Supplemental information

Supplemental Table S1

Criteria for HC intolerance/resistance

Criteria for HC intolerance/resistance (Barosi, *et al*. 2007, Barosi, *et al*. 2010)	
Any ONE of the following:	Platelet count >600 x 109/L after 8 weeks of at least 2 g/day or Maximum Tolerated Dose (MTD) of HC (2.5 g/day in patients with a body weight>80 kg
	Platelet count >400 x 109/L and WBC < 2.5 x 109/L at any dose of HC (for a period of at least 8 weeks
	Platelet count >400 x 109/L and Hb < 110 g/L at any dose of HC (for a period of at least 8 weeks)
	Platelet count persistently <100 x 109/L at any dose of HC (for a period of at least 8 weeks)
	Progressive splenomegaly or hepatomegaly i.e. enlargement by more than 5cm or appearance of new splenomegaly or hepatomegaly on HC treatment
	Not achieving the desired reduction of hematocrit or packed cell volume with the addition of HC in patients who do not tolerate frequent venesections after 8 weeks of at least 2 g/day of HC (2.5 g/day in patients with a body weight>80 kg)
	Not achieving the desired stable reduction of WBC when leukocytes are a target of therapy after 8 weeks of at least 2 g/day or MTD of HC (2.5 g/day in patients with a body weight>80 kg)
	Thrombosis or hemorrhage (including Transient Ischemic Attack (TIA)) while on therapy
	Presence of leg ulcers or other unacceptable HC-related non-haematological toxicities, such as unacceptable mucocutaneous manifestations, gastrointestinal symptoms, pneumonitis or fever at any dose of HC. OR Cycling platelet counts on therapy
	Disease related symptoms not controlled by hydroxycarbamide

Supplemental Table S2

Criteria for high-risk ET

Criteria for high-risk ET	
High-risk is defined as any ONE of the following:	Age > 60 years
	Platelet count > 1500 x 109/L (at any point during the patient's disease)
	Previous documented thrombosis (including Transient Ischemic Attack (TIA)), erythromelalgia or migraine (severe, recurrent, requiring medications, and felt to be secondary to the MPN) either after diagnosis or within 10 years before diagnosis and considered to be disease related
	Previous hemorrhage related to ET

	Diabetes or hypertension requiring pharmacological therapy for > 6 months

Supplemental Table S3

Eligibility criteria and randomization

Eligibility criteria and randomization	
Inclusion criteria	• Male or female patient ≥ 18 years of age • A confirmed diagnosis of high risk ET (as per Supplemental Table 2) • Either intolerant or resistant to Hydroxycarbamide (as per Supplemental Table 1), having met any one of the criteria at any point in their disease whilst on Hydroxycarbamide
Exclusion criteria	• Pregnant and lactating patients (patients of childbearing potential must have a negative • pregnancy test prior to study entry) • Patients and partners of childbearing potential not willing to use effective contraception • ECOG Performance Status Score ≥ 3 • Uncontrolled rapid or paroxysmal atrial fibrillation, uncontrolled or unstable angina, recent (6 • months) myocardial infarction or acute coronary syndrome or any clinically significant cardiac • disease > NYHA Class II (Appendix 5) • Patients who have transformed to myelofibrosis • Previous treatment with Ruxolitinib • Previous (within the last 12 months) or current platelet count <100 x 10^9/L or neutrophil count • < 1 x 10^9/L not due to therapy. • Inadequate liver function as defined by ALT/AST >1.5 x ULN • Inadequate renal function as defined by GFR < 30 mls/min • Unable to give informed consent
Randomization	• Patients were randomly assigned to treatment on a 1:1 ratio based on a minimization algorithm prepared and run by the CRCTU • Randomization was stratified by type of disease (PV or ET) and JAKV617F status (negative: positive) for ET patients

Supplemental Table S4

List of CALR and MPL mutations detected

Gene	Mutation detected	No. of patients
CALR NM_004343.3	Type 1: 52bp deletion	19
	Type 2: 5bp insertion	10
	c.1100_1147delinsGC p.(Leu367Argfs*48)	1
	c.1093_1139del p.(Gln365Argfs*8)	1
	c.1099_1135del p.(Leu367Argfs*51)	1
	c.1111_1141del p.(Glu371Argfs*49)	1
	c.1124_1133del p.(Lys375Argfs*52)	1
MPL NM_005373.2	c.1544G>T (Trp515Leu)	4
	c.1543_1544delinsAA,p.(Trp515Lys)	2

Supplemental Table S5

Univariate and multivariate analysis of factors influencing complete response

Variable	OR (UV)	95% CI (UV)	*P* (UV)	OR (adj)	95% CI (adj)	*P* (adj)
JAK2 V617F *allele burden* (%) (More than 50%)	.63	(.16, 2.57)	.52			
HC resistance or intolerant (intolerant)	1.66	(.77, 3.55)	.19	1.17	(.53, 2.58)	.70
Disease duration (greater than 5 years)	.82	(.37, 1.79)	.61			
White blood cells (greater or equal to 10×10^9/L)	1.24	(.45, 3.41)	.67	1.45	(.50, 4.96)	.50
*Platelets (*lower 0-33.3 %)						
Median (33.3% -66.6%)	.58	(.23, 1.46)	.25	.58	(.22, 1.52)	.27
Upper (66.6% -100%)	.43	(.17, 1.10)	.08	.44	(.16, 1.20)	.11
Hemoglobin (Hem≥100)	1.52	(.42, 5.52)	.53	1.82	(.45, 7.29)	.40
Allele (*JAK2* V617F) CALR	.77	(.32, 1.84)	.55	.90	(.32, 5.60)	.69

Neither	.74	(.56, 1.65)	.55	.79	(.27, 4.84)	.85
Previous Therapies						
2	1.89	(.71, 4.96)	.20			
3	2.48	(.75, 8.17)	.14			
>3	.98	(.28, 3.46)	.98			
Treatment (Ruxolitinib)	1.10	(.52, 2.33)	.81	1.14	(.52, 2.52)	.74
*Hematocrit (*lower 0-33.3 %)						
Median (33.3% -66.6%)	.92	(.37, 2.29)	.86			
Upper (66.6% -100%)	1.62	(.64, 4.11)	.306			

OR Odds Ratio; CI Confidence Intervals; UV Univariate; adj adjusted
The adjusted analysis has been modelled using: treatment, resistance or intolerance, white cell count, platelets, hemoglobin, *JAK2* V617F or *CALR* or neither, and *JAK2* V617F allele burden (%).

