Response definition

Clinical response (CR) was defined as complete resolution of clinical symptoms. Complete (CBR) and partial (PBR) biological responses were defined as complete normalization and ≥ 50% reduction of C-reactive protein (CRP) level from baseline respectively. Red blood cell (RBC) transfusion dependency characterized patients having received at least 4 unit of RBCs every 8 weeks for hemoglobin level below 90 g/L, as recommended by guidelines for MDS management (Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127(20):2391–2405). Response was assessed after 1, 3, and 6 months of JAKi treatment, and at the time of last follow-up. Time to next treatment was defined as the time between the onset of JAKi and of subsequent treatment for the VEXAS syndrome (steroids dose increase was not considered as an event).

Statistical analysis

Comparative descriptive statistics were used to characterize patients' characteristics. Continuous variables were reported as median ± range. Continuous variables comparison was made by a t test if the distribution was normal in both groups (evaluated by Fisher's test) or by a Mann-Whitney test in cases of non-normal distribution. For discrete and qualitative variables, the difference between groups was assessed with a Pearson Chi-square test. Two-way ANOVA was used to evaluate the impact of qualitative variables (time and type of treatment) on mean level of quantitative variables (hemoglobin and platelet count). Probabilities of survival (time to next line of treatment) was estimated by the Kaplan-Meier method, and the log-rank test evaluated differences between survival probabilities. Univariate and multivariate analyses including baseline demographic, biological and clinical

features were assessed by Cox regressions. Only significant variables in univariate analysis were included in the Cox regression model. Statistical results were two-sided with a p-value < 0.05 considered as statistically significant.

Hematological tolerance

Three patients, all treated with ruxolitinib, developed transient neutropenia (grade 3, n=2, grade 4, n=1) which resolved after ruxolitinib dose reduction (Supplemental Figure 6A, blue line). Conversely, three patients with MN and mild neutropenia prior to ruxolitinib had increased absolute neutrophil count (ANC) with treatment (Supplemental Figure 5A, red line). No treatment related neutropenia or significant improvement in ANC was observed in patients treated with other JAKi (Supplemental Figure 5B).

