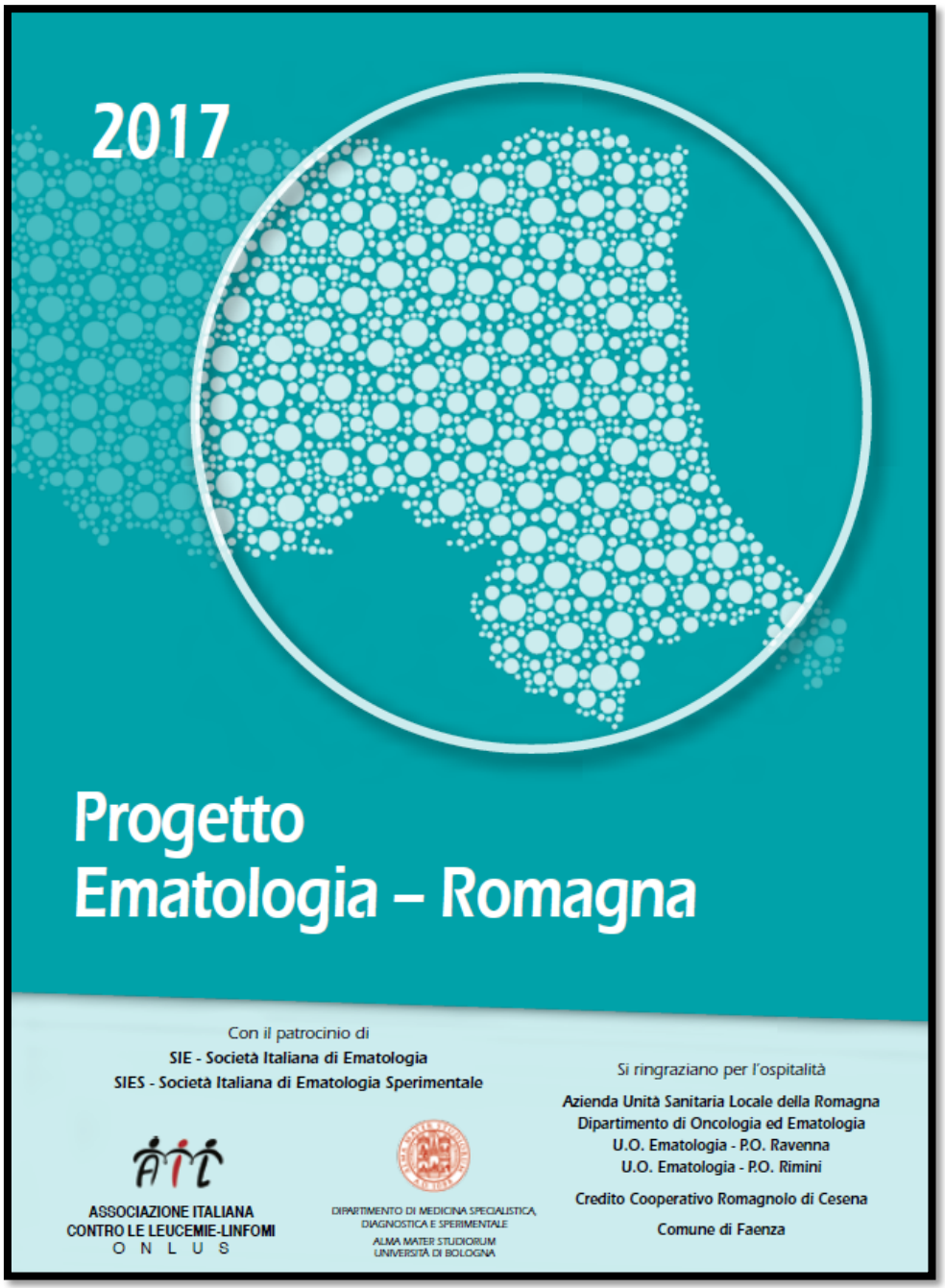

Mielofibrosi: i nuovi farmaci "target"

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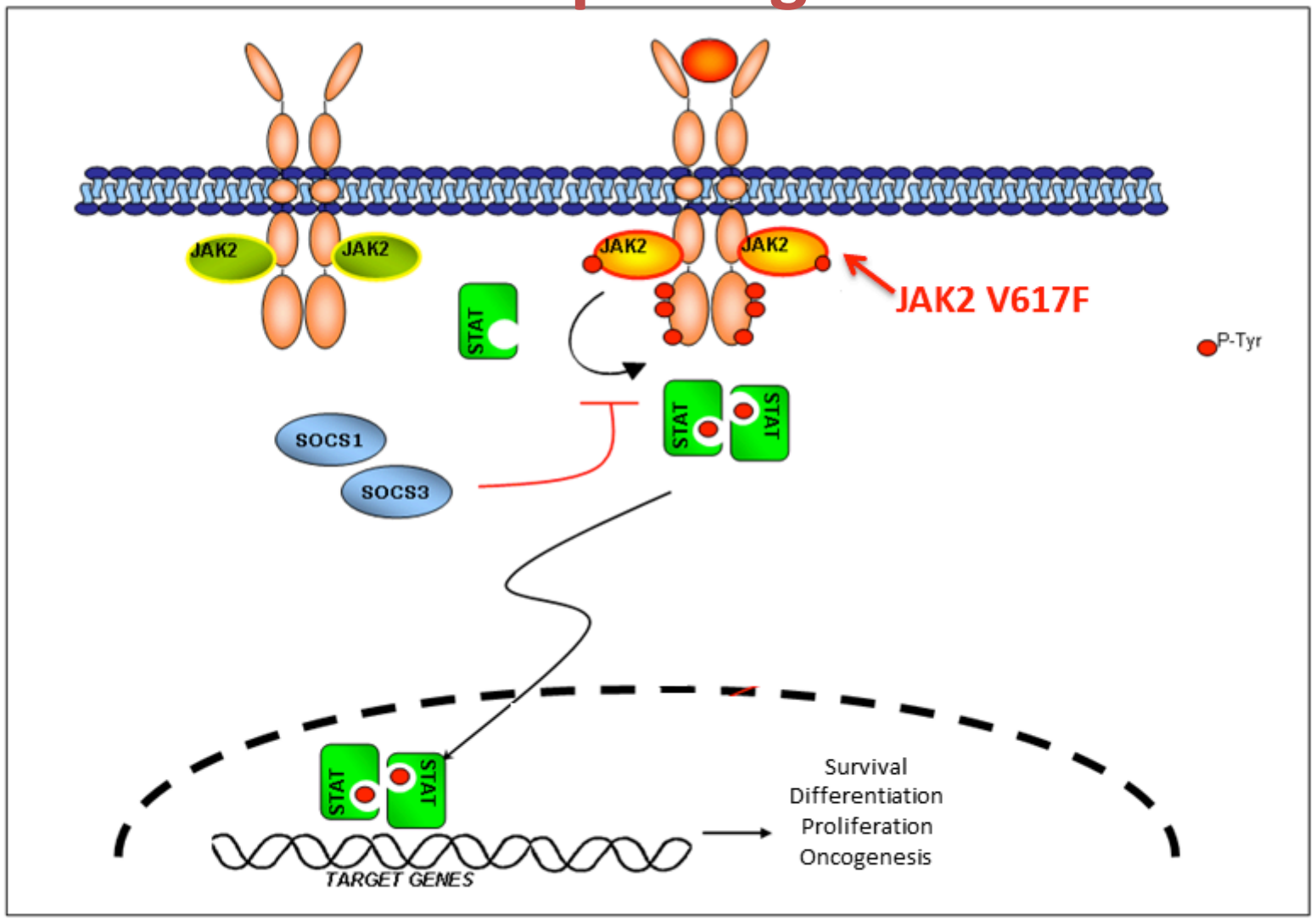
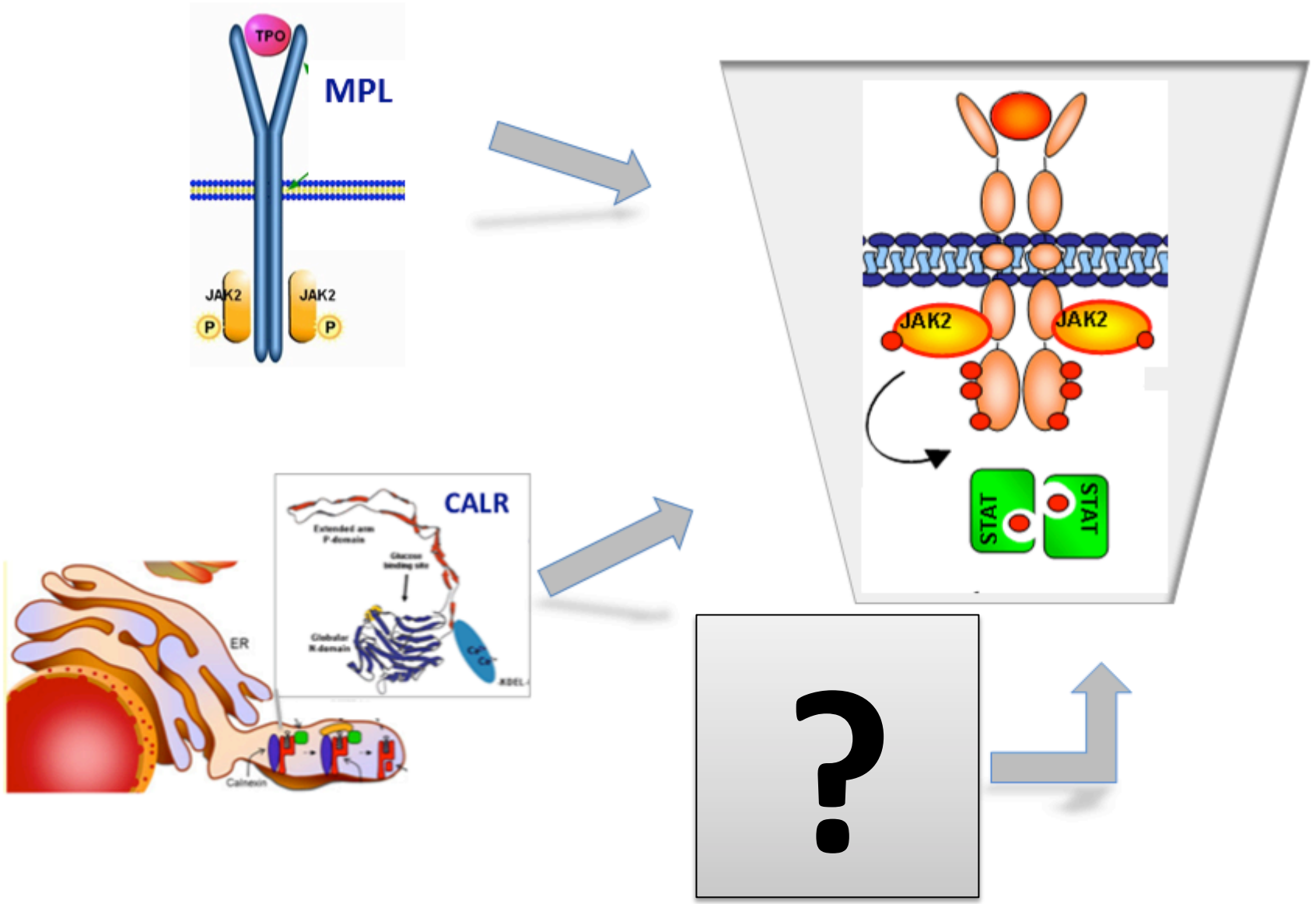
"L.e A. Seràgnoli"
Ospedale S. Orsola-Malpighi