Supplemental Table S6

Univariate and multivariate analysis of factors influencing development of PET-MF

Variable	**OR** (UV)	**95% CI** (UV)	***P*** (UV)	**OR** (adj)	**95% CI** (adj)	***P*** (adj)
JAK2 V617F allele burden (%)* (More than 50%)	3.92	(.82, 18.6)	.09			
HC Resistance or intolerant (intolerant)	.76	(.24, 2.4)	.64			
Disease duration (greater than 5 years)	1.93	(.5, 7.5)	.34			
White blood cells ł (greater or equal to 10×10^9/L)	1	-	-			
*Hematocrit (*lower 0-33.3 %) Median (33.3% -66.6%) Upper (66.6% -100%)	.39 .29	(.09, 1.65) (.06, 1.48)	.20 .14			
*Platelets (*lower 0-33.3 %) Median (33.3% -66.6%) Upper (66.6% -100%)	1.37 .75	(.34, 5.54) (.16, 3.60)	.66 .72			
Hemoglobin (Hem≥100)	.27	(.07, 1.25)	.10	.33	(.74, 1.51)	.15
Allele (*JAK2* V617F) CALR Neither	.73 1.60	(.17, 3.11) (.41, 6.29)	.67 .50			
Previous Therapies 2 3 >3	1.63 3.47 1.37	(.29, 9.01) (.57, 21.23) (.17, 10.60)	.58 .18 .76			
Treatment (Ruxolitinib)	2.39	(.69, 8.26)	.17	2.14	(.60, 7.55)	.24

OR Odds Ratio; CI Confidence Intervals; UV Univariate; adj adjusted

* Data to be interpreted with caution. Reduced power due to lack of data

Ł No events were recorded in this group, therefore no *P* value can be estimated

The final model has adjusted for hemoglobin and allele type.

Supplemental Table S 7

Grade 3 and 4 adverse events by grade and by NCI CTCAE Category

CTC Category		Best Available Therapy		Ruxolitinib		Overall
		Grade 3	Grade 4	Grade 3	Grade 4	
		N	N	N	N	N
Blood and lymphatic system disorders	Anemia	0	0	23	0	23
	Leukocytosis	1	0	4	0	5
Cardiac disorders		2	0	4	0	6
Eye disorders		0	0	1	0	1
Gastrointestinal disorders		1	0	6	0	7
General disorders and administration site conditions		2	0	0	0	2
Infections and infestations	Lung infections	0	0	5	0	5
	Tooth infections	1	0	1	0	2
	Bronchial infection	0	0	1	0	1
	Kidney infection	0	0	1	0	1
	Sepsis	0	1	0	0	1*
	Skin infection	0	0	1	0	1
Injury, poisoning and procedural complications		1	0	4	0	5
Investigations		1	0	7	1	9
Metabolism and nutrition disorders	Hyperkalemia	2	0	3	0	5
	Hyponatremia	0	0	10	0	10
	Other	0	0	1	1	2
Musculoskeletal and connective tissue disorders		0	0	1	0	1
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)		3	0	2	0	5*ł
Nervous system disorders		3	0	6	0	9
Renal and urinary disorders		1	0	4	0	5
Respiratory, thoracic and mediastinal disorders		3	0	4	0	7
Skin and subcutaneous tissue disorders		3	0	1	0	4
Vascular disorders		2	0	8	1	11
Total		*26*	*1*	*98*	*3*	*128*

*A sepsis and pancreatic cancer related death of 1 ruxolotinib patient occurred more than 30 days post treatment discontinuation and was therefore not counted as an AE.

ł A cancer related death of 1 ruxolitinib patient occurred more than 30 days post treatment end and was therefore not recorded as an AE

Supplemental Table S8

Univariate and multivariate analysis of factors influencing development of anemia or thrombocytopenia