Detailed adverse events

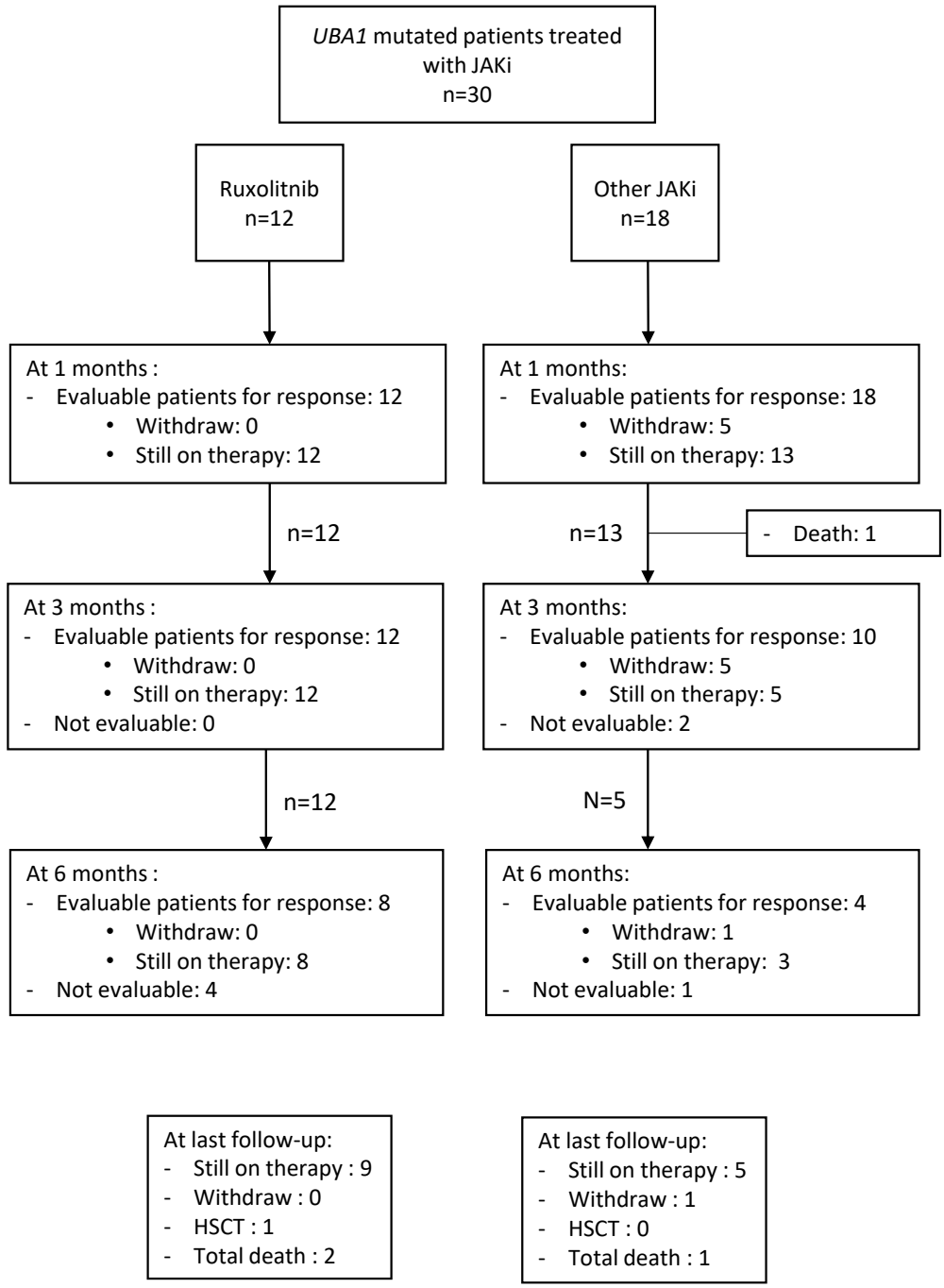
Pneumonia was the most frequent infection (n=9; 4 undocumented, 3 cases of COVID-19, one legionellosis, and one RSV infection). We observed one case of herpetic keratitis in a patient treated with upadacitinib). Patients who experienced infections had received a greater number of previous lines of treatment (median, 3.5 vs 2; p=0.04). Six VTE occurred after a median of 5.5 months since the start of JAKi (2 with ruxolitinib, 4 with other JAKi).

Practical considerations regarding the use of ruxolitinib in VEXAS patients

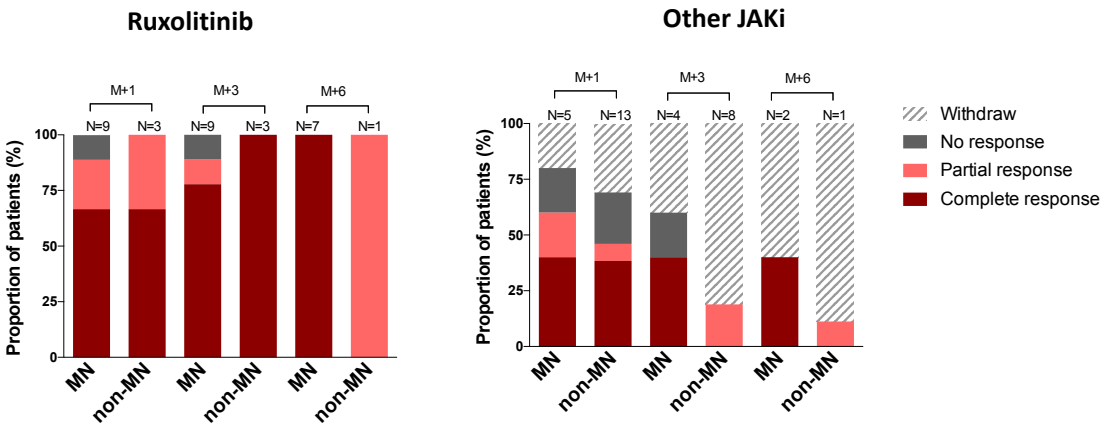
The use of ruxolitinib in patients with VEXAS syndrome is associated with a learning curve. We advise to start ruxolitinib at 10mg twice daily (TD) or 5mg TD in elderly or neutropenic patients. Dose increase should be based on clinical and biological response kinetics being mindful of hematological tolerance. Ruxolitinib should be increased progressively up to 20mg TD, until clinical response and eventually biological response are reached. We do not recommend tapering steroids prior to achieving at least 3 months of stable clinical response at fixed dosage. Loss of biological response during steroid taper can occur and does not

