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Ruxolitinib Induced Meaningful and Directional Changes in the Bone Marrow Microenvironment of Patients With Myelofibrosis Enrolled in the COMFORT-I Study

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Background

- Myelofibrosis (MF) is characterized by clonal proliferation of hematopoietic progenitor cells (HPCs) and proliferation of cytokine-producing atypical megakaryocytes and macrophages[1,2]
  - Evidence suggests that the proinflammatory microenvironment, fostered by the hematopoietic clone, results in bone marrow stromal alterations (including bone marrow fibrosis and osteosclerosis), which can, in turn, influence the hematopoietic niche[2,3]
- The oral Janus kinase (JAK) 1/JAK2 inhibitor ruxolitinib has been shown to reduce bone marrow fibrosis in patients with MF compared with conventional therapies or placebo[4]

Objective

- To evaluate bone marrow changes in order to characterize the long-term effects of ruxolitinib on bone marrow stromal alterations, cytokine-producing cells (i.e., megakaryocytes and macrophages), and plasma cells (surrogates of inflammation) in a cohort of patients with MF who were enrolled in the phase 3 COMFORT-I study

Methods

Study Design and Patients

- COMFORT-I (NCT00952289) was a randomized, double-blind, phase 3 study evaluating the efficacy and safety of ruxolitinib compared with placebo in patients diagnosed with intermediate-2 or high-risk primary MF, post–polycythemia vera MF, or post–essential thrombocythemia MF[5]
  - Starting doses of ruxolitinib were based on platelet count (15 mg twice daily [BID] for platelets ≥100 × 10^9/L to ≤200 × 10^9/L; 20 mg BID for platelets >200 × 10^9/L) with dose adjustments as needed for suboptimal efficacy or toxicity
  - Once all patients had completed the Week 24 visit and ≥50% of patients completed the Week 36 visit, the study was unblinded and patients randomized to placebo were permitted to cross over to receive ruxolitinib
- This analysis included 57 patients from COMFORT-I (36 originally randomized to ruxolitinib and 21 crossed over from placebo)
  - All patients in the analysis had a baseline bone marrow biopsy and ≥1 subsequent bone marrow biopsy
  - For patients who crossed over to ruxolitinib before Week 36, the Week 0 (study baseline) biopsy was used as the baseline bone marrow assessment; for patients crossing over after Week 36, the Week 48 biopsy was used as baseline

Assessments

- For fibrosis and osteosclerosis assessments, sections were stained according to the World Health Organization (WHO) recommendations with hematoxylin and eosin, Gomori's silver impregnation, and trichome[4,6,7]
- WHO guidelines were used to grade bone marrow fibrosis[6] and European consensus guidelines were applied for the analysis of bone marrow cellularity[8]
- Evaluations of HPCs included immunohistochemistry (IHC) with CD34 and morphometric assessment **(Table 1)**

Table 1. Semiquantitative Assessment of CD34+ HPCs

Categories	Definition
Normal	CD34+ frequency 0%–2% / no clustering
Normal/regeneration	CD34+ frequency 3%–5% / no clustering
Biologically abnormal/progressive disease	CD34+ frequency 3%–5% / + clustering
Abnormal	CD34+ frequency 0%–2% / + clustering
Abnormal/accelerated phase	CD34+ frequency >5%

HPC, hematopoietic progenitor cell.

- Specific IHC stains were used to assess megakaryocytes (CD61); plasma cells (MUM1); and activated macrophages, including CD68 to identify M1 and anti-inflammatory M2 subtypes, and CD163, a very specific marker for the M2 subtype
- Each parameter was graded, by consensus, based on independent review by 3 expert hematopathologists (HMK, JT, CEB-R)