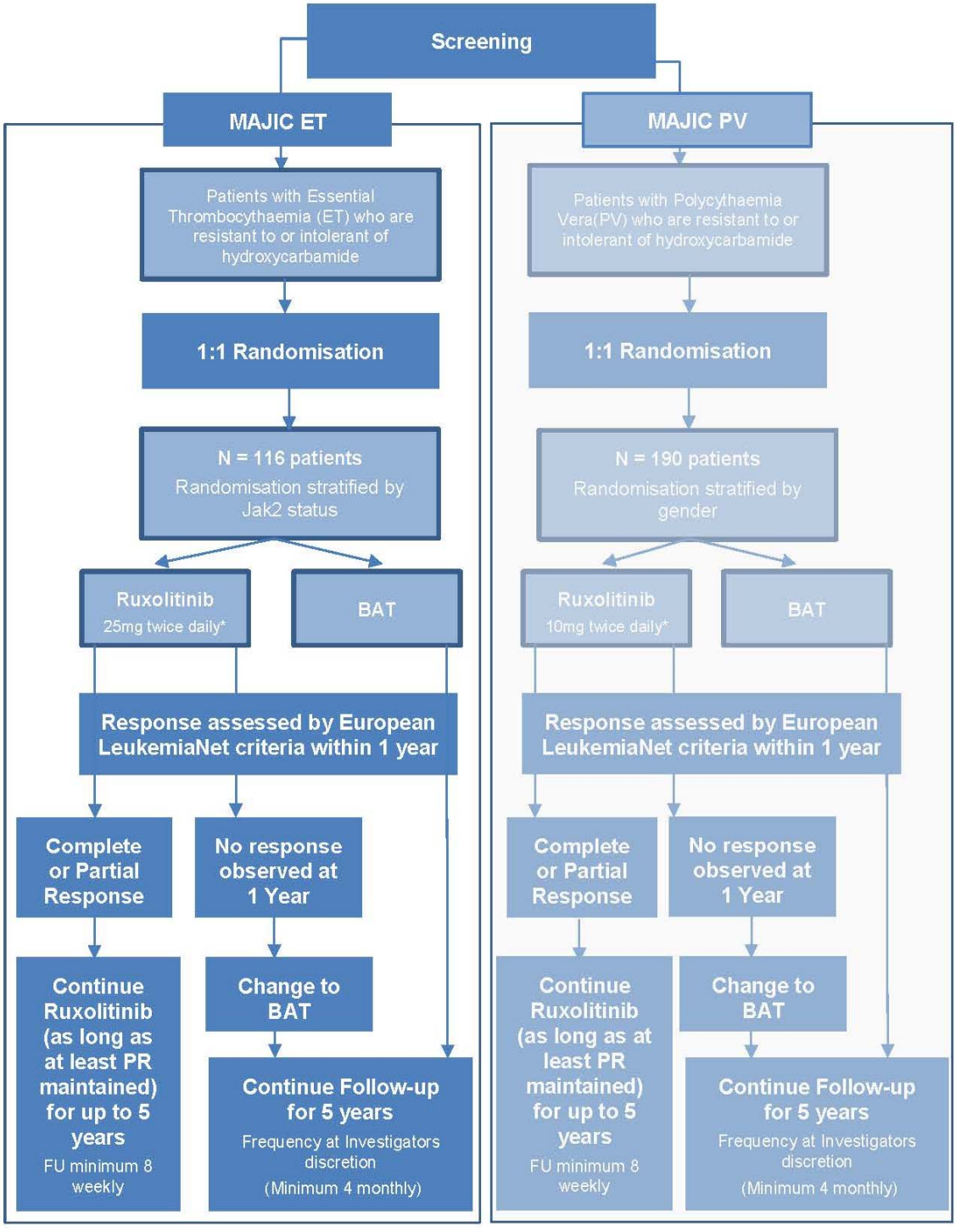
Variable	**OR** (UV)	**95% CI** (UV)	***P*** (UV)	**OR** (adj)	**95% CI** (adj)	***P*** (adj)
JAK2 V617F *allele burden* (%)* (More than 50%)	4.29	(.78, 23.42)	.09			
HC Resistance or intolerant (intolerant)	.91	(.27, 3.00)	.87			
Disease duration (greater than 7 years)	.92	(.28, 3.06)	.90			
White blood cells (greater or equal to 10×10^9/L)	1.79	(.44, 7.35)	.42			
*Hematocrit (*lower 0-33.3 %)						
Median (33.3% -66.6%)	.39	(.09, 1.65)	.20			
Upper (66.6% -100%)	.29	(.06, 1.48)	.14			
*Platelets (*lower 0-33.3 %)						
Median (33.3% -66.6%)	1.37	(.34, 5.54)	.66			
Upper (66.6% -100%)	.75	(.16, 3.60)	.72			
Hemoglobin (Hem≥100)	.15	(.04, 0.61)	.01	.17	(.04, .72)	.016
Allele (*JAK2* V617F)						
CALR	.40	(.08, 2.02)	.27	.52	(.1, 2.80)	.21
Neither	.96	(.23, 4.08)	.96	.97	(.21, 4.38)	.16
Previous Therapies						
2	1	-	-			
3	3.08	(.35, 27.3)	0.31			
>3	7.14	(.75, 27.34)	0.09			

OR Odds Ratio; CI Confidence Intervals; UV Univariate; adj adjusted

* Data to be interpreted with caution. Reduced power due to lack of data

The multivariate model has adjusted for treatment and hemoglobin. White cell could not be estimated as there are no events in one of the groups hence the odds ratio could not be estimated.

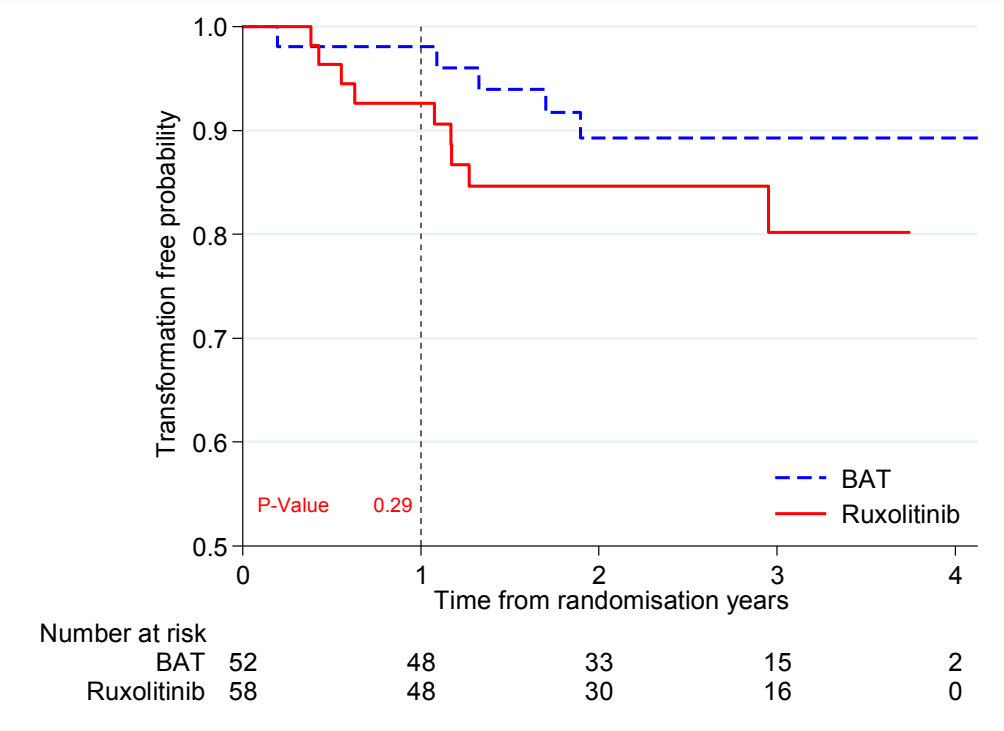
Supplemental Figure S1 Trial Schema



*Patients with platelet count between 100 and 200 x 10^9/L will be started on a reduced dose