The JAK-STAT pathway plays a central role in MPN pathogenesis

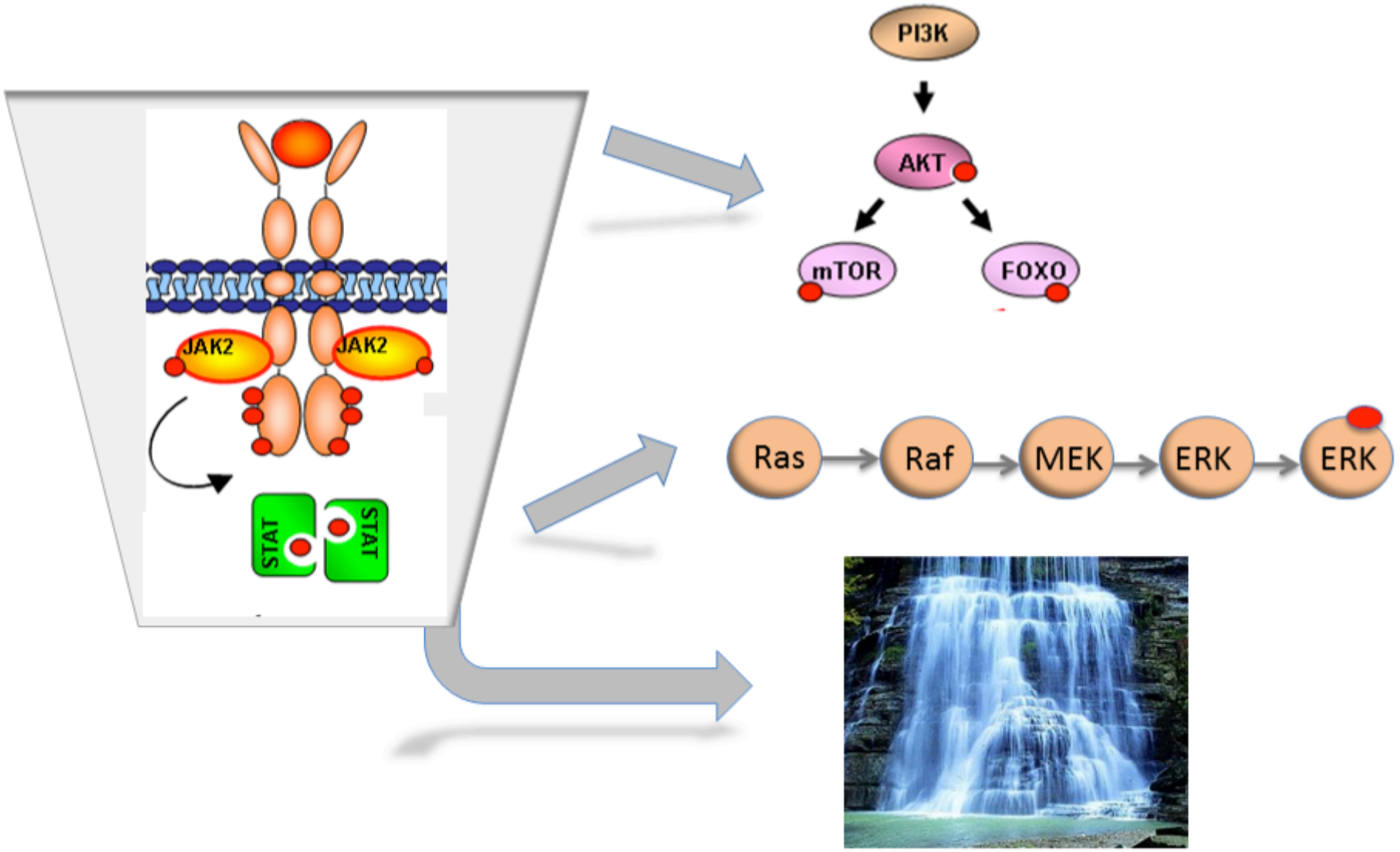


Vannucchi AM et al., CA Cancer J Clin. 2009; 59:171-91

The JAK-STAT pathway is activated also by MPL and CALR mutations

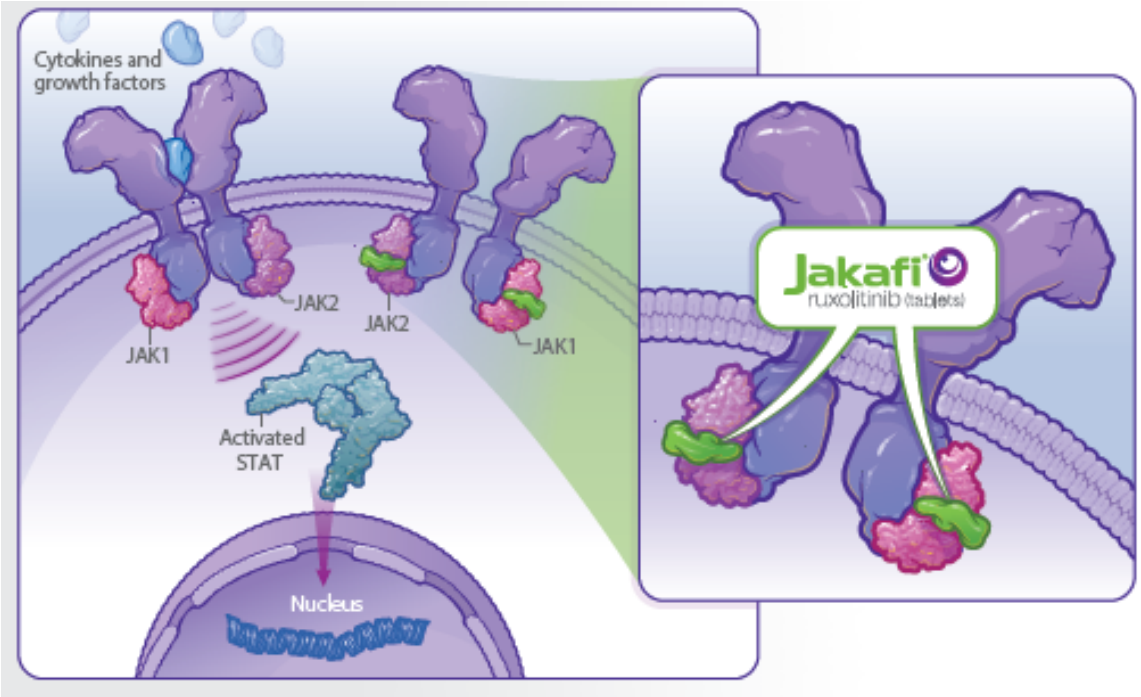


The JAK-STAT pathway activate many other pathways

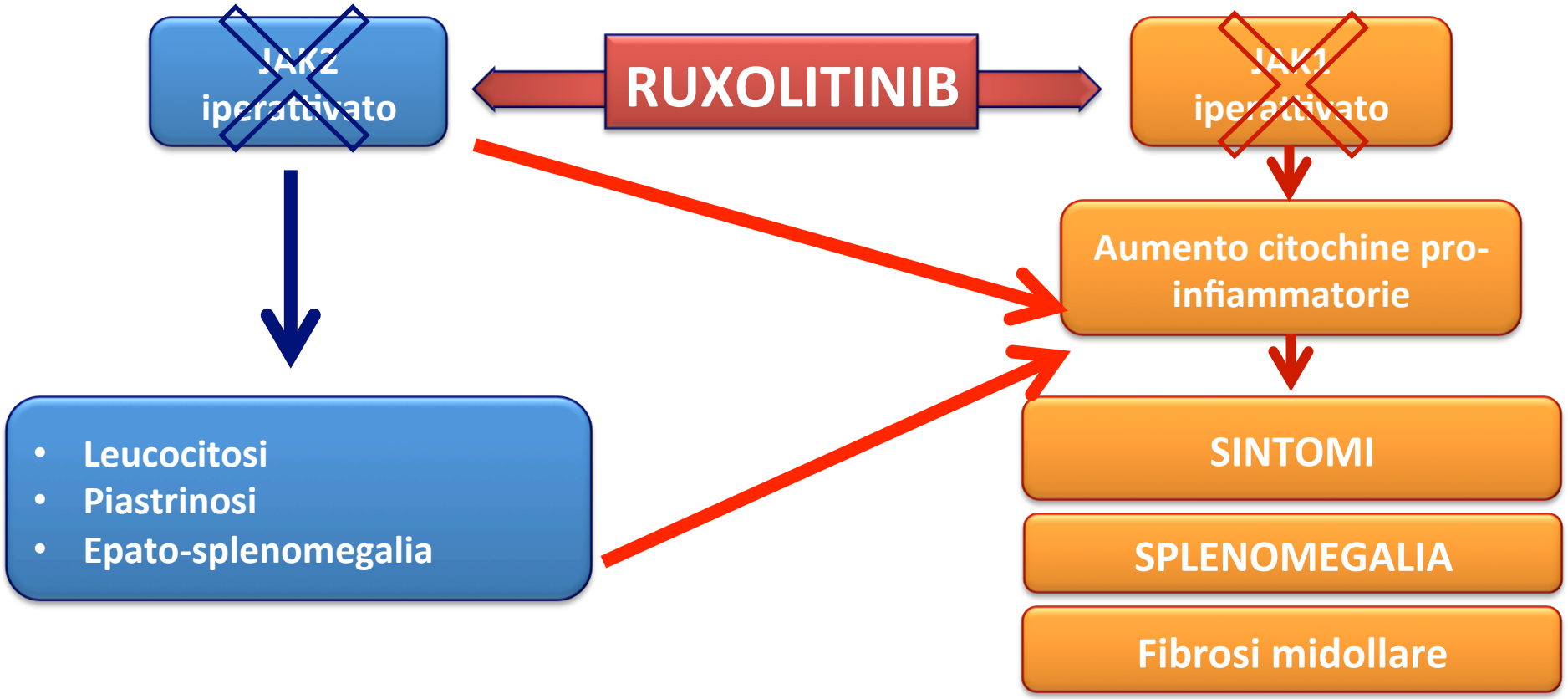


The big question in MF:
One target is enough?
Which is the right target?

The first big target: JAK1 & JAK2 - Ruxolitinib



Ruxolitinib: mechanism of action

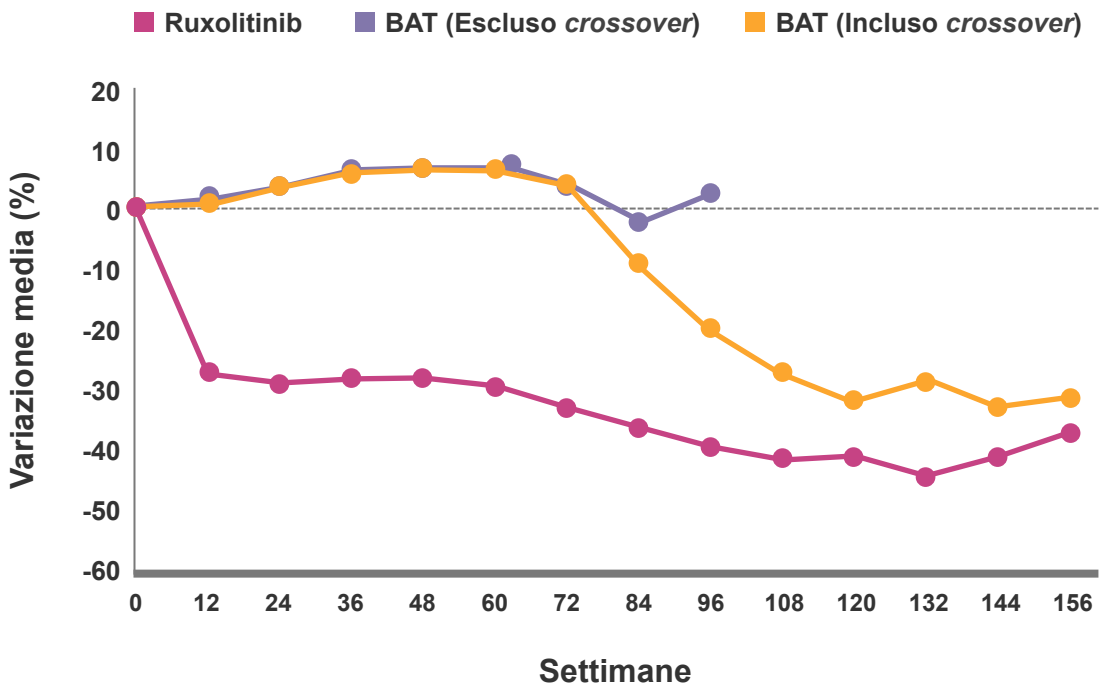


- *JAK2 è fondamentale per regolare la proliferazione cellulare, in particolare delle cellule emopoietiche (globuli rossi, globuli bianchi, megacariociti e piastrine)*
- *JAK1 è il principale mediatore della produzione di sostanze (citochine) che si liberano normalmente durante le infezioni e le infiammazioni*

Ruxolitinib is Active on spleen:

- *60% achieve >35% MRI-reduction anytime*
- *50% lose response at 3 years*

Variazione media percentuale del volume splenico nel corso del tempo[1]



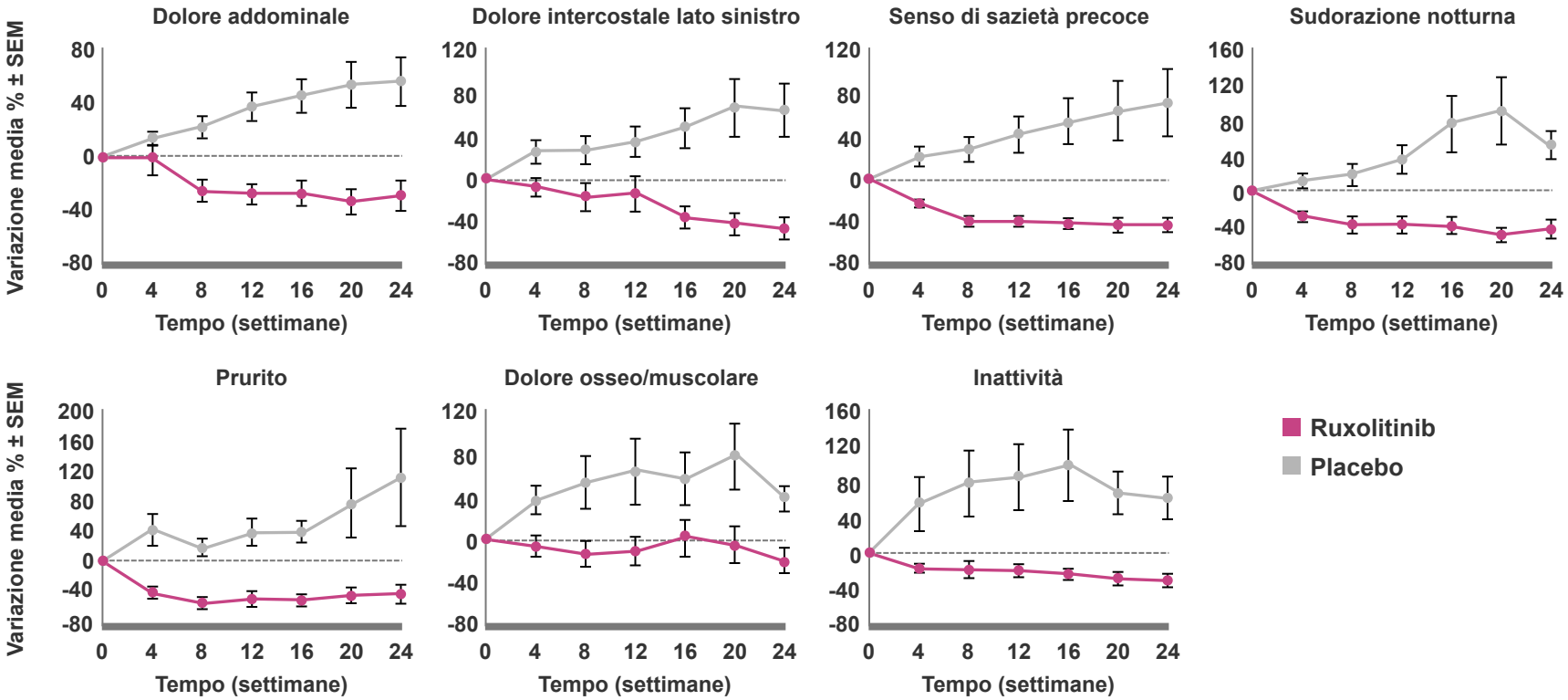
Studio COMFORT-II verso BAT
Dati a 3 anni