Statistical Analyses

- Changes in bone marrow parameters described in the assessments were summarized using descriptive statistics
  - A 0–3 grading system was used for fibrosis, osteosclerosis, plasma cells, megakaryocyte clustering/atypia, and CD68/163 macrophages
  - Changes from baseline to last bone marrow observation were reported and categorized as improved, stable, or worsened
    - Improvement/worsening was defined as ≥1 reduction/increase from baseline or a change in abnormal/normal status
    - Improvement was assessed in patients with baseline values of 1–3 or abnormal, stability in patients with baseline values 0–2 or normal, and worsening in patients with baseline values 0–2 or normal

Results

Patient Demographics and Clinical Characteristics

- Characteristics of the 57 patients included in this analysis are summarized in **Table 2**

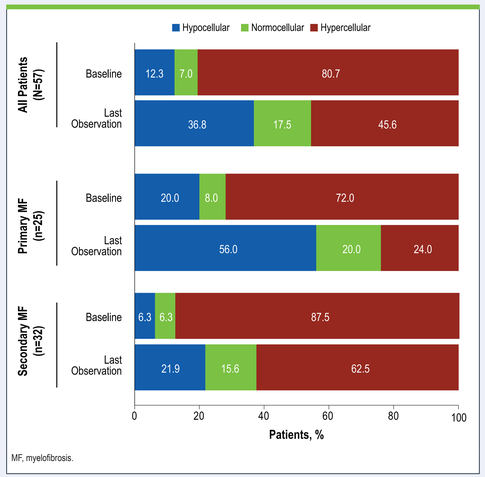
Table 2. Patient Characteristics at the Time of Randomization

Characteristic	Total (N=57)
Age, y	
Mean (SD)	67.2 (8.6)
Min, max	43, 83
Male, n (%)	31 (54.4)
Disease duration, y	
Mean (SD)	4.2 (6.2)
Min, max	0, 32
Risk category at screening, n (%)	
Intermediate risk (2 factors)	20 (35.1)
High risk (≥3 factors)	37 (64.9)
Disease type, n (%)	
Primary myelofibrosis	25 (43.9)
Post–polycythemia vera myelofibrosis	19 (33.3)
Post–essential thrombocythemia myelofibrosis	13 (22.8)
Cohort, n (%)	
Randomized to ruxolitinib	36 (63.2)
Crossed over to ruxolitinib	21 (36.8)

Cellularity and the Bone Marrow Microenvironment

- With respect to age-adjusted cellularity at baseline, most marrows were hypercellular, with a notable increase in the proportion of normocellular and hypocellular marrows by the last observation **(Figure 1)**
- A higher proportion of patients with secondary MF (87.5%) were hypercellular at baseline compared with patients with primary MF (72.0%; **Figure 1**)

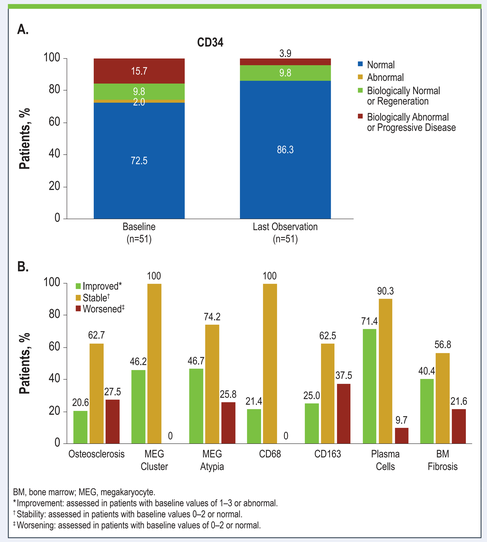
Figure 1. Cellularity Distribution Over Time



MF, myelofibrosis.