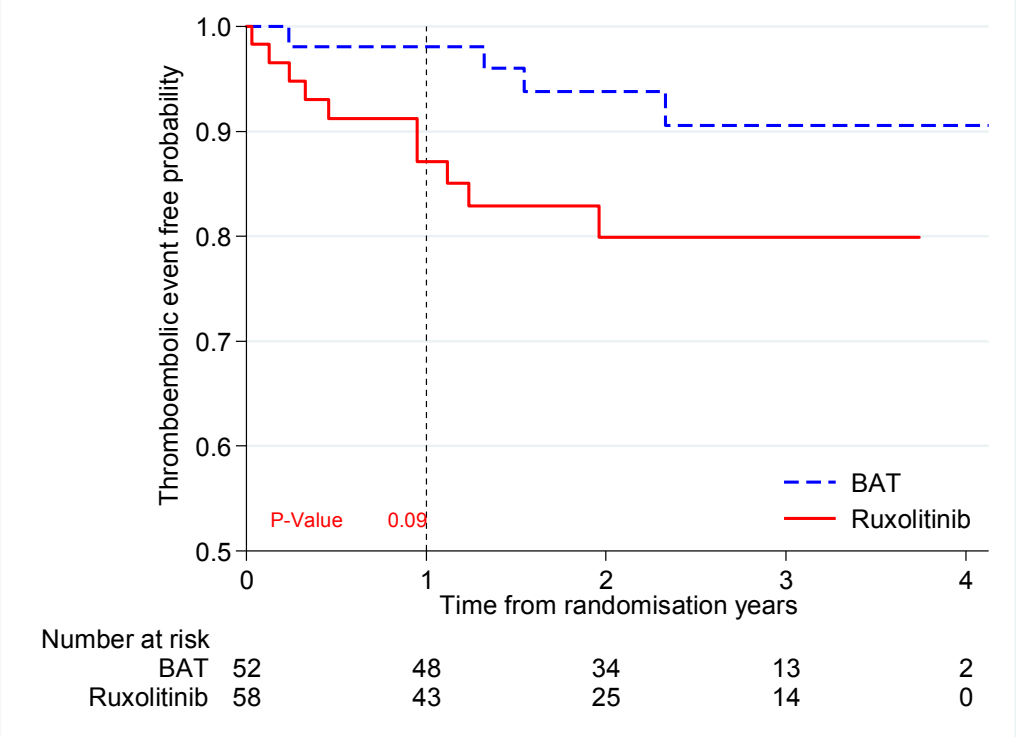
Supplemental Figure S2 A. Transformation event free probability by treatment arm

Transformation event free probability has been measured from date of randomization to first transformation. Events appeared more frequently for Ruxolitinib treated patients but in fact the difference between the treatment arms was not significant (P=0.29). Transformation event free probability estimates at one year were: BAT .96 (.85, .99) and ruxolitinib .98 (.87, .99); and at 2 years: BAT .91 (.80, .97) and ruxolitinib .98 (.87, .99)



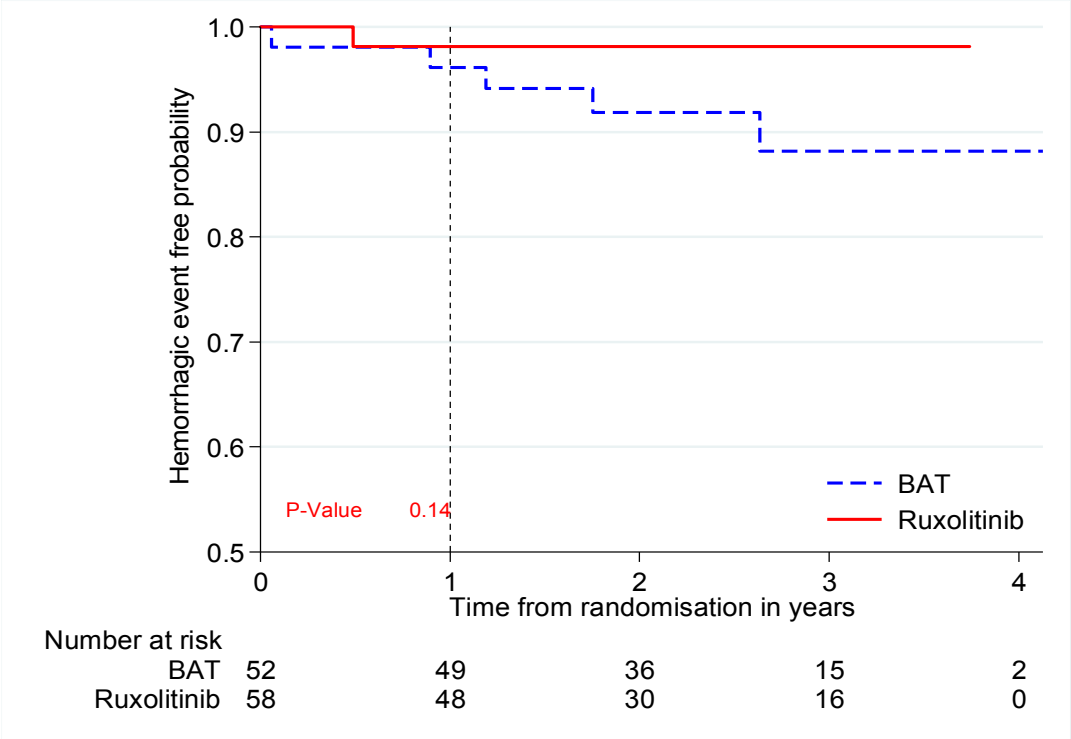
Supplemental Figure S2 B. Thromboembolic event free probability by treatment arm