Ruxolitinib	146	136	125	118	113	102	93	79	61	49	58	46	50	16
BAT, inclusi pazienti passati a ruxolitinib	73	60	46	41	38	29	23	20	16	16	14	15	13	6

1. Cervantes F, et al. Blood 2013; 122(25): 4047-53.

Ruxolitinib is Active on symptoms:

- *>80% response rate*
- *Responses per single symptom*

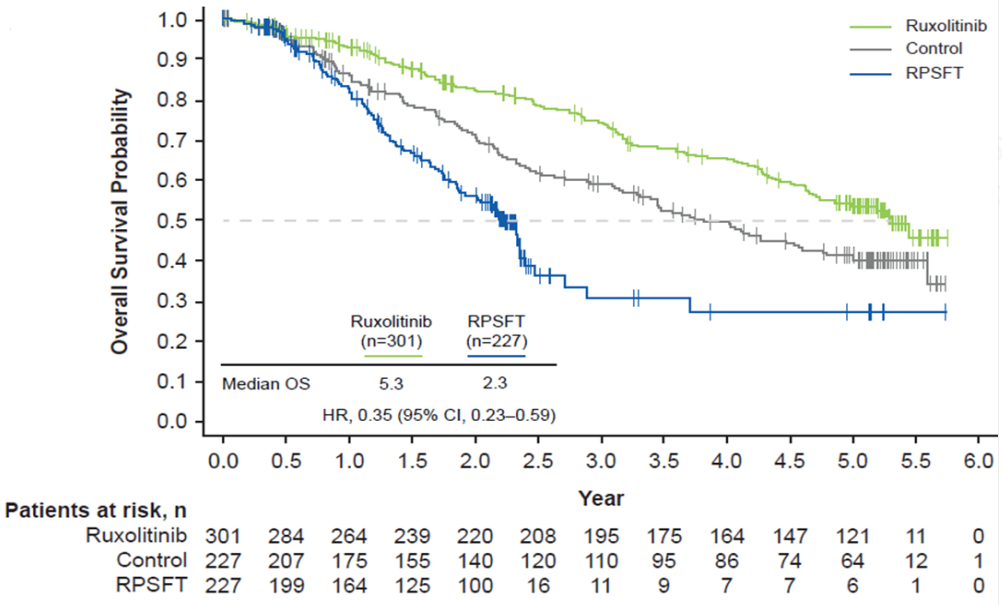


Studio COMFORT-I verso placebo[1]

1.Mesa RA, et al. J Clin Oncol 2013; 31(10): 1285-92.

Pooled analysis of 5-yr data from COMFORT trials

Ruxolitinib was associated with 30% reduction in the risk of death



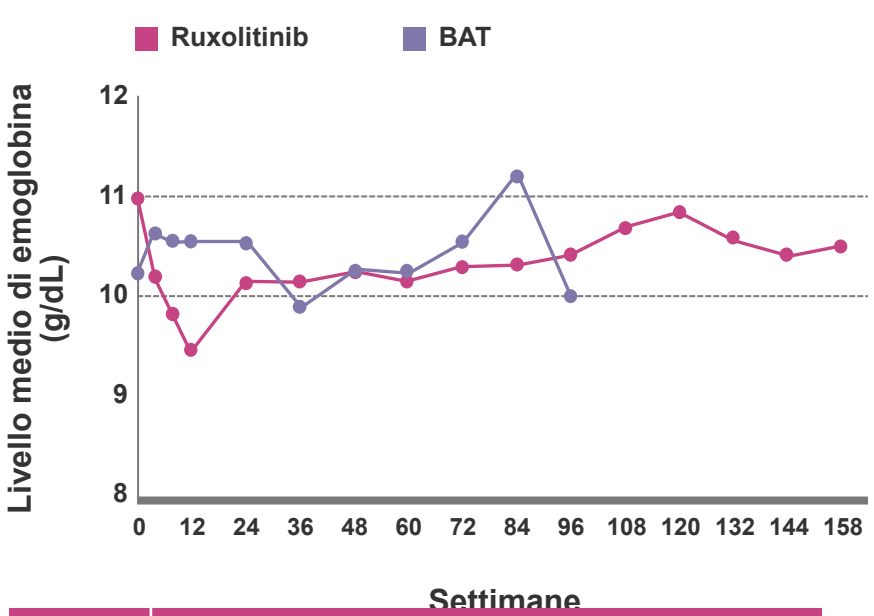
- Significantly fewer patients died in the JAKAVI group than in the control group[1]
  - 42.5% vs. 51.5%; median OS: 5.3 vs. 3.8 yrs
  - HR 0.70, 95%CI 0.54–0.91; *P* = 0.0065)
- Correction for crossover (RPSFT model):[1]
  - Median OS: 5.3 years with JAKAVI and 2.3 years with control
  - HR 0.35, 95% CI 0.23–0.59
- Corrections for patients who crossed over to ruxolitinib suggested that the separation between ruxolitinib and control OS curves was primarily caused by a delay in ruxolitinib treatment.

Reference: Verstovsek et al., 2016

L’emoglobina e la conta piastrinica si stabilizzano in corso di terapia con ruxolitinib

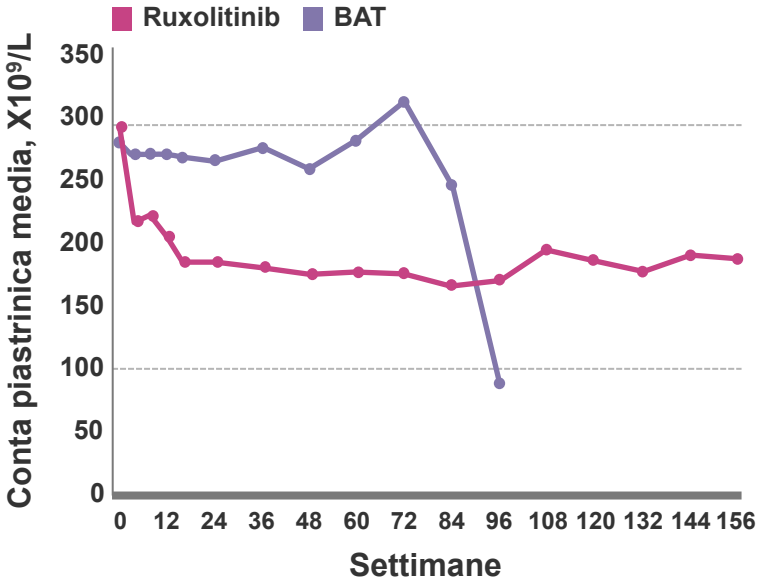
Studio COMFORT-II - *Follow-up* a 3 anni[2]

Variazione dei livelli di emoglobina nel tempo



Ruxolitinib	146	127	121	101	101	96	90	78	71	64	61	59	49	50	22
BAT	73	59	53	37	31	29	19	10	6	1					

Variazione dei livelli di piastrine nel tempo



N di pazienti															
Ruxolitinib	146	126	116	98	96	91	89	77	68	63	61	57	48	49	22
BAT	73	60	51	40	28	27	19	10	6	1					

1. Verstovsek S, et al. Haematologica. 2015 Jan 23. pii: haematol.2014.115840. [Epub ahead of print], 2.Cervantes F, et al. Blood. 2013;122(25):4047-53.

Infezioni osservate nei primi 6 mesi di terapia con ruxolitinib

Studio COMFORT-II - F*ollow-up* a 3 anni[1]

Ruxolitinib	**Settimana**						
Randomizzazione + estensione, %	0–24 (n=146)	24–48 (n=134)	48–72 (n=116)	72–96 (n=101)	96–120 (n=93)	120–144 (n=81)	**144–168 (n=72)**
SMQ - Parametro	**Percentuale di pazienti**						
Infezioni	50,0	35,1	37,9	25,7	43,0	33,3	25,0
Bronchite	3,4	6,7	8,6	3,0	10,8	4,9	4,2
Gastroenterite	5,5	3,0	0,9	1,0	2,2	1,2	0
Rinofaringite	13,7	5,2	7,8	4,0	10,8	3,7	4,2
Infezione delle vie urinarie	**4,8**	**2,2**	**5,2**	**4,0**	**5,4**	**3,7**	2,8

- **Negli studi registrativi, le infezioni erano generalmente più comuni nel braccio di trattamento con ruxolitinib rispetto ai bracci controllo.**
- **Le infezioni più severe si sono manifestate con una frequenza sovrapponibile nel braccio placebo e *Best Available Therapy* (BAT).**

1.Cervantes F, et al. Blood. 2013;122(25):4047-53.

Very (very) sum of Ruxolitinib

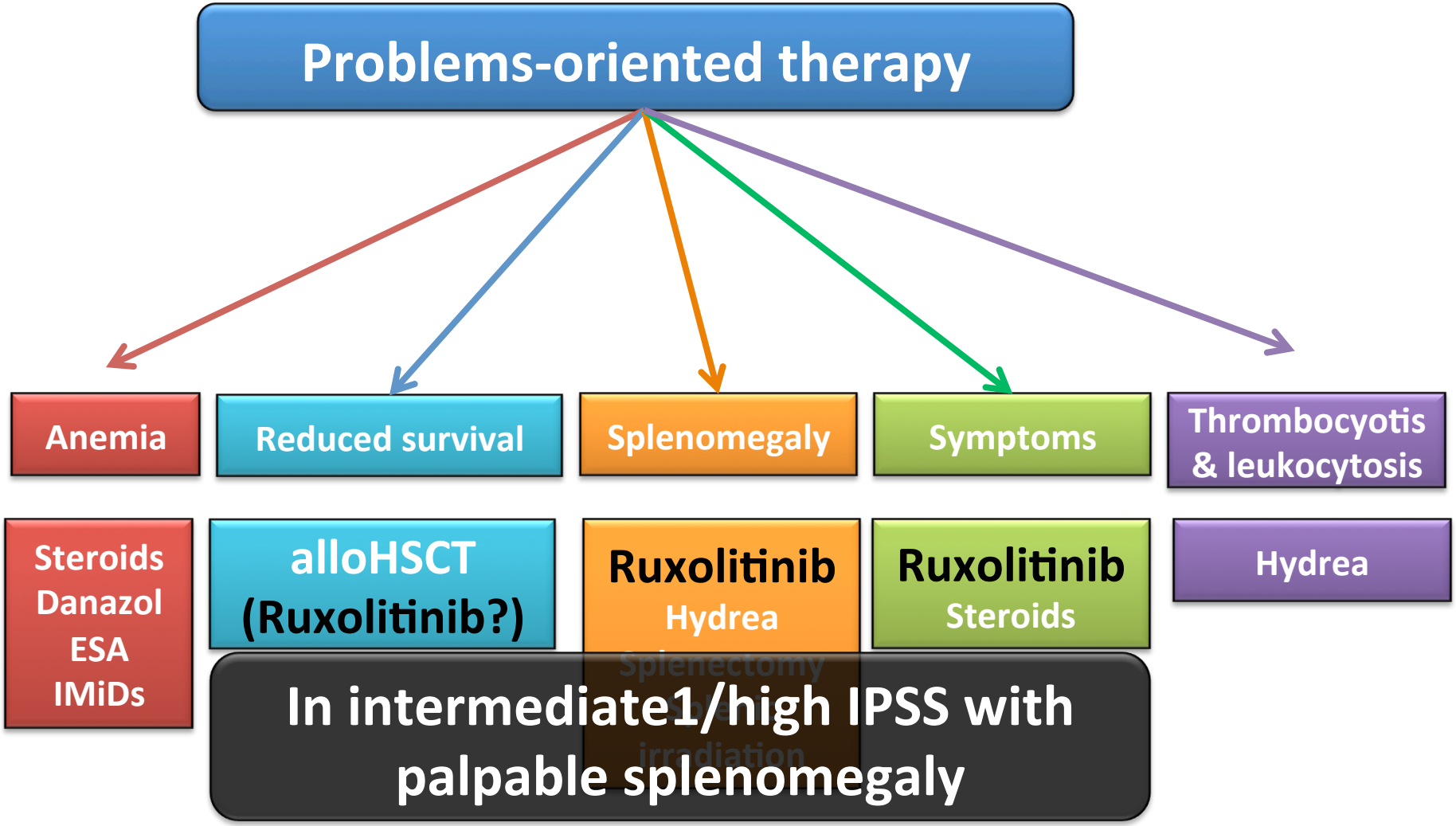
- 53% of patients receiving RUX achieved spleen response at any time
- The probability of maintaining a spleen response was 0.51 at 3 years and 0.48 at 5.0 years

- One-third of evaluable *JAK2* V617F-positive patients had a >20% reduction in allele burden
- 16% improved fibrosis; 32% had stable fibrosis, 18% had a worsening at their last assessment