necessarily predict a clinical inflammatory flare. If clinical response is lost during tapering, steroids should be transiently increased and ruxolitinib dosage should be further increased if possible. Worsening cytopenia, especially anemia, during the first weeks of treatment is common. Erythropoietin might be considered and used safely based on our experience. For treatment related neutropenia, grade 1 and 2 should be tolerated, but ruxolitinib dosage should be reduced in cases of grade 3-4 neutropenia. Granulocyte-colony stimulating factor (G-CSF) might be considered in cases of febrile neutropenia. Based on limited observations, we did not observe inflammatory flare after G-CSF use. Due to its hematological safety profile, ruxolitinib might not be a suitable option for patients with profound cytopenias, especially severe neutropenia.

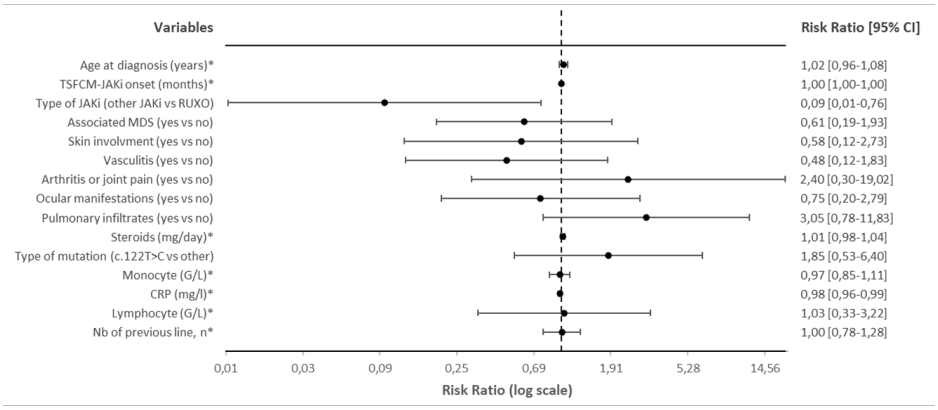
Supplemental Figure 1 : **Flow chart schematic representation of the follow-up of patients included in the study.** Patients without long-enough follow-up at a given timepoint are considered as "Not evaluable"



Supplemental Figure 2 : **Clinical response in patients with or without myeloid neoplasm (MN) treated with ruxolitinib or other JAKi**

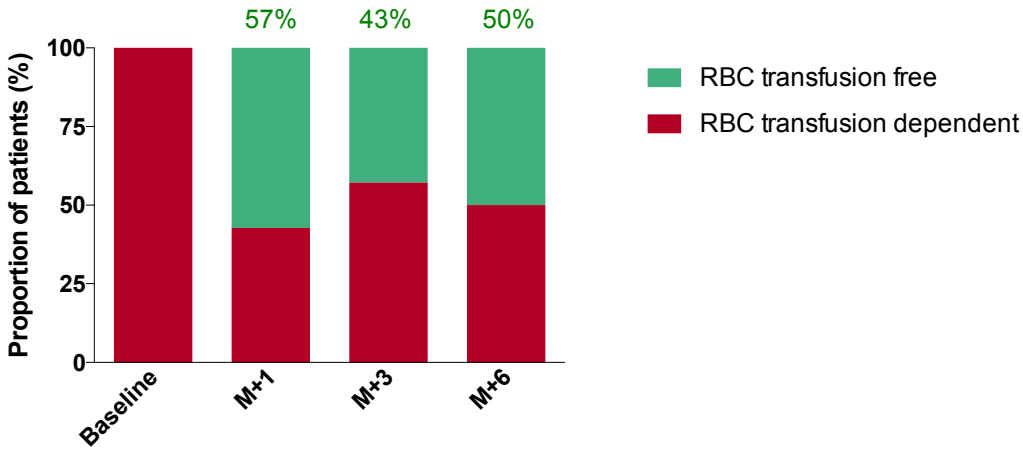


Supplemental Figure 3 : **Forest plot** representation of the univariate analysis of variables influencing the probability of clinical response.