Thrombosis event free probability has been measured from date of randomization to first thrombotic event. Differences between the treatment arms approached but were not statistically significant (*P* =.09). Thrombosis free probability at one year for BAT was .98 (95% CI: .87, .99) and for ruxolitinib .92 (95% CI: .81, .97); after a further year these figures for BAT were .89 (95% CI:.76, .95) and for ruxolitinib .85 (95% CI:.72, .92)



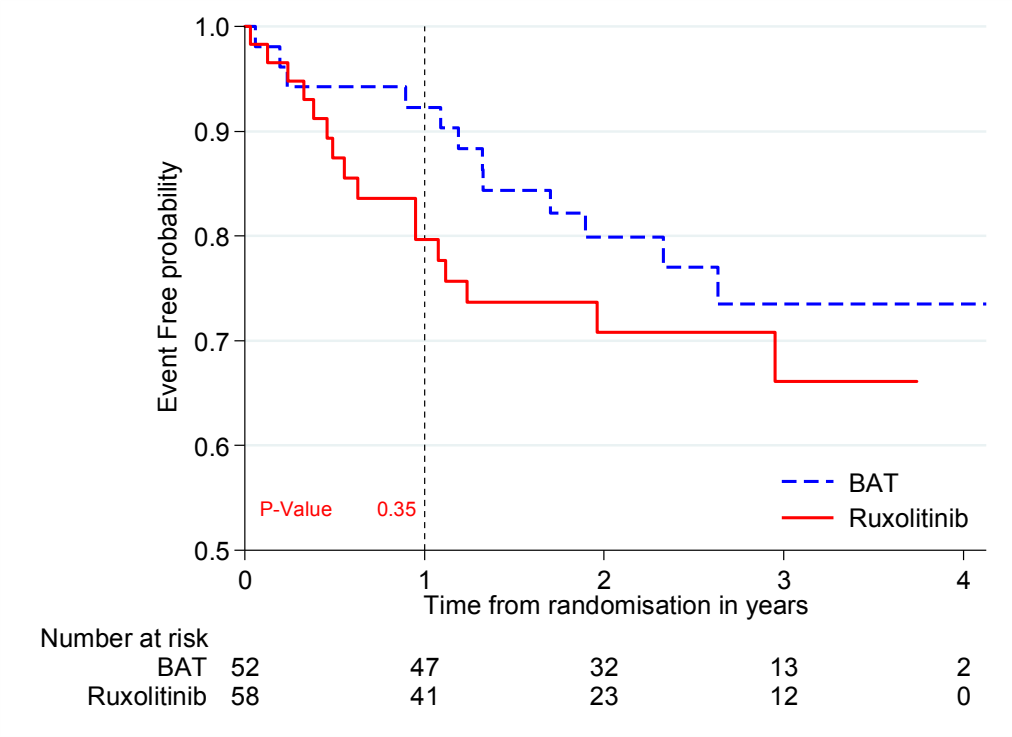
Supplemental Figure S2 C. Hemorrhagic event free probability by treatment arm

Hemorrhage event free probability has been measured from date of randomization to first hemorrhagic event. Hemorrhage was less frequent for patients treated with ruxolitinib but again this difference was not significant (*P*=.14). Hemorrhage-free survival estimates at one year were: BAT .98 (95% CI:.87, .99), and ruxolitinib .87 (95% CI:.75, .94); and at 2 years: BAT .94 (95% CI:.82, .98) and ruxolitinib .80 (95% CI:.65, .88



Supplemental Figure S2 D. Time to first hemorrhagic, thromboembolic and transformation event by treatment arm

Event free probability has been measure from date of randomisation to date of first event (haemorrhage, thrombosis or transformation).There was no statistical significant difference between the two arms of the MAJIC-ET trial for this combined endpoint (P=0.35). Event free survival estimates at one year were: BAT .92 (.81, .97) and ruxolitinib .80 (.65, .88); and two years: BAT .80 (.66, .89) and ruxolitinib .64 (.56, .81)

