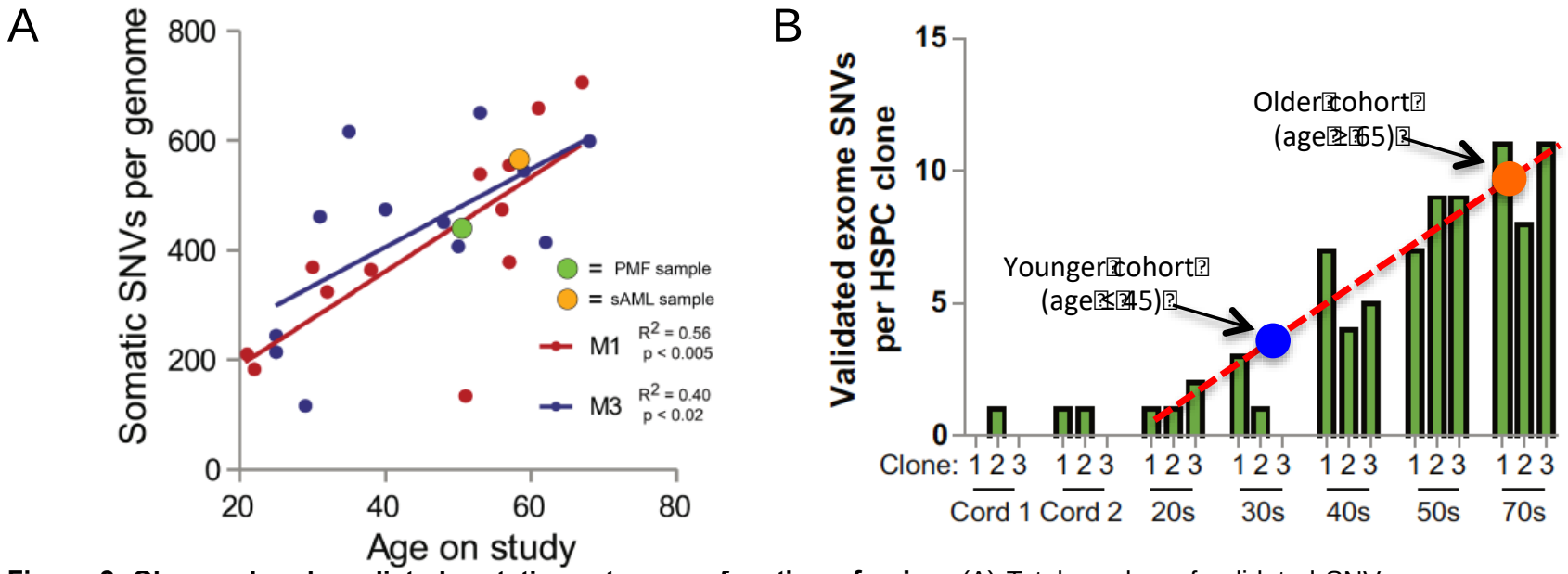


Figure 2. Observed and predicted mutation rates as a function of aging. (A) Total number of validated SNVs per genome versus age of patient in *de novo* AML; M1 AML (red), M3 AML (blue), adapted from Welch et al.[18] Green and yellow circles depict total SNVs identified in a patient with PMF transformed to sAML.[17] (B) Number of validated SNVs per exome identified in each of three clones derived from individual HSPCs from seven healthy donors, adapted from Welch et al.[18] Blue and orange circles represent predicted mutation rates in younger (age ≤ 45) and older (age ≥ 65) PV patients.