Legends : TSFCM = time since first clinical manifestations; * continuous variables

Supplemental Figure 4 : **Evolution of the proportion of patients with RBC transfusion dependency under JAKi**



Supplemental table 1: **Clinical and biological characteristics of the patients**.

IPSS-R = Revised International Prognostic Scoring System; MDS = myelodysplastic syndrome, MPN = myeloproliferative neoplasm, RBC = red blood cell

Patients characteristics	**N=30**
Median age at first VEXAS manifestation (range)	67.9 (45.2-89.5)
Median time (years) between first clinical manifestation and JAKi onset (range)	2.66 (0.55-8.3)
VEXAS syndrome clinical manifestations. n (%)	
- Skin involvement	26 (86.6)
- Arthritis or arthralgia	25 (83.3)
- Fever	24 (80)
- Pulmonary infiltrates	17 (56.6)
- Vasculitis	12 (40)
- Ocular manifestations	7 (23.3)
- Venous thromboembolism	10 (33)
Type of *UBA1* mutation. n (%)	
c.122T>C (p.Met41Thr)	15 (50)
c.121A>C (p.Met41Leu)	8 (26.6)
c.121A>G (p.Met41Val)	4 (13.3)
c.118-1G>C (splice variant)	2 (6.6)
c.118-2T>C (splice variant)	1 (3.3)
Associated MN (according to 2016 WHO classification). n (%)	14 (46.6)
- MDS	12 (40)
- Atypical MDS/MPN	1 (3.3)
- Essential thrombocytemia	1 (3.3)
MDS characteristics. n (%)	
- IPSS-R very low/low	8 (61.5)
- IPSS-R low	3 (23.1)
- IPSS-R intermediate	2 (15.4)
- IPSS-R high	0
- IPSS-R very high	0
Median number of previous lines prior JAKi initiaton (range)	2.5 (0-9)
Concomittant steroids at JAKi initiation. n (%)	26 (86.6)
- Median posology. mg/day (range)	30 (5-60)
Biological characteristics at JAKi initiaiton	
- Median C reactive protein. mg/L (range)	50 (10-138.2)
- Median hemoglobin, g/L (range)	94 (62-140)
- Median platelets, 10^9/L (range)	160.5 (13-264)
- Median leukocytes, 10^9/L (range)	3.9 (0.28-8.8)

Supplemental table 2 : **Summary of cytogenetic and molecular findings**

	Patients	Cytogenetics	MDS subtype	UBA1 mutation	Other somatic mutations by NGS (VAF %)
MN	Patient #1	46,XY,del(7)(q22q35)[18]/ 46,XY[2]	MDS	c.122T>C	No additional mutation
	Patient #3	46,XY[20]	MDS/MPN	c.118-2T>C	JAK2 p.V617F (13%)
	Patient #4	46,XY[20]	MDS-EB1	c.121A>G	No additional mutation
	Patient #6	46,XY[20]	MDS-SLD	c.122T>C	No additional mutation
	Patient #7	46,XY[20]	MDS-SLD	c.122T>C	No additional mutation
	Patient #12	46,XY[20]	MDS-MLD	c.122T>C	No additional mutation
	Patient #13	Monosomy 7 (7% by FISH)	ND	c.121A>C	DNMT3A p.R882H (38%) CALR p.L367fs*46 (29%)
	Patient #17	46,XY [20]	MDS-MLD	c.122T>C	TET2 p.T1884A (3%)
	Patient #18	46,X, -Y[10]; 46,XY [10]	MDS-EB1	c.121A>G	DNMT3A p.R882H (1.3%)
	Patient #26	46,XY [20]	MDS-MLD	c.122T>C	DNMT3A p.R882H (36%)
No MN	Patient #2	Not assessed	-	c.122T>C	No additional mutation
	Patient #11	Not assessed	-	c.122T>C	No additional mutation
	Patient #14	46,XY [20]	-	c.118-1G>C	No additional mutation
	Patient #27	46,XY [20]	-	c.122T>C	No additional mutation
	Patient #28	46,XY [20]	-	c.122T>C	DNMT3A p.R882H (37%). KDM6A p.L1375Qfs*10 (5%)
	Patient #29	46,XY [20]	-	c.121A>C	No additional mutation
	Patient #30	46,XY [20]	-	c.121A>G	No additional mutation