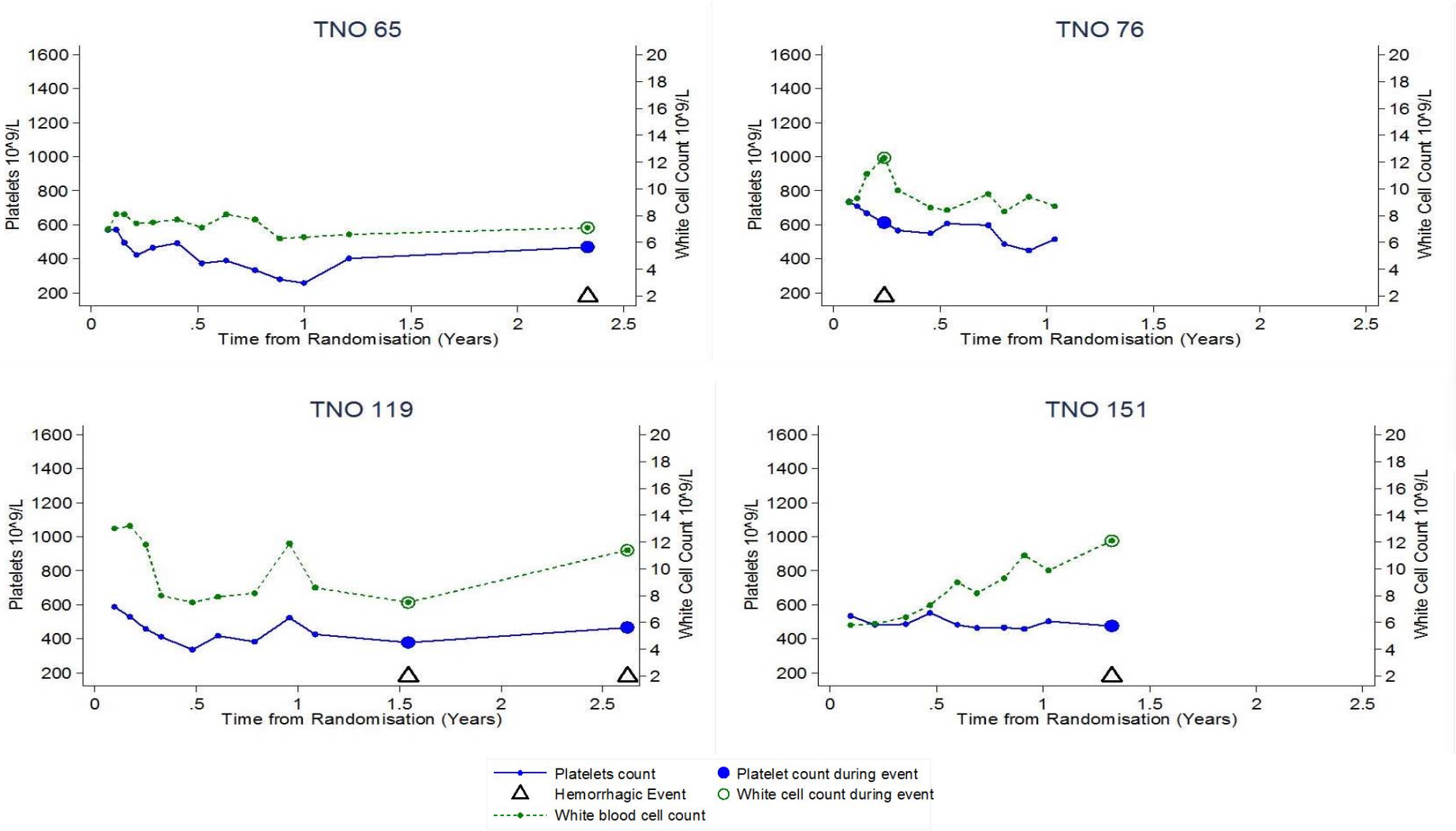


Supplemental Figure S3

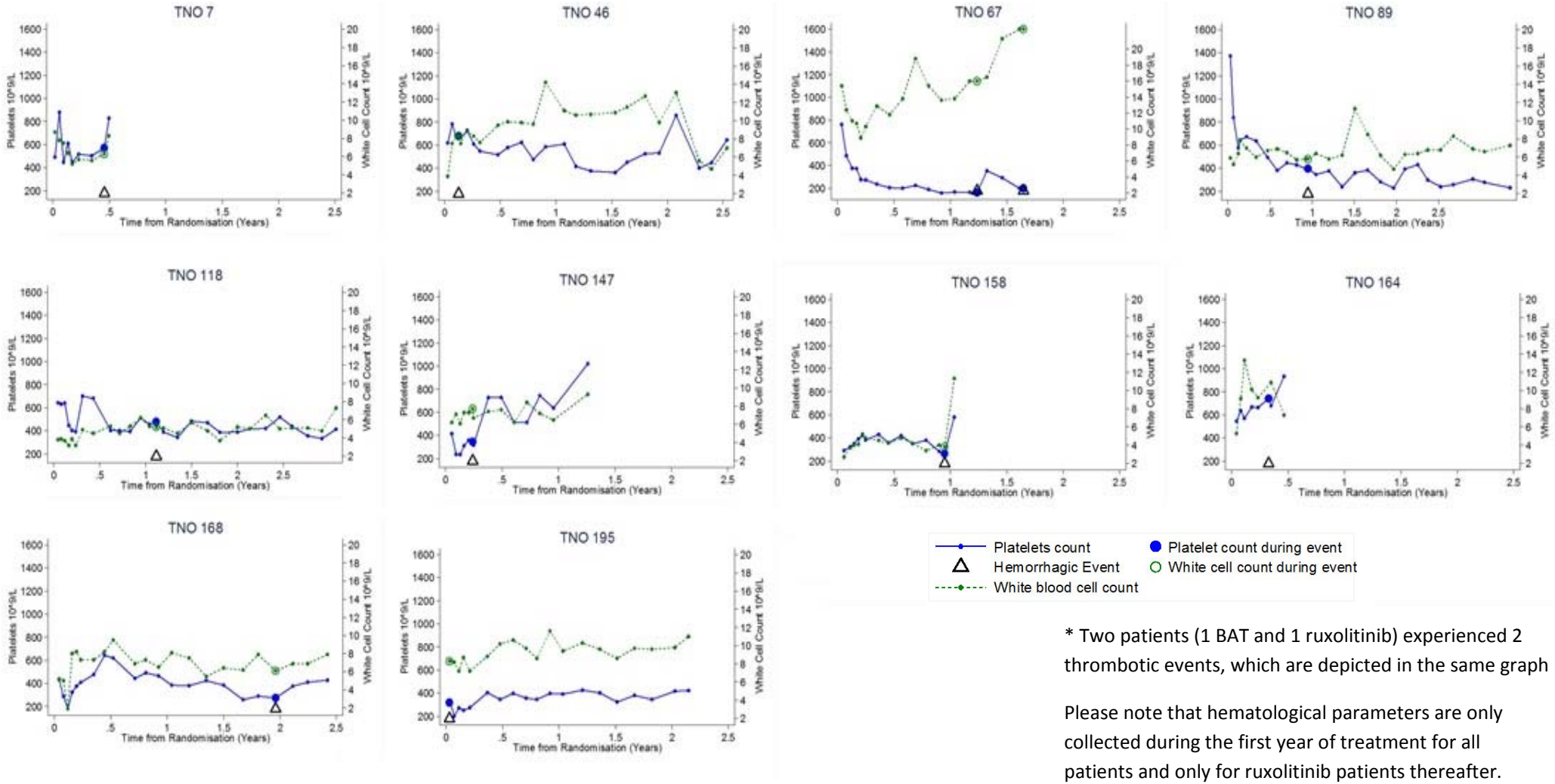
Control of White Blood Cell and Platelet counts in correlation with hemorrhagic and thrombotic events