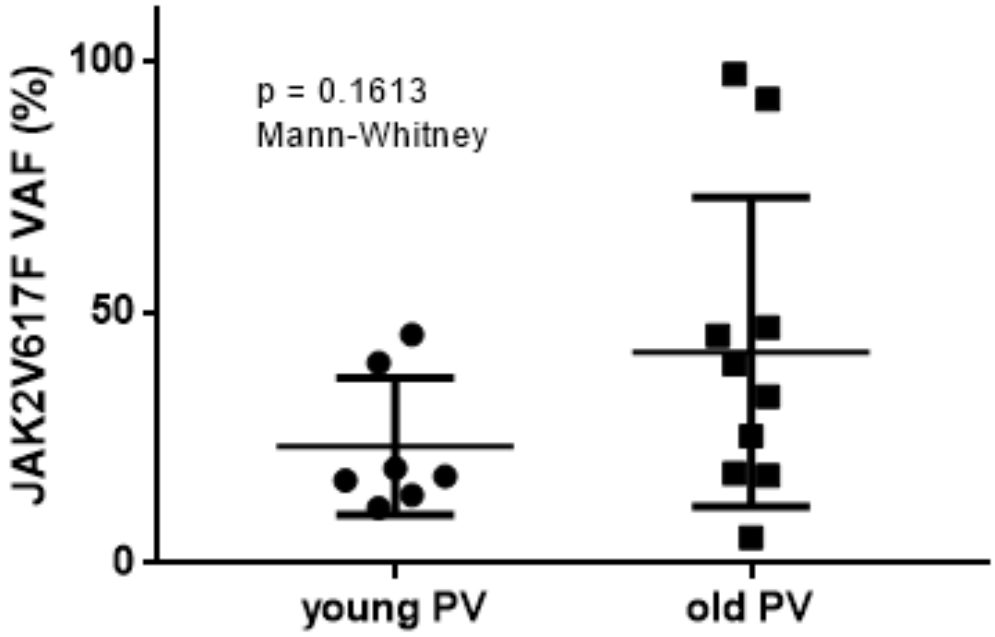
Age-Related Differences in Disease Phenotype in PV

Objective: To identify differences in the spectrum of genetic changes present in younger and older patients with polycythemia vera

- Exome capture sequencing of 10 younger (age ≤ 45) and 11 older (age ≥ 65) PV patient samples
- Identify somatic mutations in both cohorts
- Validate mutation hierarchy by genotyping single cell-derived clones