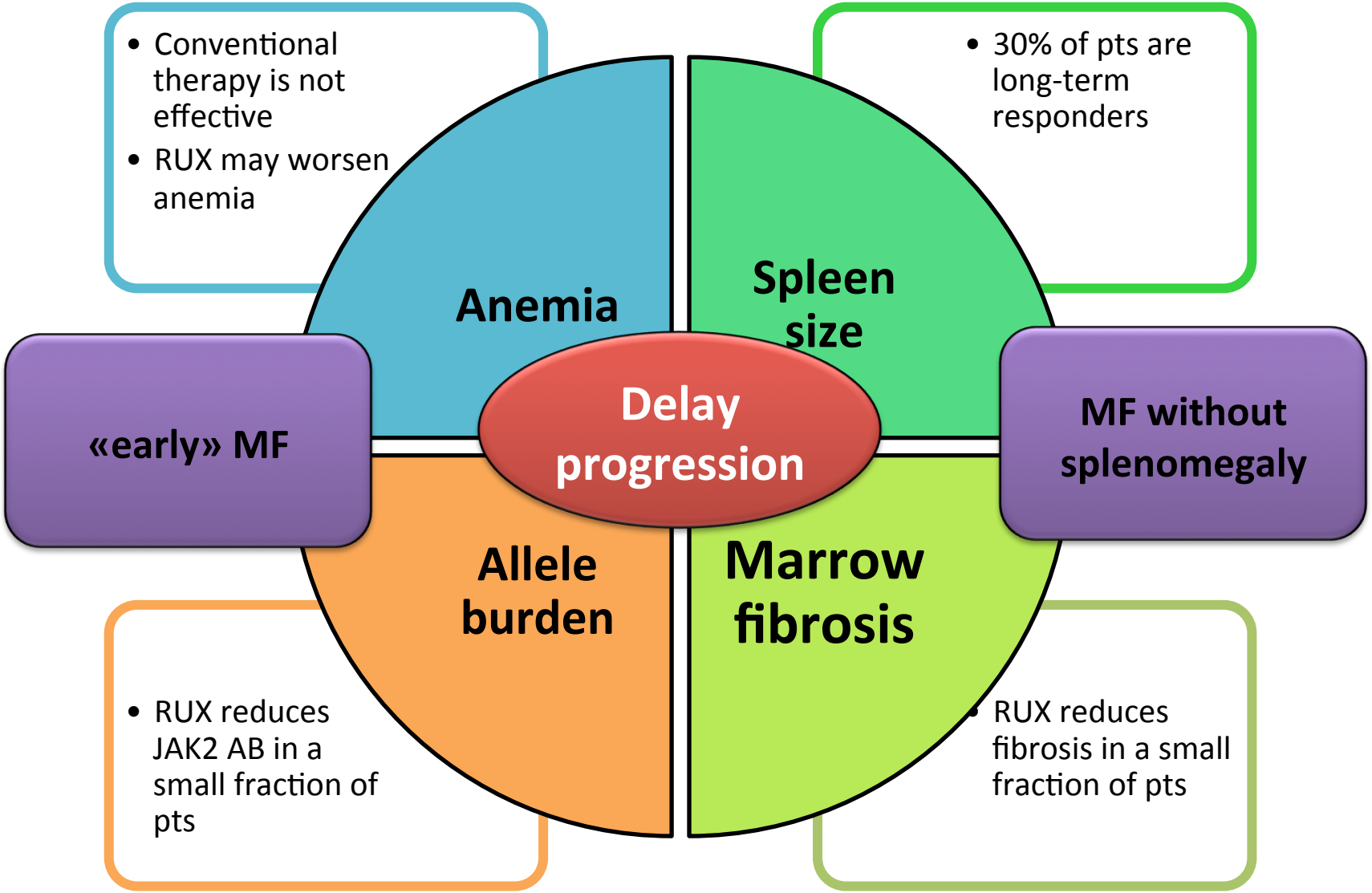
- Adverse events grade 3-4: anemia (22%), thrombocytopenia (15%), pneumonia (6%)
- Ruxolitinib-associated anemia, which occurs predominantly during early therapy, is not predictive of shortened survival

Harrison et al; Leukemia. 2016 Aug;30(8):1701-7; Gupta et al, Haematologica. 2016 Dec;101(12):e482-e484

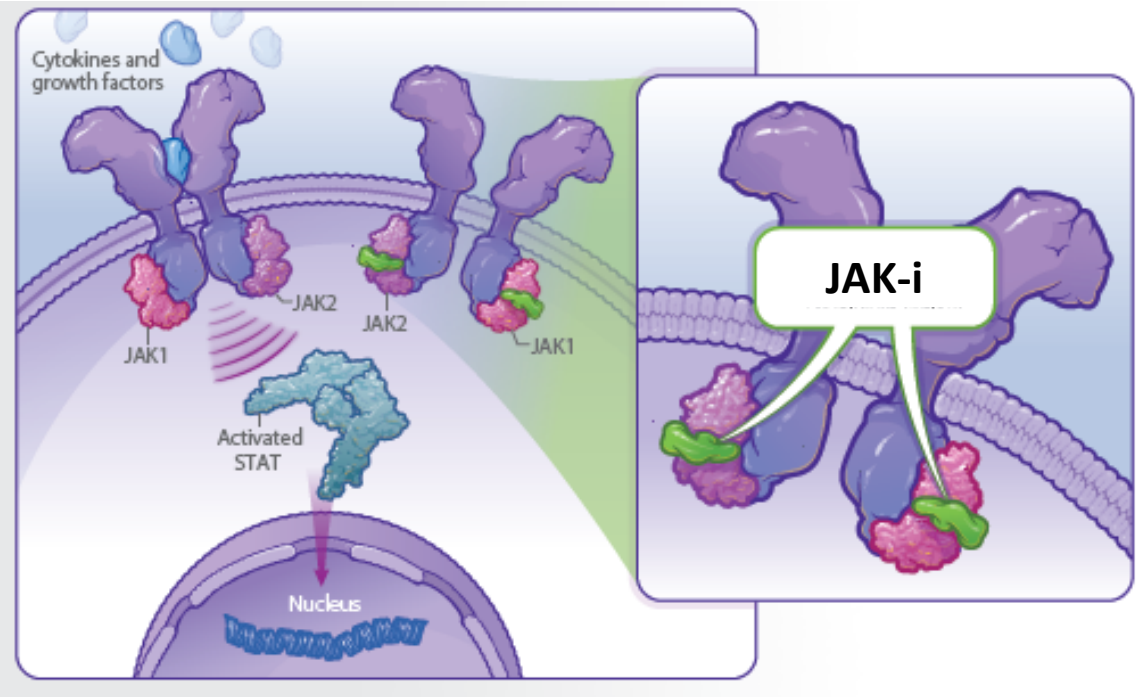
Conventional treatment of MF (Italy 2017)



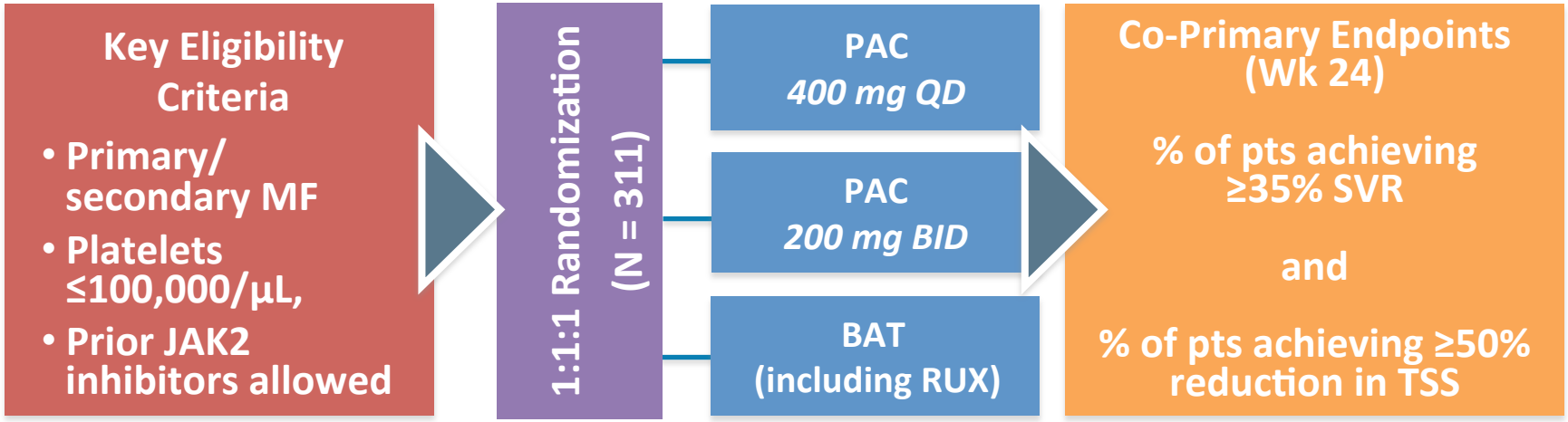
Unmet clinical needs in MF



Targeting one big target (again): JAK2 inhibitors other than Ruxolitinib



Pacritinib - PERSIST2 Phase 3 Study



- Crossover from BAT allowed after progression (any time) or at Wk 24
- Study Objectives: Efficacy of pooled QD and BID PAC vs BAT & efficacy of QD PAC or BID PAC separately vs BAT
- 221 pts enrolled; 405 received prior RUX in any arm; BAT was RUX in 30% of pts

PAC on full clinical hold by the FDA on 2/8/2016 based on concerns around excess deaths and cardiac and hemorrhagic events in PERSIST-1.

Mascarenas J et al. Blood 2016 128:LBA-5

Courtesy Dr J. Mascarenas

Pacritinib - Efficacy Summary

Endpoint	Statistics	PAC QD+BID (n=149)	PAC QD (n=75)	PAC BID (n=74)	BAT (n=72)
Patients with ≥35% SVR from BL to Wk 24	n (%)	27 (18.1)	11 (14.7)	16 (21.6)	2 (2.8)
	95% CI*	12.3-25.3	7.6-24.7	12.9-32.7	0.3-9.7
	P value vs BAT	***0.001***	***0.017***	***0.001***	-
Patients with ≥50% reduction in TSS from BL to Wk 24	n (%)	37 (24.8)	13 (17.3)	24 (32.4)	10 (13.9)
	95% CI*	18.1-32.6	9.6-27.8	22.0-44.3	6.9-24.1
	P value vs BAT	***0.079***	***0.652***	***0.011***	-

Mascarenas J et al. Blood 2016 128:LBA-5

Courtesy Dr J. Mascarenas

Momelotinib in MF, phase 1-2 study at Mayo on 100 patients

Dose escalation (100/400 mg), dose confirmation (300 mg OAD) phases

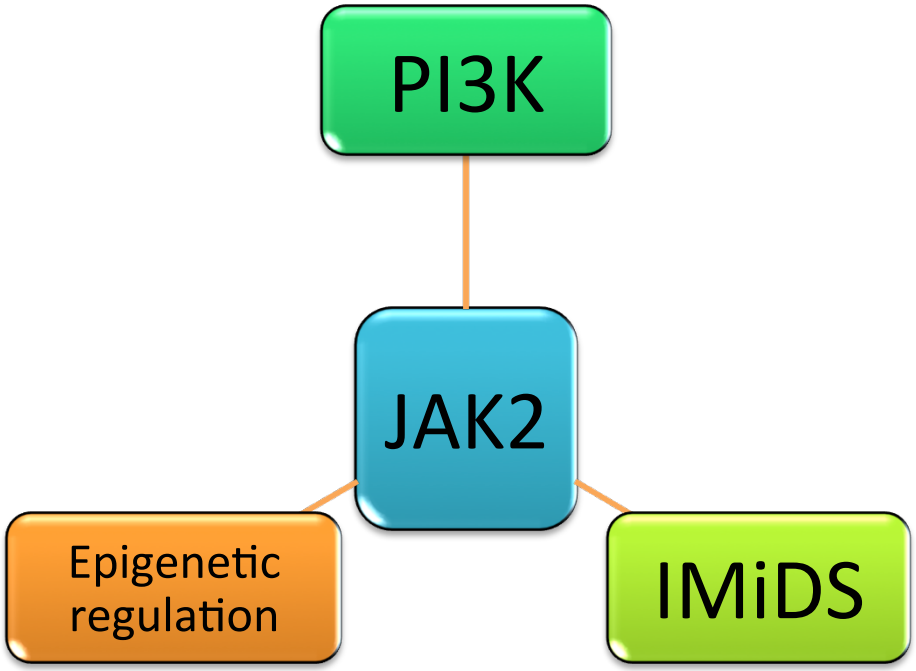
- 43% Spleen response
- **44% Anemia response**
- **51% of transfusion-dependent patients became transfusion independent**
- 34% G3-G4 thrombocytopenia; 5% G3-G4 anemia; 7% increased lipase; 4% increased AST/ALT; 47% G1-G2 peripheral neuropathy

ACD - rat model to explain anemia improvement with MOME

- MOME inhibits Activin A receptor type 1 (ACVR1)-mediated hepcidin expression in the liver, leading to increased mobilization of sequestered iron from cellular stores resulting in erythropoiesis stimulation

Tefferi A et al. Blood 2016 128:1123: Asshoff et al, Blood. 2017 Feb 10. pii: blood-2016-09-740092