A. Thrombotic events *

i) BAT Patients



ii) Ruxolitinib patients

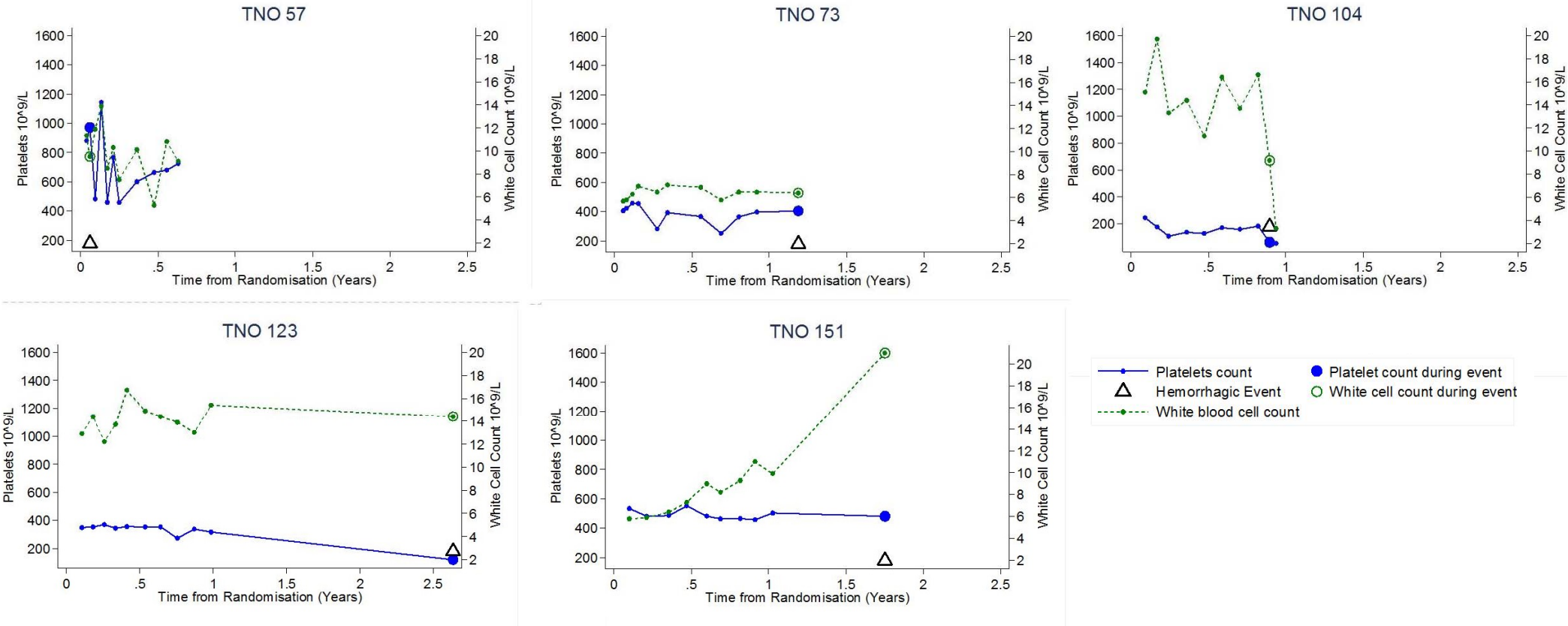


* Two patients (1 BAT and 1 ruxolitinib) experienced 2 thrombotic events, which are depicted in the same graph

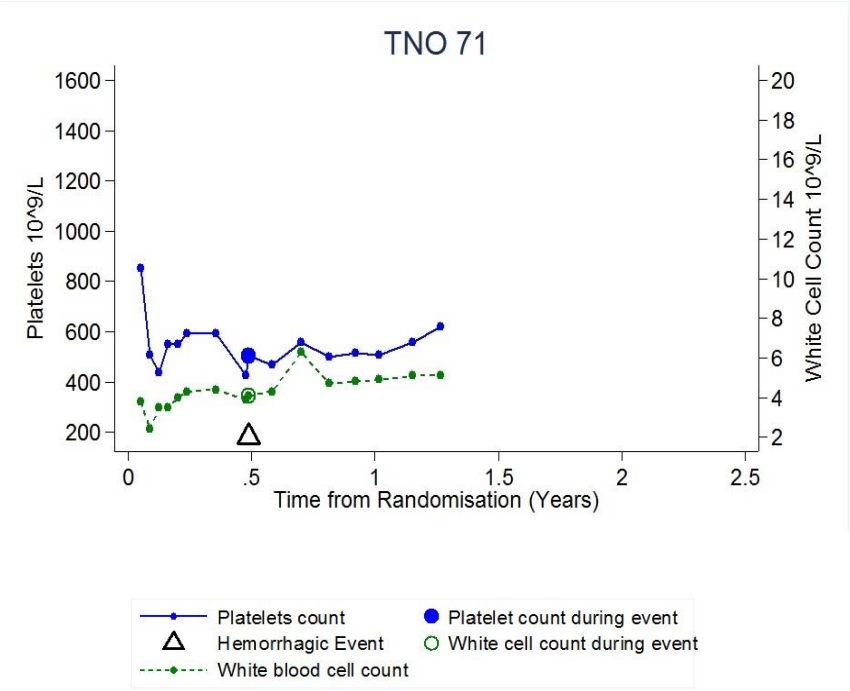
Please note that hematological parameters are only collected during the first year of treatment for all patients and only for ruxolitinib patients thereafter.