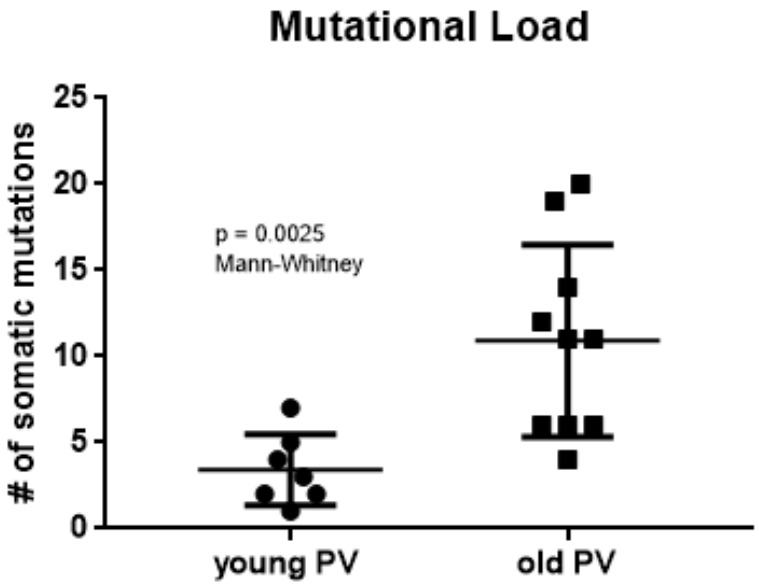
LONGER LIFE FOUNDATION

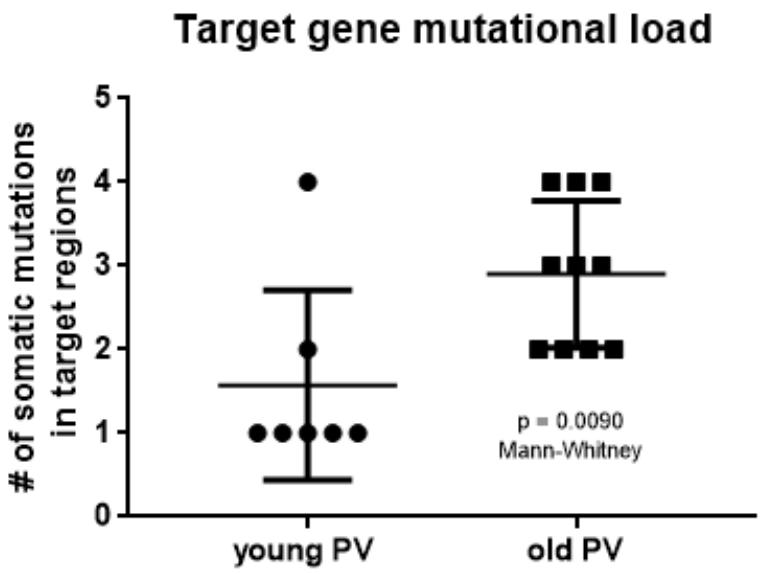
JAK2 V617F allele burden in young vs old PV patients