Adding a target: Combinations of JAK2 inhibitors with other agents



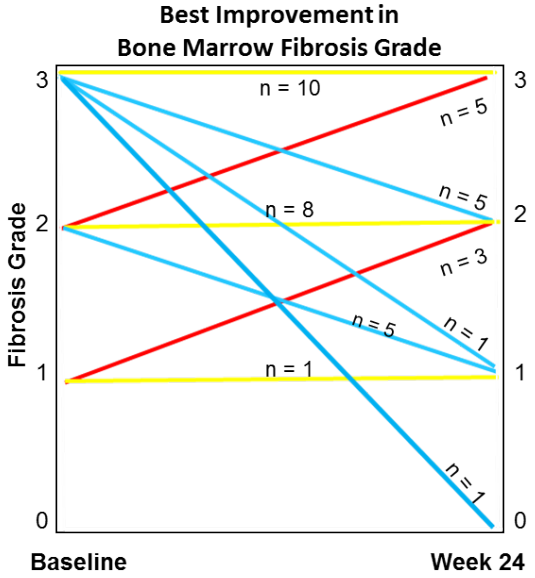
RUX + AZACYTIDINE

- Primary or secondary MF, Int-1/Int-2/ High-Risk per DIPSS
- **RUX 15-20 mg BID** (cycles 1-3)
- **AZA 25 mg/m^2 IV daily x 5 days** (starting cycle 4 day 1)
- Azacytidine could be increased to 50mg/m^2 and 75 mg/m^2
- Cycles repeated every 4-6 weeks

- 44 patients enrolled
- 39 patients evaluable for response
- New onset cytopenia in 16 (36%)
- 1 pt off study due cytopenia

- BM fibrosis was reduced in 30.7%
- JAK2V617F burden was reduced in 63%

Baseline spleen ≥ 5 cm	29
>50% palpable spleen length reduction at 24 weeks	14 (48%)
>50% reduction any time	23 (79%)
Median time to CI spleen	1.8 mo (0.9-16.8)



JAK2 allele reduction (24 evaluable)

13% > 50%
21% 20-50%
29% 0-20%

17% stable/increase

Daver et al. Blood 2016 128:1127

Courtesy Dr N. Daver

RUX + TGR-1202 (PI3Kδ inhibitor)

- PI3Kdelta is highly expressed in MF patients and its inhibition leads to reduced proliferation and increased apoptosis

- Phase I Dose Escalation Trial enrolling 12 MF pts with suboptimal responses to RUX (mainly, persistence of splenomegaly and/or symptoms)
- Treatment plan:
  - Highest tolerated dose of ruxolitinib without change for ≥ 8 weeks PLUS
  - escalating doses of TGR-1202 in a standard 3+3 algorithm (400 mg/600mg/800 mg)

- The maximum tolerated dose (MTD) of TGR-1202 + RUX was not established
- 11 of 11 evaluable patients had improvement in hematologic parameters and 10 had reduced MF symptoms with a median 33% decrease in TSS. One patient achieved a CR (regression of marrow fibrosis)
- 7 pts off-study (2 patients due to G3 amylase/lipase elevation; 1 patient due to G3 diarrhea; 4 for lack of response/MF progression)

Moyo et al. Blood 2016 128:1125

Courtesy Dr T. Moyo

RUX + Pomalidomide

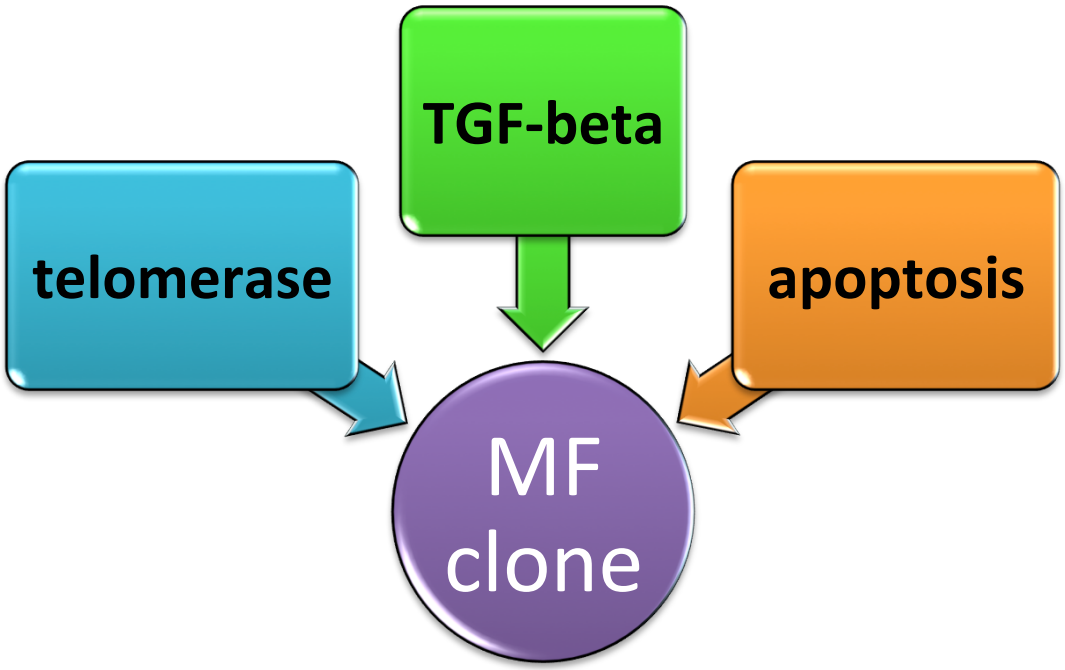
- Phase-Ib/-II combination study of RUX plus POM (MPNSG-0212 trial, NCT01644110)
- MF pts with anemia (Hb <10 g/dL and/or RBC transfusion dependence) and splenomegaly
- POM is given at 0.5 mg/d QD. RUX is started at 10 mg BID with dose modifications to optimize efficacy and to manage toxicity

- 37 patients DIPSS intermediate-2/high risk were enrolled
  - 11 subjects (30%) were RBC-transfusion-dependent
  - Median spleen size by ultrasound was 18 cm (range, 13-28 cm)

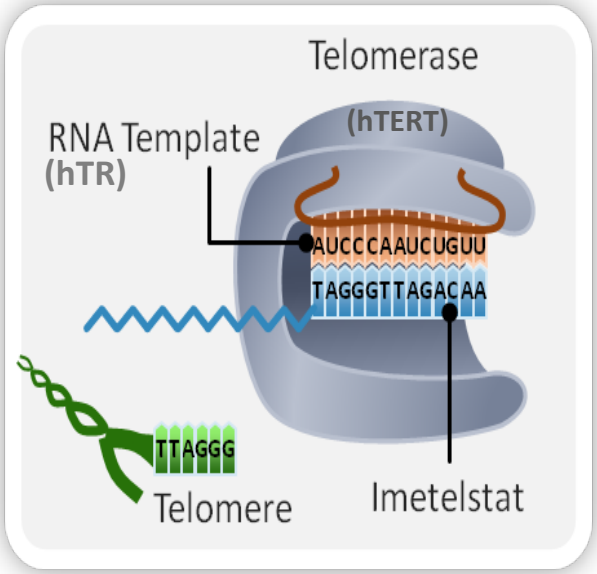
- 6 subjects (16.2%) responded with spleen reduction or ≥2 g/dL hemoglobin increase / RBC transfusion independence (N=3)
- 12 subjects (32%) continued treatment after cycle 12 because of response or SD plus clinical benefit
- Worsening of anemia was the most frequent AE (35%). Two SAE (G4 anemia and G3 neuropathy) were considered therapy-related.

Stegelman F et al. Blood 2016 128:1939

Moving the target: drugs alternative to JAK-inhibitors



Imetelstat: targeting telomerase activity



13-mer oligonucleotide sequence with lipid tail as a competitive and potent inhibitor of telomerase enzyme activity

While normal human somatic cells do not or only transiently express telomerase and therefore shorten their telomeres with each cell division, MF cells typically express high levels of telomerase and show unlimited cell proliferation.

High telomerase expression allows cells to proliferate and expand long-term and therefore supports tumor growth.

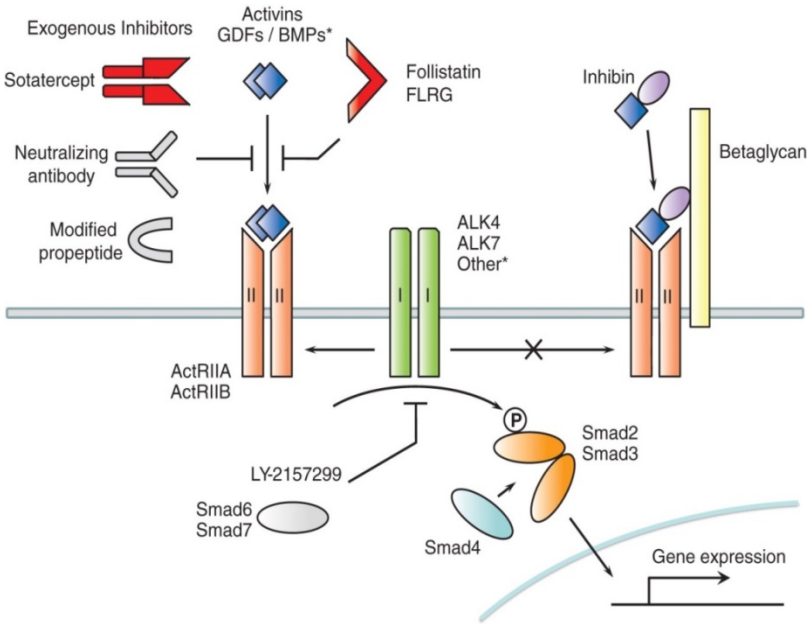
- long half-life in bone marrow, spleen and liver (estimated t½ = 41 hr with doses 7.5-11.7 mg/kg)
- Administered by two hour i.v. infusion
- sterile, lyophilized powder, designed to deliver 200mg per 10 mL vial; final volume 500mL NaCl
- premedication with diphenhydramine and hydrocortisone, or equivalent, are recommended to decrease chance of infusion reactions

Efficacy of Imetelstat in MF

- 33 pts accrued, 76% of patients discontinued IME, median duration of treatment, 8.6 months
- Complete or partial response: 21% (4 CR; 3 PR)
  - median time to onset: 3.5 months
  - median duration of response: 18 months (CR), 10 months (PR)
  - **4 CR had reversal of BM fibrosis**
  - 3 of 4 CR had molecular remission
  - response: **27% in JAK2+**, 0% in JAK2-; 0% in ASXL1+ and **32% in ASXL1-**; **38% in U2AF1/SF3B1+** and 4% in U2AF1/SF3B1-
- 8/10 with WBC> 25 $x10^9$/L normalized (3) or halved WBC (5)
- 11 with PLT > 400 $x10^9$/L normalized (10) or halved PLT (1)
- 35% reduces the spleen size

Tefferi et al., NEJM 2015

Sotatercept- TGF beta inhibitor



Sotatercept binds to and sequesters ligands of the transforming growth factor beta (TGF-ß) superfamily, relieving their blockade of terminal erythroid differentiation

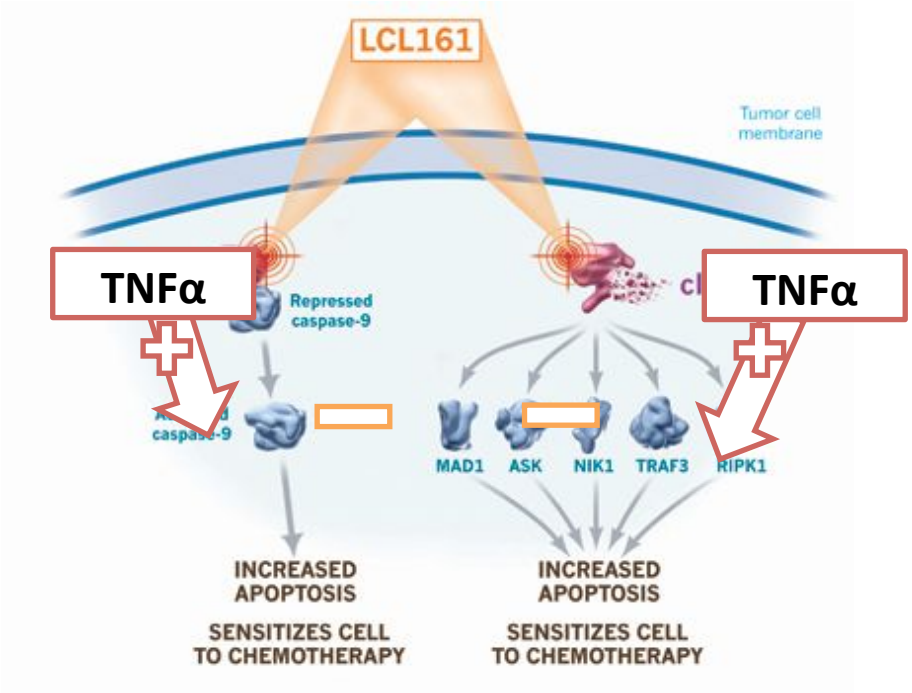
Phase 2 study including 19 MF patients with ANEMIA

- **5 of 14 (36%) evaluable pts have responded; all female**
- **13 have discontinued, mainly due to lack of efficacy (5 had no response, 2 went to SCT, 2 had MF progression, 1 transformed to AML, 1 withdrew consent, 1 had unrelated medical problems)**
- **Only AEs possibly attributable to sotatercept: grade 3 HYPERTENSION, injection site reaction, pain in extremity, bone pain, myalgia (all grade 1)**