Supplemental table 3 : **Clinical and biological characteristics of patients with or without myeloid neoplasm (MN).** RBC: red blood cell

Patients characteristics	**MN (N=14)**	**non-MN (N=16)**	**p-value**
Median age at first VEXAS manifestations (range)	70.1 (58-89)	66.6 (45-76)	0.13
VEXAS syndrome clinical manifestations, n (%)			
- Skin involvement	13/14 (92.8)	13/16 (81.2)	0.56
- Arthritis or arthralgia	11/14 (78.6)	14/16 (87.5)	0.41
- Vasculitis	6/14 (42.8)	5/16 (31.2)	0.39
- Fever	8/14 (57.2)	15/16 (93.7)	0.041
- Ocular manifestations	3/14 (21.4)	4/16 (25)	0.87
- Pulmonary infiltrates	6/14 (42.8)	11/16 (68.7)	0.43
- Venous thromboembolism	3/14 (21.4)	8/16 (50)	0.034
Type of *UBA1* mutation, n(%)			
c.122T>C (p.Met41Thr)	6/14 (42.8)	9/16 (56.3)	
c.121A>C (p.Met41Leu)	3/14 (21.4)	5/16 (31.2)	
c.121A>G (p.Met41Val)	3/14 (21.4)	1/16 (6.2)	ns
c.118-1G>C (splice)	1/14 (7.1)	1/16 (6.2)	
c.118-2T>C (splice)	1/14 (7.1)	0/16	
Median number of previous lines prior JAKi initiation (range)	2.5 (1-9)	1.5 (0-8)	0.17
Concomittant steroids at JAKi initiation, n (%)	13/14 (92.8)	11/12 (91.6)	0.86
- Median posology, mg/day (range)	18.5 (8-60)	30 (5-60)	0.37
Biological characteristics at JAKi initiaiton			
- Median C reactive protein. mg/L (range)	50 (1.5-138)	49.8 (6.5-80)	0.89
- Median hemoglobin, g/L (range)	94.5 (62-126)	97.5 ()	0.77
- Median platelets, 10^9/L (range)	154.5 (13-264)	199 (80-247)	0.27
- Median leukocytes, 10^9/L (range)	3.45 (0.3-9.6)	4.3 (1.6-4.9)	0.47
RBC transfusion dependency, n (%)	7/14 (50)	2/16 (16.6)	0.074
Type of JAKi received, n (%)			
- Ruxolitinib	9/14 (64.2)	3/16 (18.7)	
- Tofacitinib	3/14 (21.4)	8/16 (50)	0.01
- Upadacitinib	1/14 (7.2)	2/16 (12.5)	
- Baricitinib	1/14 (7.2)	3/16 (18.8)	

Supplemental table 4 : **Description of the previous lines of treatment received before JAKi**

Drugs, n	**N=30**
Tocilizumab	13
Anakinra	5
Anti-TNF	
- Adalimumab	4
- Etanercept	2
- Golimumab	3
- Infliximab	3
Colchicine	4
Hydroxychloroquine	2
Potassium iodure	1
Disulone	1
Methotrexate	8
Azacitidine	4
Leflunomide	2
Ciclosporine	3
Mycophenolate mofetil	3
Belimumab	1
Thalidomide	2
Cyclophosphamide	1
Rituximab	1
Patients without information	5