LONGER LIFE FOUNDATION

Mutational load in young vs old PV patients

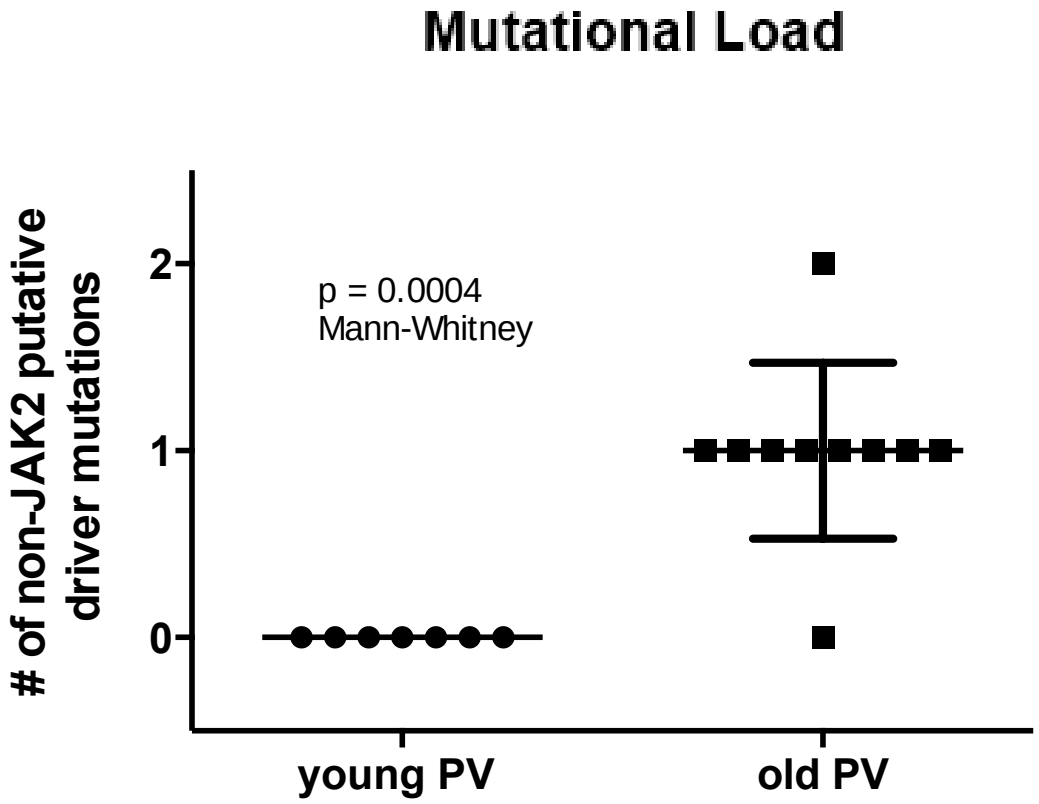




LONGER LIFE FOUNDATION

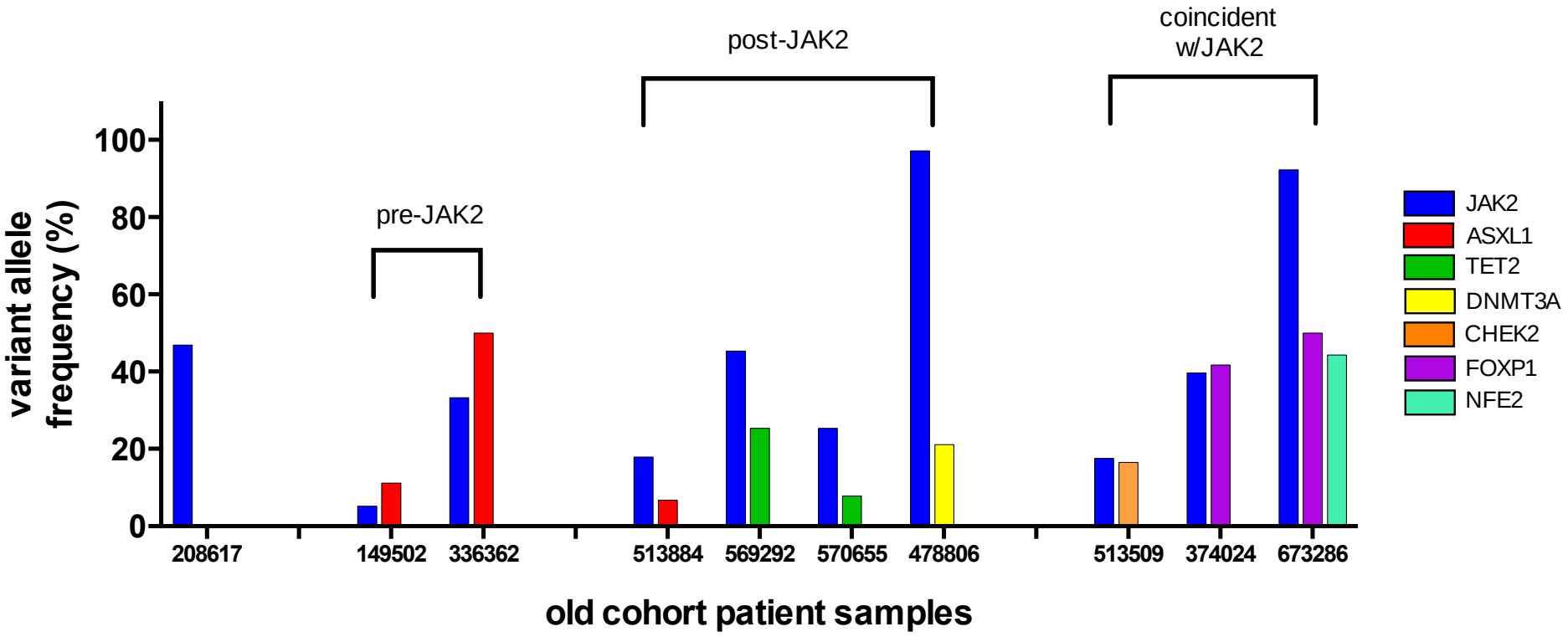
Mutational load in young vs old PV patients

- 0/7 young PV patients with likely cooperating mutations
- 9/10 old PV patients with likely cooperating mutations