Bose P et al. Blood 2016 128:478

Courtesy Dr P. Bose

LCL161, a SMAC Mimetic



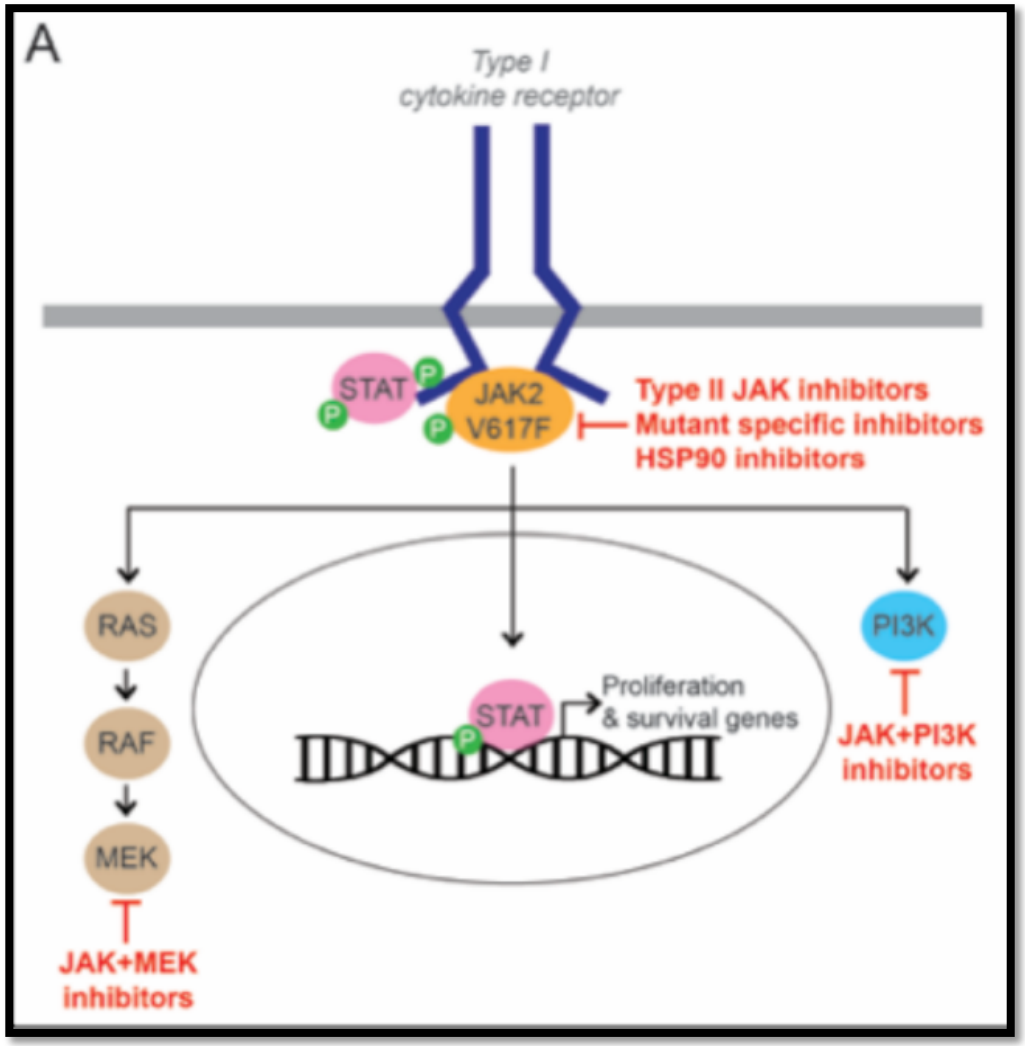
- Second mitochondria-derived activator of caspases (SMAC) mimetics, or **inhibitors of apoptosis (IAP) antagonists**, lead to increased apoptotic cancer cell death, especially in high TNFα-expressing tumor models like MF
- 25 pts intm2/high IPSS risk resistant/ intolerant to JAK-i

- **Responses in 7/21 evaluable (clinical improvements in anemia and/or symptoms and/or splenomegaly)**
- **Mainly hematological toxicity**

Pemmaraju N et al. Blood 2016 128:3105

The future:
Refining the target

Second-generation JAK2 inhibitors

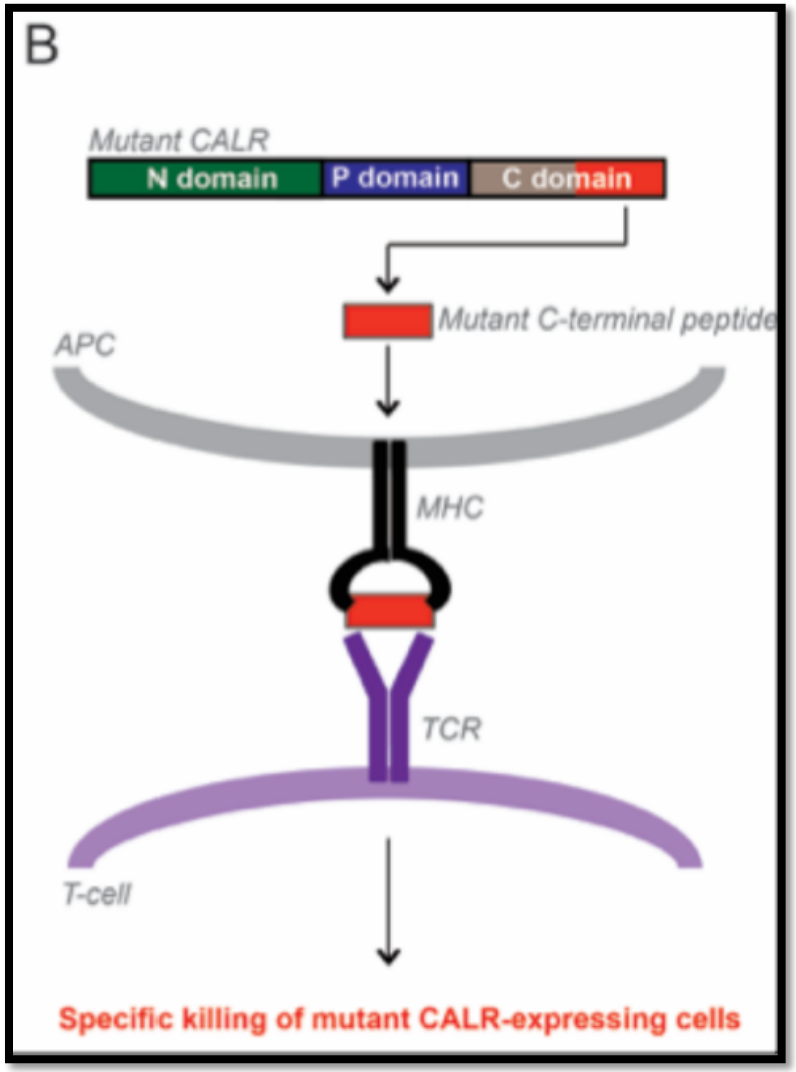


- **Type II JAK inhibitors** inhibit JAK2 in its inactive form, and preferentially inhibit JAK2V617F over wild type JAK2.

- **Mutant specific JAK2 inhibitors** are being developed to specifically target the JAK2V617F mutant protein.

Meyer SC, Keller MD, Chiu S, Koppikar P, et al. CHZ868, a Type II JAK2 Inhibitor, reverses type I JAK inhibitor persistence and demonstrates efficacy in myeloproliferative neoplasms. Cancer Cell 2015;28:15-28

Immunological targeting of mutant CALR



Potential mechanism for immune responses to be generated against mutant CALR.

Derivatives of the mutant C-terminal peptide of mutant CALR have been shown to elicit T-cell responses in *CALR*-mutant MPN patients following *ex vivo* peptide stimulation, suggesting that the mutant CALR C-terminus contains tumor-specific neo-epitopes that are targeted by T-cells.

CALR C-terminus is immunogenic, and may represent a promising immunotherapeutic target in CALR-mutant MPN patients

Holmström MO, Riley CH, Svane IM, et al. 2016. The CALR exon 9 mutations are shared neoantigens in patients with CALR mutant chronic myeloproliferative neoplasms. Leukemia 2016;30:2413-6

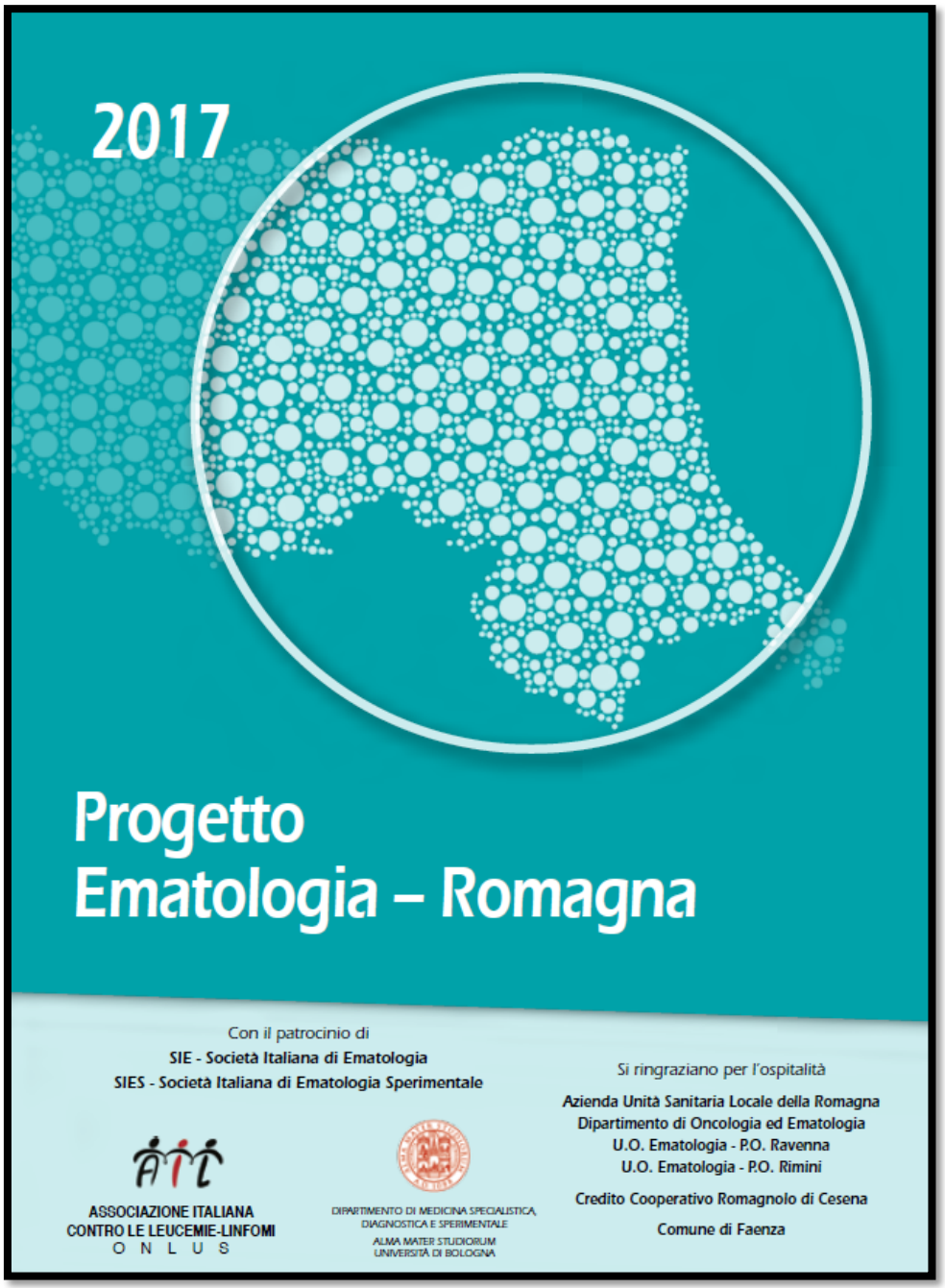
Conclusions

- Ruxolitinib has paved the way for target therapy in MF and is the most promising option for clinical practice in symptomatic patients

- The pathogenesis of MF is complex and many players contribute to disease phenotype (molecular abnormalities, epigenetic dysregulation, chronic inflammation). It is therefore unlikely that targeting one target may induce the remission of the disease.

- The possible future:
  - Pacritinib
  - Momelotinib
  - Imetelstat
  - New combo trials: AZA / PI3Kdelta-i

- Issues to investigate:
  - Immunological targets (CALR)
  - Type II & mutant-specific JAK2-inhibitors



Grazie!