B. Hemorrhagic events

i. BAT patients



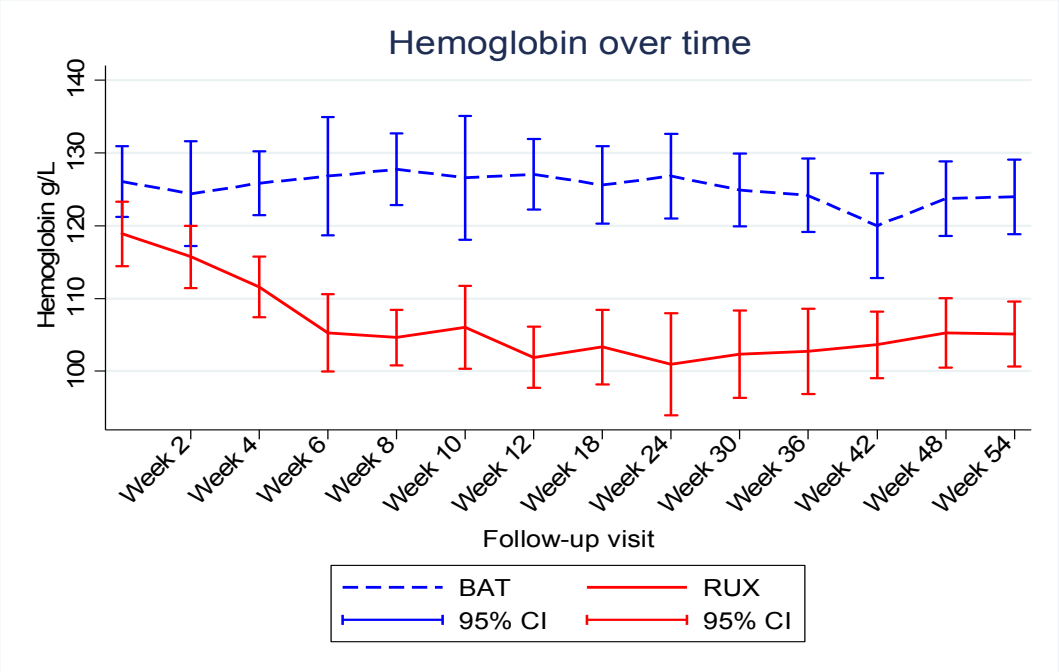
ii. Ruxolitinib patient



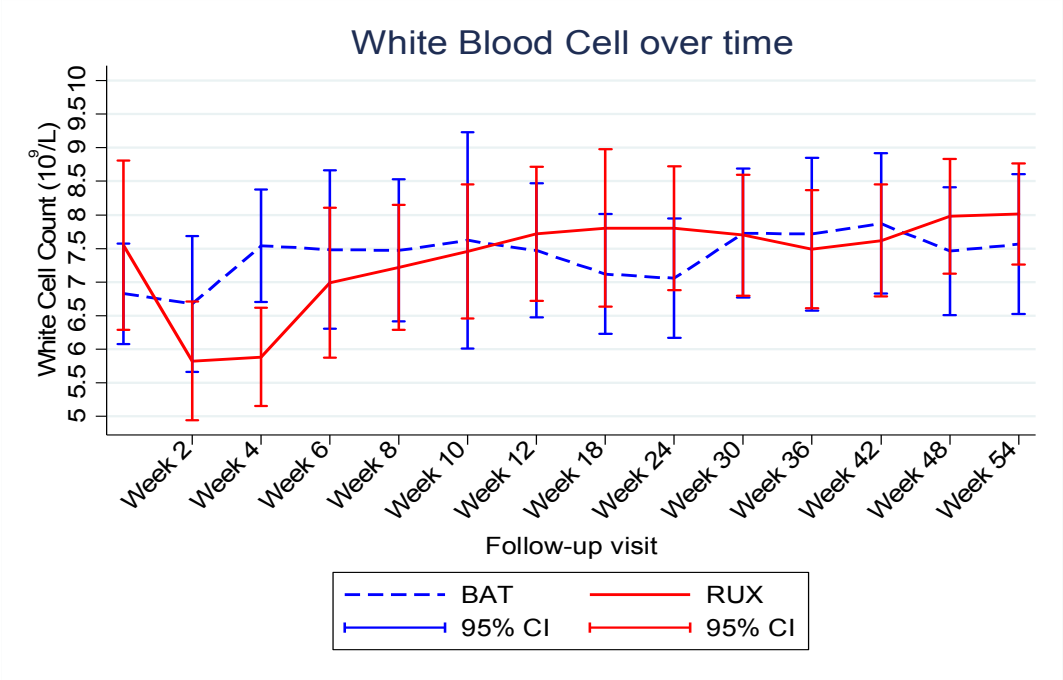
Please note that hematological parameters are only collected during the first year of treatment for all patients and only for ruxolitinib patients thereafter.

Supplemental Figure S4. Hematological variables during the first year of the MAJIC-ET trial

Supplemental Figure S4A shows hemoglobin values (mean and 95% CI) according to whether the patient received BAT or ruxolitinib. Ruxolitinib treated patients had lower hemoglobin values which became most marked and persisted after week 4.



Supplemental Figure S4B shows white cell count during the first year of MAJIC-ET, there were no significant differences between the allocated treatment arms.


White Blood Cell over time
White Cell Count (10^9/L)
5 5.5 6 6.5 7 7.5 8 8.5 9 9.5 10
Week 2
Week 4
Week 6
Week 8
Week 10
Week 12
Week 18
Week 24
Week 30
Week 36
Week 42
Week 48
Week 54
Follow-up visit
BAT
95% CI
RUX
95% CI

Supplemental Figure S4C shows the platelet count during the first year of MAJIC-ET, these do not differ between the two treatment arms.