LONGER LIFE FOUNDATION

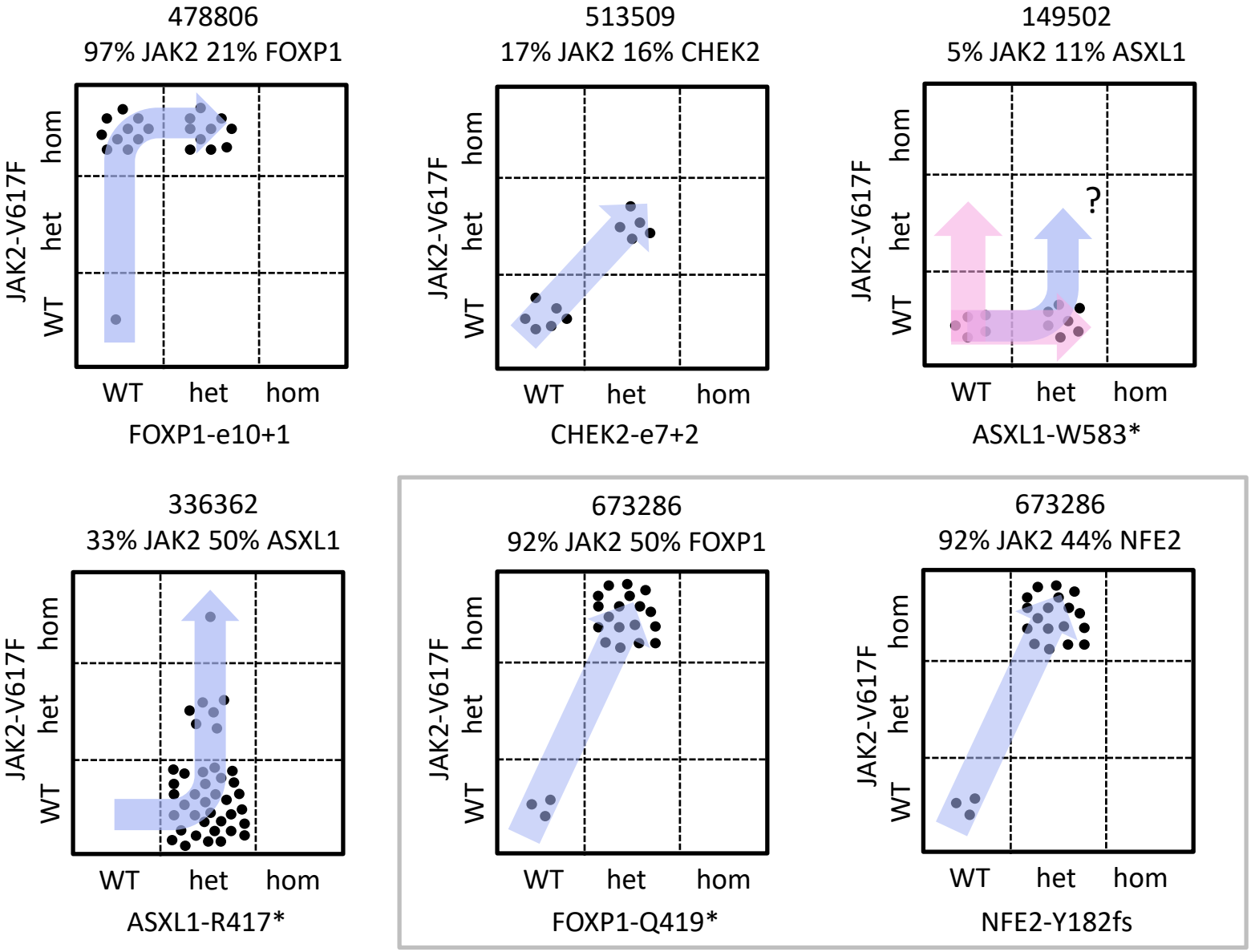
Clonal hierarchy in old PV patients



- 2/9 pts with mutations likely acquired *before* JAK2
- 4/9 pts with mutations likely acquired *after* JAK2
- 3/9 pts with mutations likely acquired *coincident* with JAK2

LONGER LIFE FOUNDATION

Clonal hierarchy in old PV patients



LONGER LIFE FOUNDATION

Ongoing studies/outstanding questions

- Germline analysis ongoing – are there variants that modify the likelihood of acquiring PV at young vs old age?
- ET patients also have low *JAK2* V617F allele burden – what factors differentiate ET from young PV patients?
- Does the inflammatory milieu play a role in determining age-associated PV phenotype?
- Validation in a larger cohort of young vs old PV patients