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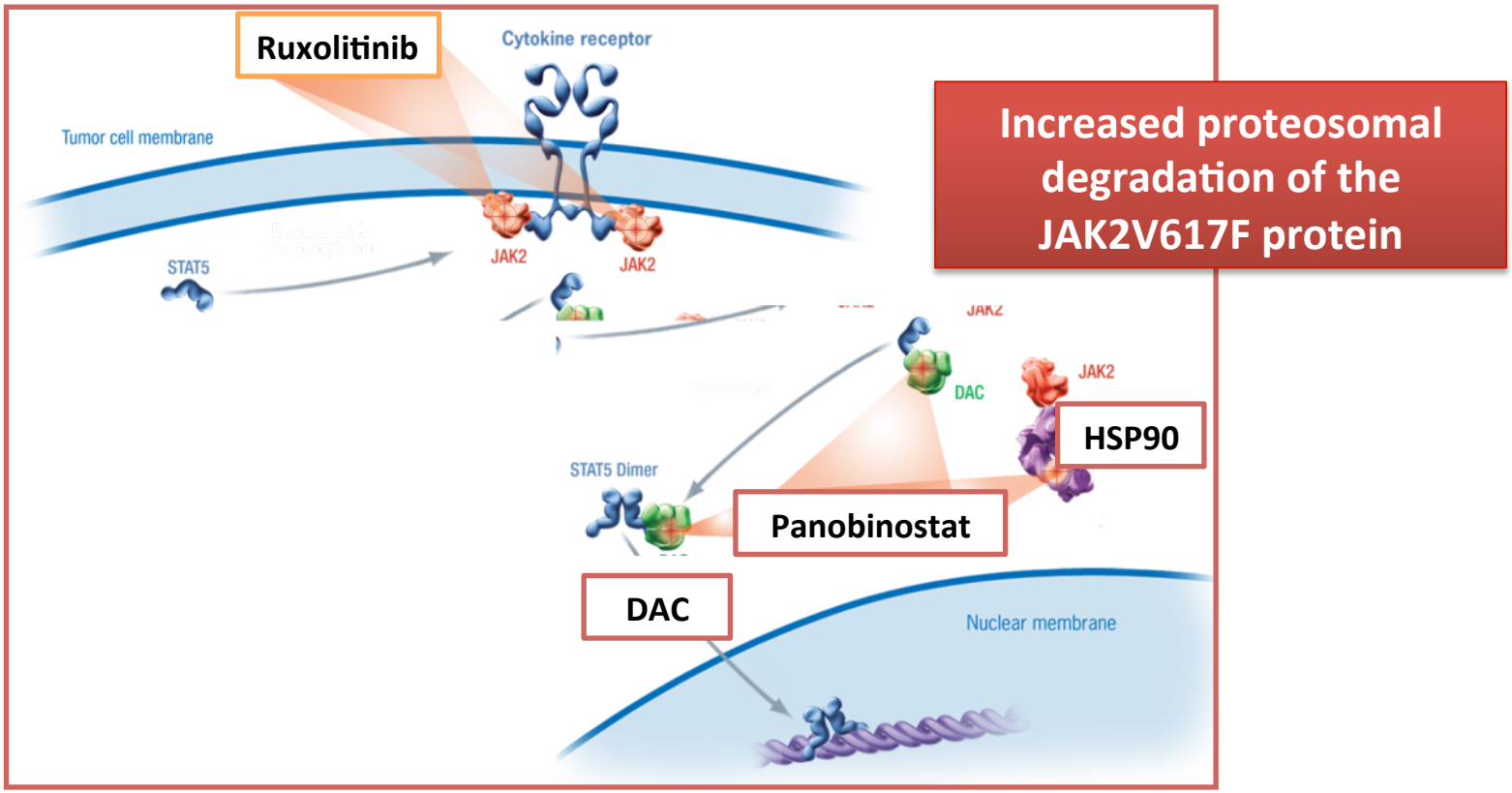
RUX & PANOBINOSTAT
(hystone deacetylase inhibitor)



The down-regulation of the JAK2 V617F protein occurs through **inhibition of the association with HSP90, leading to proteasomal degradation of the JAK2 V617F protein**

RUX and Panobinostat

Phase 1b, open-label, multicenter, dose-finding study (NCT01433445)

Patients

- PMF or PPV/PET-MF
- ≥1 IPSS risk factor[1]
- Palpable spleen ≥5 cm

Dose-escalation phase

- **Determine MTD/RP2D (n = 9, minimum)**
- **Enroll cohorts until MTD and/or RP2D reached**

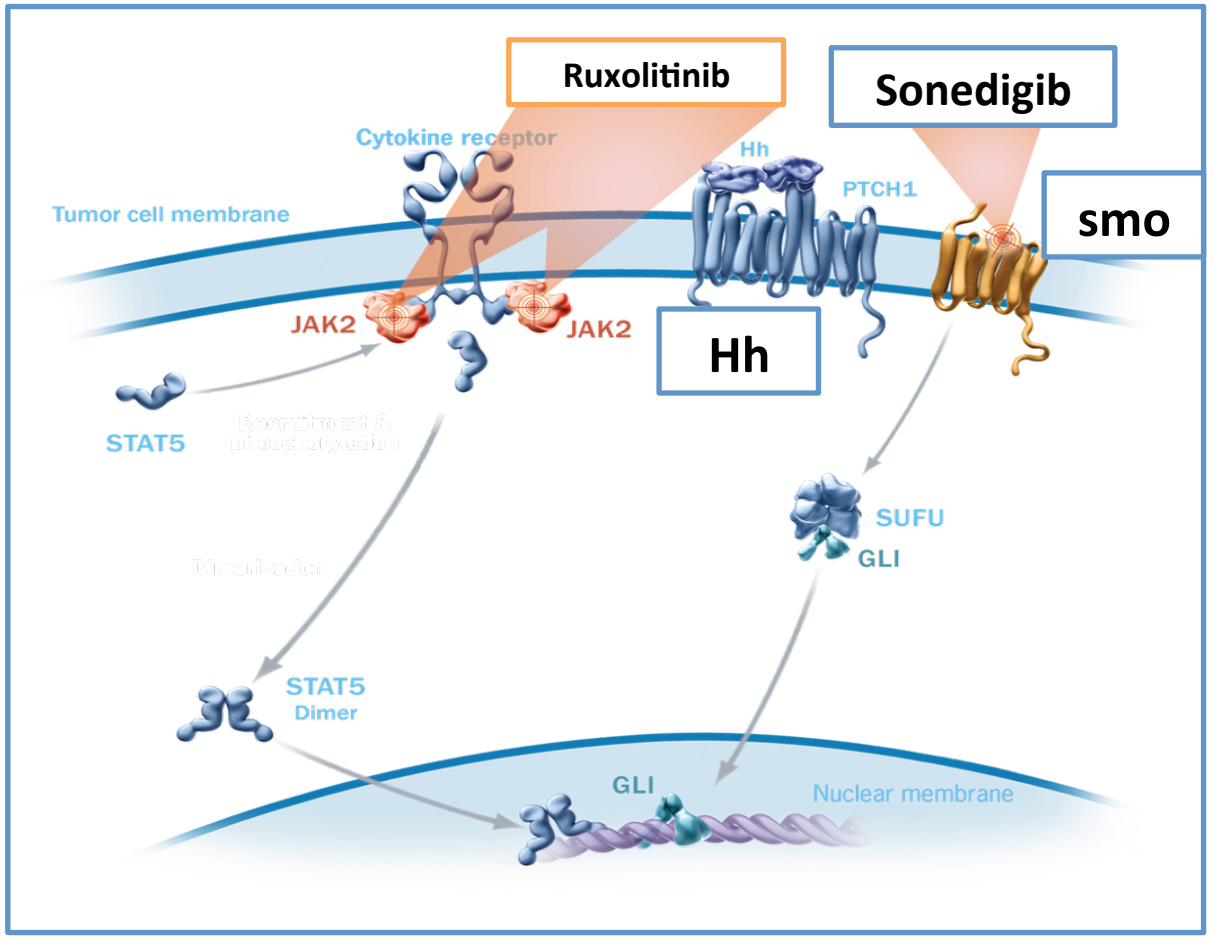
Safety-expansion phase

- **23 additional patients at RP2D**
- **2 x 28-day cycles**
- **Confirm RP2D**

Dose Level	Combination Treatment Dose	
	INC424	LBH280
1	5 mg BID	10 mg TIW/QOW
2	10 mg BID	10 mg TIW/QOW
3	15 mg BID	10 mg TIW/QOW
4	15 mg BID	15 mg TIW/QOW
5	15 mg BID	20 mg TIW/QOW
6 (RP2D)	**15 mg BID**	**25 mg TIW/QOW**

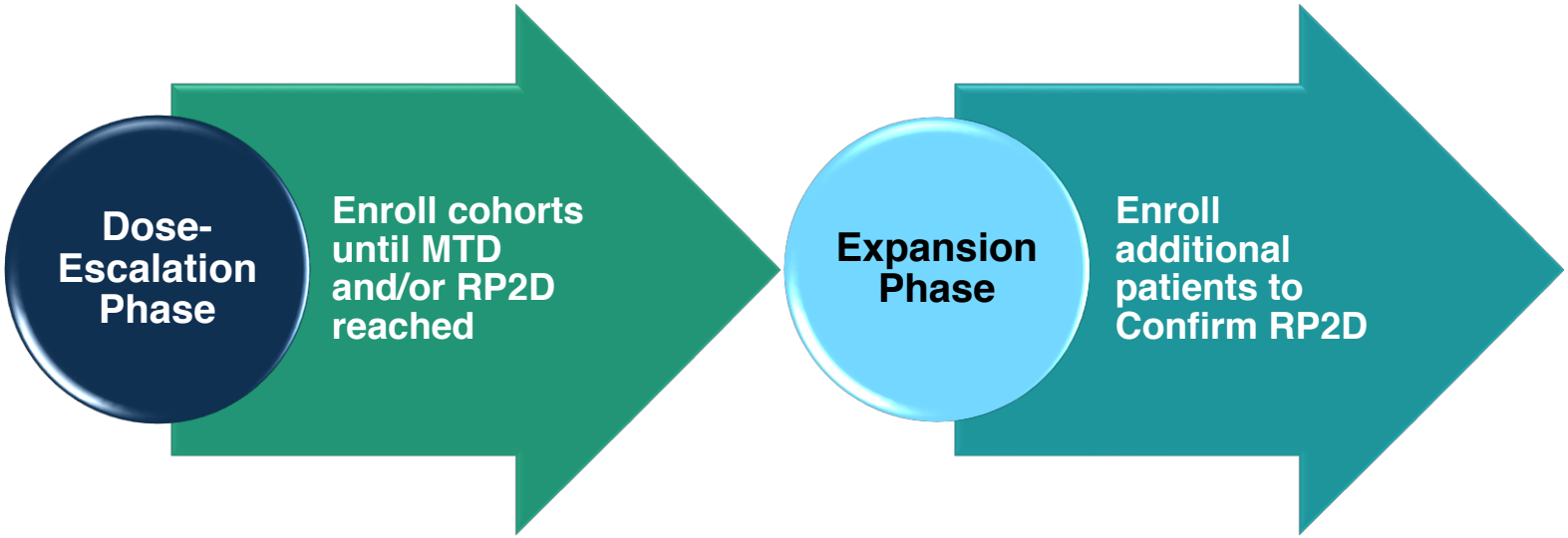
Kiladjian et al, ASH 2014

RUX & SONEDIGIB (LDE225)
(smo/hedgehog inhibitor)



HedgeHog signaling pathway is involved in numerous stages and processes of hematopoiesis via STAT5 pathway activation

RUX and Sonedegib (LDE225)



Dose Level	Combination Treatment Dose
-1	INC424 10 mg BID + LDE225 200 mg QD
1	INC424 10 mg BID + LDE225 400 mg QD
2	INC424 15 mg BID + LDE225 400 mg QD
3	INC424 15 mg BID + LDE225 800 mg QD
4	INC424 20 mg BID + LDE225 800 mg QD
RP2D	**INC424 20 mg BID + LDE225 400 mg QD**

Primary objective

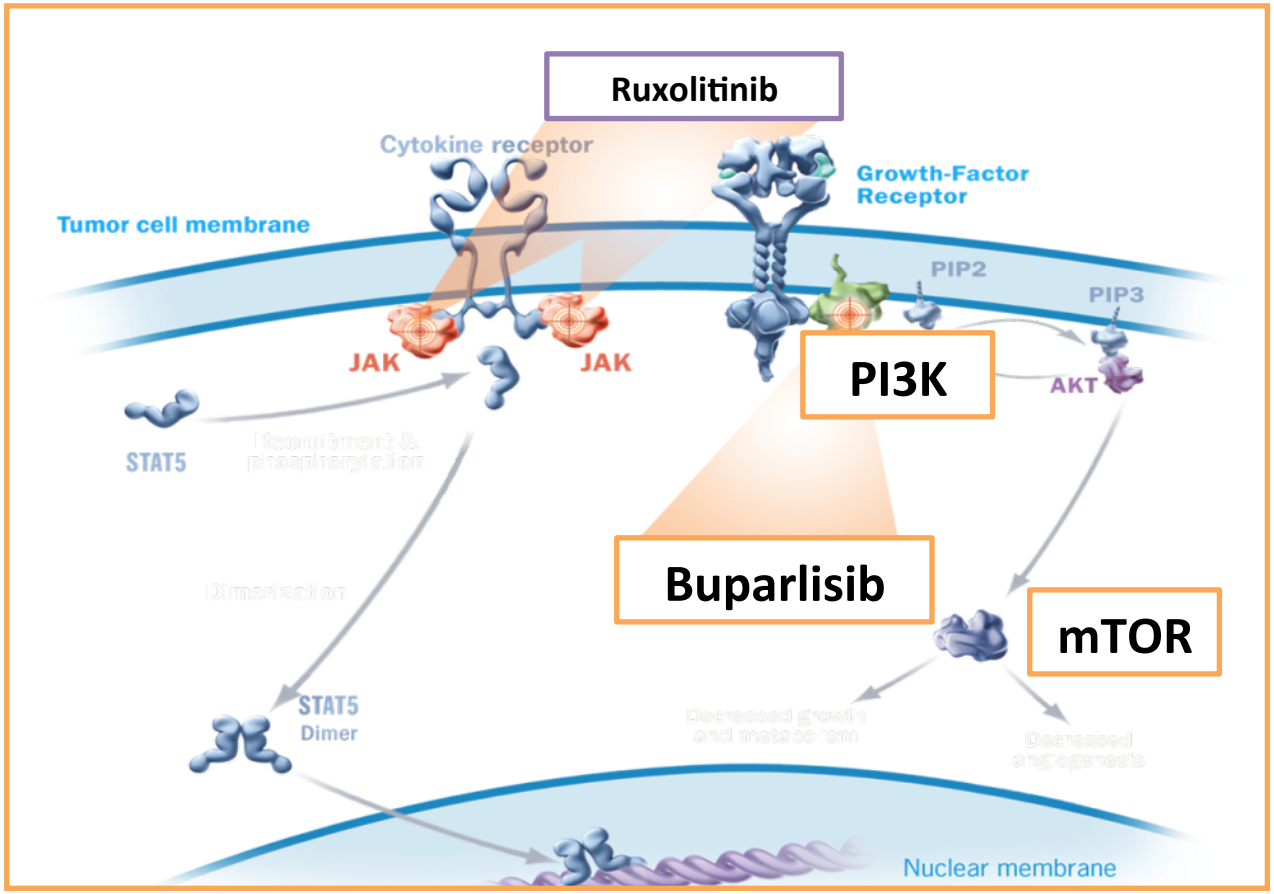
- Determine the MTD or RP2D

Secondary objectives

- Safety and tolerability
- Pharmacokinetics
- Efficacy

Gupta et al, ASH 2014

RUX and Buparlisib (BKM120) (PI3K inhibitor)