- The proportion of patients with normal CD34+ clustering/frequency increased from 72.5% at baseline to 86.3% at the last observation **(Figure 2A)**
  - 89.2% (33/37) of patients who had normal CD34 at baseline remained at a normal state
  - Of the 8 patients who had CD34 levels consistent with biologically abnormal/progressive disease at baseline, 5 (62.5%) became normal **(Table 1)**
  - No patient transformed to an accelerated phase
  - The proportion of patients with no clustering increased from 82.4% at baseline to 96.1% at the last bone marrow observation
    - The proportion of patients with grade 1 clustering decreased from 17.6% to 3.9%
    - No patient had grade 2 or higher clustering at the last observation
- Similarly, the majority of patients had stable (62.7%) or improved (20.6%) osteosclerosis at the last observation compared with baseline **(Figure 2B)**
- Assessments of megakaryopoiesis revealed stabilization or improvement of megakaryocyte clustering (MC) in all patients **(Figure 2B)**
  - A similar proportion of patients with primary and secondary MF had stable MC
  - The proportion of patients with improved MC was slightly higher in patients with primary MF than in those with secondary MF
  - No patient had grade 3 MC at the last observation
- Megakaryocyte atypia was improved or stable in the majority of patients **(Figure 2B)**
  - At the last observation, a higher proportion of patients with primary MF had improvement in atypia and a lower proportion had worsening compared with patients with secondary MF
  - A similar proportion of patients with primary and secondary MF had stable levels of atypia
- Additionally, ruxolitinib resulted in normalization of CD68+ and CD163+ macrophages in 21.4% and 25.0% of patients, respectively **(Figure 2B)**
- With respect to evidence of decreased inflammation in the bone marrow microenvironment, the majority of patients (71.4%) with abnormal plasma cells at baseline had a normal level of plasma cells by the last observation **(Figure 2B)**
  - Only 9.7% of patients with a normal plasma cell grade at baseline had evidence of worsening at the last observation
  - A similar proportion of patients with primary and secondary MF (91.7% and 89.5%, respectively) had stability in plasma cells at the last observation
  - A similar proportion of patients with primary and secondary MF (8.3% and 10.5%, respectively) had worsened plasma cells at the last observation
  - A lower proportion of patients with primary MF had improved plasma cells (57.1%) compared with patients with secondary MF (85.7%)
- In most patients, bone marrow fibrosis was either stable or improved from baseline to last observation; 21.6% of patients had a worsening of fibrosis **(Figure 2B)**
  - No patient had a 2-grade worsening from baseline in fibrosis
  - 20% (8/41) of patients with grade 2 or 3 bone marrow fibrosis at baseline had a 2-grade improvement by the last observation **(Table 3)**
  - All 8 patients who had a worsening grade at the last observation had never improved from baseline

Figure 2. Bone Marrow Changes Observed With Ruxolitinib Treatment (Baseline vs Last Observation)



BM, bone marrow; MEG, megakaryocyte.
*Improvement: assessed in patients with baseline values of 1–3 or abnormal.
†Stability: assessed in patients with baseline values 0–2 or normal.
‡Worsening: assessed in patients with baseline values of 0–2 or normal.

Table 3. Change in Bone Marrow Fibrosis Grade From Baseline to Last Observation

	Last Result, n (%)			
Baseline Result	0	1	2	3
0 (n=5)	5 (100.0)	0 (0)	0 (0)	0 (0)
1 (n=11)	3 (27.3)	4 (36.4)	4 (36.4)	0 (0)
2 (n=21)	1 (4.8)	4 (19.0)	12 (57.1)	4 (19.0)
3 (n=20)	1 (5.0)	6 (30.0)	6 (30.0)	7 (35.0)
	Worsened	Improved	Stable	Unchanged

Conclusions

- **These results extend previous observations on the effect of ruxolitinib on the bone marrow in patients with primary and secondary MF**
- **Ruxolitinib treatment resulted in improvements in the HPCs, atypical megakaryocytes, and activated macrophages that are classically thought to produce inflammatory cytokines that drive bone marrow stromal alterations**
- **These improvements were associated with improvement/stabilization of bone marrow fibrosis and sclerosis in the majority of patients**
- **Directional improvements in bone marrow plasma cells, a surrogate of inflammation in the bone marrow microenvironment, were also observed**
- **The disease-modifying properties of ruxolitinib are likely attributable to its ability to address not only the myeloproliferation through inhibition of JAK2 but also the secondary inflammatory state through inhibition of JAK1**

Disclosures

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