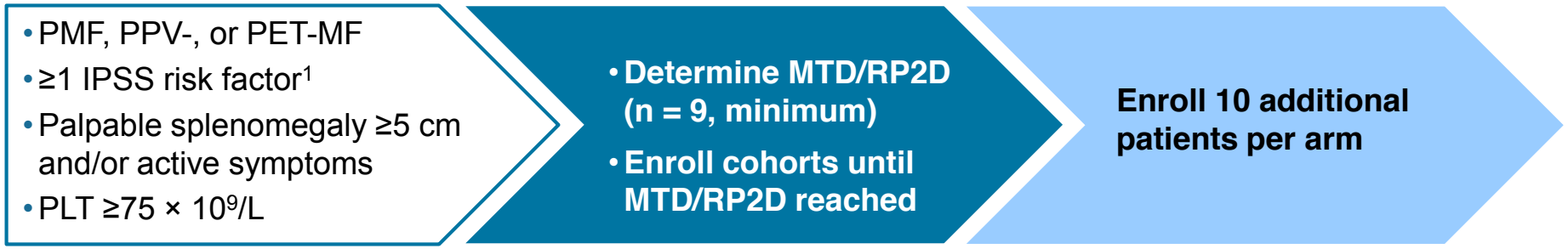
Buparlisib is a pan-PI3K inhibitor with specific and potent activity against class I PI3Ks. JAK signaling can activate the PI3K/mTOR pathway, and constitutive PI3K activation has been implicated in numerous cancers.

Kiladjian; et al, ASH 2014 Durrant; et al, ASH 2014; Gupta et al, ASH 2014

RUX and Buparlisan (BKM120)

Phase 1b, open-label, multicenter, 2-arm, dose-finding study (NCT01730248)

- **Arm A: no prior JAK inhibitor therapy**
- **Arm B: prior JAK inhibitor therapy**



Cohort (≥3 patients each)	Combination
Dose level 1	INC424 10 mg BID / BKM120 60 mg QD
Dose level 2 (RP2D)	**INC424 15 mg BID / BKM120 60 mg QD**
Dose level 3	INC424 15 mg BID / BKM120 80 mg QD
Dose level 4	INC424 20 mg BID / BKM120 80 mg QD
Dose level 5	INC424 20 mg BID / BKM120 100 mg QD

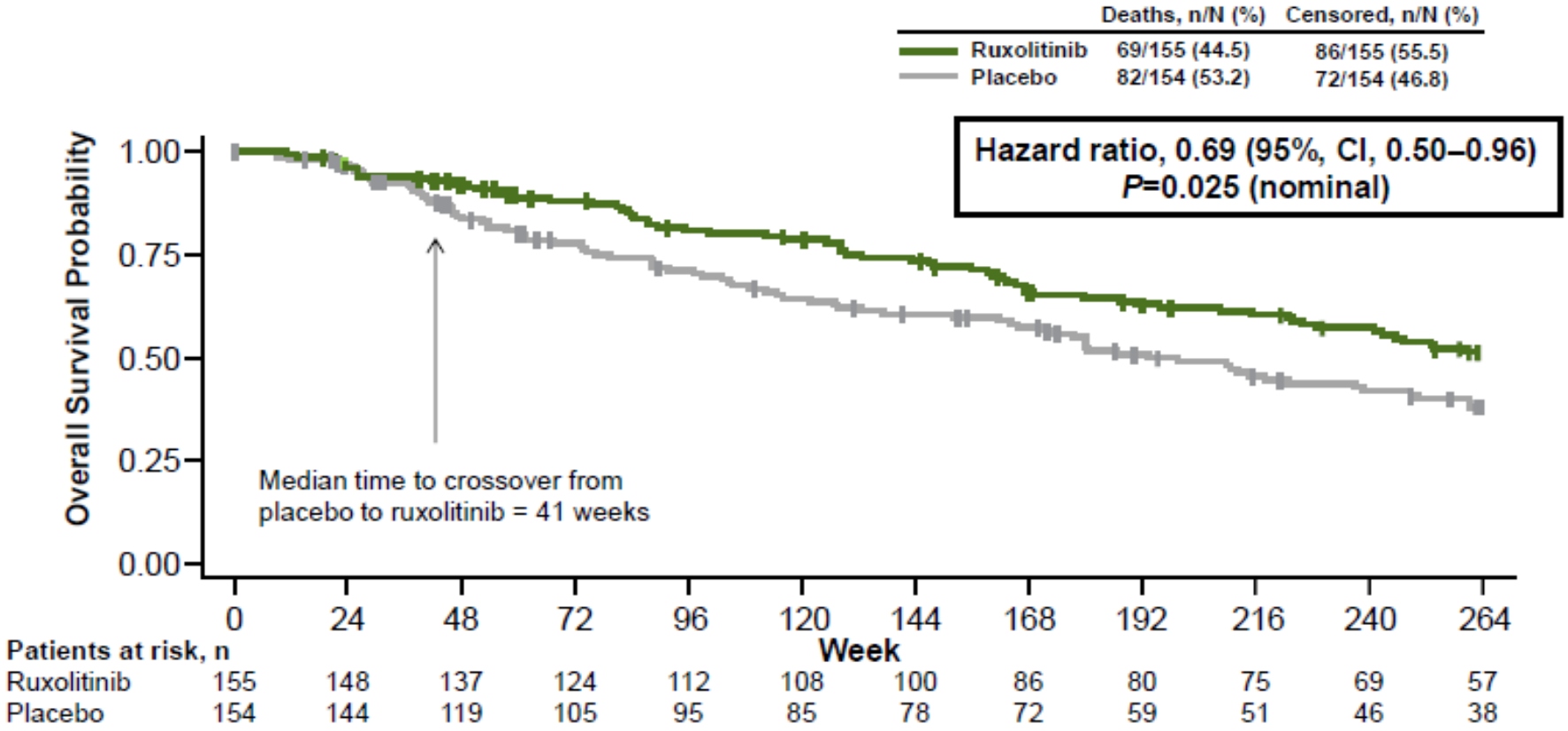
The aims of HARMONY are to evaluate the safety of co-administration of RUX and buparlisib in patients (pts) with MF and determine a recommended phase 2 dose (RP2D).

Durrant et al, ASH 2014

COMFORT I Trial: 5 Year Overall Survival

Risk of death is 31% lower with Ruxolitinib on ITT Analysis

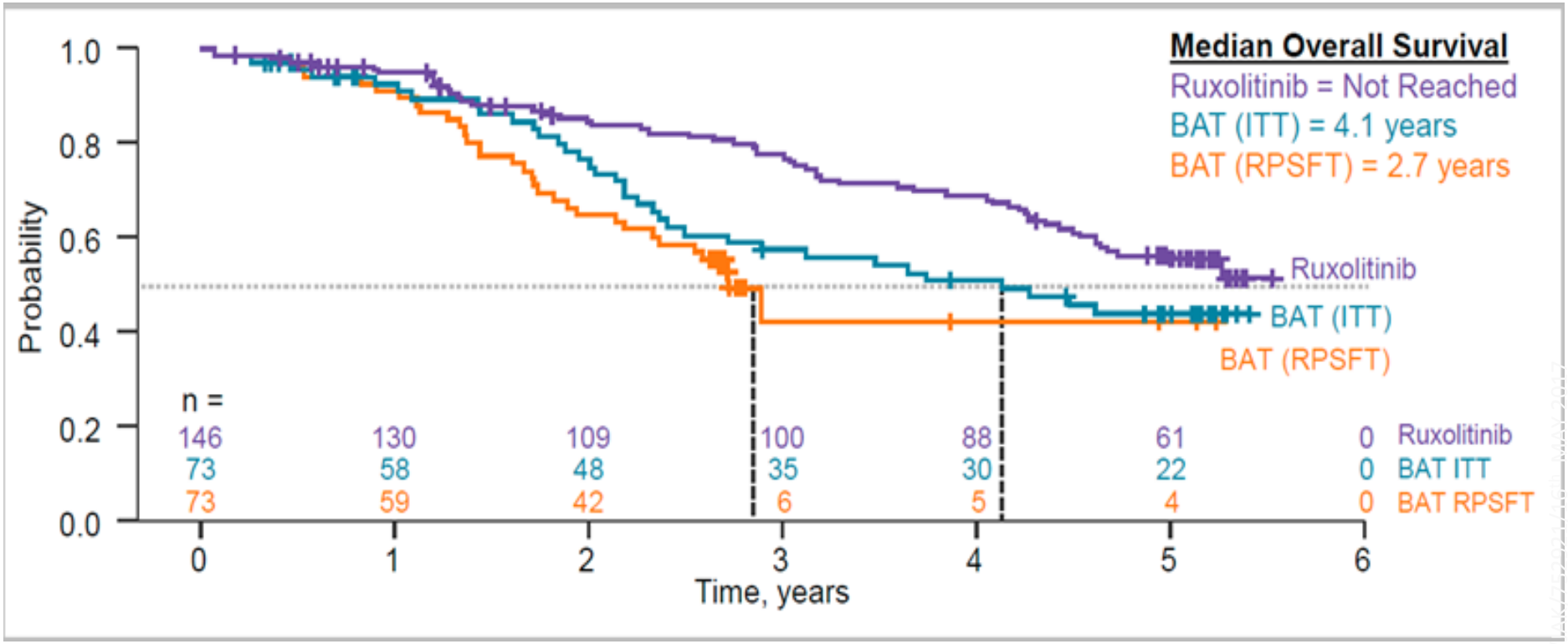
- Median OS was not reached for patients randomized to ruxolitinib (median follow-up 268 weeks) and was 200 weeks for patients in the placebo arm (median follow-up 269 weeks)



Verstovsek S. COMFORT I. EHA 2016

COMFORTII: 5 Year Overall Survival

Risk of death is 33% lower with Ruxolitinib on ITT Analysis



- **Median OS was not yet reached in the ruxolitinib arm (ie >5 years)**
  - ITT: HR, 0.67 (95% CI, 0.44-1.02); P = .06
  - RPSFT: HR, 0.44 (95% CI, 0.18-1.04) in favor of ruxolitinib vs BAT

Harrison et al. Leukemia. 2016 Aug;30(8):1701-7; 3

IME toxicity

- **Cause of IME discontinuation:**
  - slow response: 16
  - Death: 2
  - AE: 2
  - Other: 5
- **Typical IME toxicities:**
  - Myelosuppression
  - Liver
  - Infusion-related
- **Other AEs not on the Table**
  - Infections 21%

Tefferi et al., NEJM 2015

Table 1. Treatment-Related Adverse Events (of Any Grade) That Occurred in at Least Three Patients.*

Event	Group A (N=19)	Group B (N=14)	Total (N=33)	P Value†
	number of patients (percent)			
Thrombocytopenia				
All grades	10 (53)	5 (36)	15 (45)	0.48
Grade 3	8 (42)	1 (7)	9 (27)	
Grade 4	2 (11)	4 (29)	6 (18)	
Anemia				
All grades	8 (42)	5 (36)	13 (39)	1.0
Grade 2	3 (16)	0	3 (9)	
Grade 3	5 (26)	5 (36)	10 (30)	
Elevation in aspartate aminotransferase				
All grades	5 (26)	4 (29)	9 (27)	1.0
Grade 1	5 (26)	4 (29)	9 (27)	
Neutropenia				
All grades	3 (16)	6 (43)	9 (27)	0.12
Grade 3	3 (16)	2 (14)	5 (15)	
Grade 4	0	4 (29)	4 (12)	
Elevation in alkaline phosphatase				
All grades	5 (26)	2 (14)	7 (21)	0.67
Grade 1	4 (21)	2 (14)	6 (18)	
Grade 2	1 (5)	0	1 (3)	
Elevation in alanine aminotransferase				
All grades	4 (21)	2 (14)	6 (18)	1.0
Grade 1	4 (21)	2 (14)	6 (18)	
Fatigue				
All grades	5 (26)	1 (7)	6 (18)	0.21
Grade 1	2 (11)	0	2 (6)	
Grade 2	3 (16)	1 (7)	4 (12)	
Nausea				
All grades	6 (32)	0	6 (18)	0.03
Grade 1	5 (26)	0	5 (15)	
Grade 2	1 (5)	0	1 (3)	
Elevation in total bilirubin				
All grades	2 (11)	2 (14)	4 (12)	1.0
Grade 1	0	2 (14)	2 (6)	
Grade 2	2 (11)	0	2 (6)	
Infusion-related reaction				
All grades	2 (11)	2 (14)	4 (12)	1.0
Grade 1	1 (5)	2 (14)	3 (9)	
Grade 2	1 (5)	0	1 (3)	
Diarrhea				
All grades	2 (11)	1 (7)	3 (9)	1.0
Grade 1	0	1 (7)	1 (3)	
Grade 2	2 (11)	0	2 (6)	