Received: 26 August 2022 | Accepted: 8 October 2022

DOI: 10.1111/bjh.18526

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

ORIGINAL PAPER

Trend of circulating CD34$^+$ cells in patients with myelofibrosis: Association with spleen response during ruxolitinib treatment

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Funding information
Italian Ministry of Health - Current research IRCCS

Summary

We evaluated CD34$^+$ cells in a single-centre series of 49 consecutive patients with myelofibrosis (MF) at baseline and during ruxolitinib therapy and examined any association with spleen response. The median (range) absolute number of circulating CD34$^+$ cells was 0.0835 (0.001–1.528) $\times 10^9$/L at diagnosis, and 0.123 (0.002–1.528) $\times 10^9$/L at ruxolitinib start. With the exception of a transient increase after 3 months of ruxolitinib therapy, a progressive reduction in CD34$^+$ cells count was documented, down to a minimum of 0.063 $\times 10^9$/L after 36 months. We then assessed the association between spleen diameter expressed as the distance from the left costal margin (outcome) and log(CD34$^+$) cells count using random-intercept and random slope multivariable regression models to take into account within subject correlation: after adjusting for time and ruxolitinib dosage, we estimated a 0.7 cm increase (95% confidence interval 0.2–1.2, $p = 0.003$) in spleen length for each unit increase in log(CD34$^+$) cells count ($\times 10^9$/L). Although our study has some limitations, mainly related to its retrospective design, our approach may introduce a reproducible and simple tool that could facilitate the assessment of spleen response more objectively in patients with MF treated with ruxolitinib.

KEYWORDS
CD34$^+$, myelofibrosis, response, ruxolitinib, splenomegaly

INTRODUCTION

BCR::ABL1-negative myeloproliferative neoplasms (MPNs) are already known to be variably characterised by an increase in peripheral blood (PB) and bone marrow (BM) progenitor cells. More specifically, in patients with myelofibrosis (MF) it has already been reported that the number of circulating haematopoietic precursors is consistently high, with a relative circulating CD34$^+$ cells count of 0.015 $\times 10^9$/L as the most frequently used criterion to discriminate between MF and other MPNs.[1]

According to the 2016 World Health Organization (WHO) classification, primary MF (PMF) is defined as a haematopoietic stem-cell-derived clonal disorder,[2] in which the abnormal stem cell population releases various cytokines and growth factors in the BM microenvironment.[3] It can be subcategorised into pre-fibrotic and overt fibrotic PMF depending on specific features.[4] Furthermore, MF can also represent an advanced stage during the natural history of another pre-existing *BCR::ABL1*-negative MPN, namely polycythaemia vera (PV) or essential

thrombocythaemia (ET), globally defined as secondary MF (SMF).[5]

Symptoms related to splenomegaly such as early satiety, abdominal discomfort and pain, along with constitutional symptoms such as fatigue, night sweats, cachexia, itching, unexplained weight loss, fever and bone pain represent MF hallmarks. Other clinical manifestations may include portal hypertension and non-hepatosplenic extramedullary haematopoiesis leading to cord compression, pleural effusion, or pulmonary hypertension. Even though the most frequent cause of death is still evolution into acute myeloid leukaemia (AML), other conditions such as progression without transformation, cytopenias-related complications, thrombotic events and/or second neoplasia can also be fatal.[6,7]

Historically, PMF prognosis was based on three different scoring systems, which primarily rely on older age, constitutional symptoms, haemoglobin (Hb) level, white blood cell (WBC) count, and PB blasts percentage. The International Prognostic Scoring System (IPSS) can only be used at diagnosis[6]; on the contrary, the Dynamic International Prognostic Scoring System (DIPSS)[8] and the DIPSS-plus are also applicable at any time during follow-up, the latter incorporating three further independent risk factors, i.e. the need for red blood cell (RBC) transfusion, thrombocytopenia, and an unfavourable karyotype.[9] More modern stratification models have recently been developed for PMF (i.e. Mutation-enhanced IPSS in adults aged ≤ 70 years [MIPSS70], MIPSS70+ version 2.0, or Genetically inspired prognostic scoring system [GIPSS]), including additional information based on genetics, from karyotype to driver and other mutations.[10–12] For SMF, due to peculiar clinical-laboratory and molecular features that result in a prognosis different from PMF, a specific prognostic model has recently been proposed, i.e. the Myelofibrosis Secondary to PV and ET Collaboration-Prognostic Model (MYSEC-PM).[13]

The MF therapeutic algorithm is currently based on the European LeukemiaNet (ELN) recommendations[14]: in detail, ruxolitinib, the first Janus kinase (JAK) inhibitor to become commercially available, is approved in the USA for the treatment of splenomegaly in individuals with intermediate-/high-risk disease, and in Europe for the treatment of splenomegaly and/or constitutional symptoms in intermediate-/high-risk patients with MF.[15,16]

Ruxolitinib improves inflammation and proliferation, leading to clinically relevant control of splenomegaly and symptoms in most patients with MF, which may result in prolonged survival.[17–20] Nonetheless, after some time, patients treated with ruxolitinib may lose their spleen or symptom response, fail to tolerate ruxolitinib due to therapy-related anaemia, thrombocytopenia, or non-haematological adverse events (AEs), particularly infectious complications, or progress to the accelerated or blast phase of disease; e.g. in the COntrolled MyeloFibrosis Study with ORal JAK inhibitor Treatment (COMFORT)-II study, the treatment discontinuation rate was ~50% at 3 years and 75% at 5 years.[20] A similar discontinuation rate was also reported in the JUMP study (ClinicalTrials.gov Identifier: NCT01493414), which included >2200 patients with MF enrolled in countries with no access to ruxolitinib outside of a clinical trial.[17] In detail, only 57.5% of patients completed treatment per protocol (i.e. treated for up to 24 months after the last patient's first visit or switched to commercial drug), with the primary reasons for discontinuation being AEs (18.1%) and investigator-determined disease progression (9.1%).[21]

In addition, once ruxolitinib has been discontinued, patients' outcome is poor, particularly if it occurs after leukaemic evolution.[22]

Despite recent improvements, PB blasts count is still based on the morphological assessment of PB films and as such is largely operator-dependent, especially considering that the threshold for blasts count is between ≥1% and ≥3% in the different prognostic scores currently used in MF. As a matter of fact, general MF treatment algorithms are still based on disease risk scores rather than blast percentages. However, in a recent article, Masarova et al.[23] first reported that PB blast percentage offers additional prognostic value in patients with MF who have <5% BM blasts, particularly when referring to patients with 4% PB blasts who appeared to behave more like those with ≥5% PB blasts compared to patients with lower blasts.

Furthermore, even if the current WHO classification that forms the basis for pathology practice does not take into account increased blasts when they do not exceed 10%, Geyer et al.[24] have recently shown that patients with MPNs developing 5%–9% PB or BM blasts have worse outcomes than chronic phase-MPN patients, thus potentially representing a new parameter to be considered for future inclusion among pathological criteria for diagnosing disease progression in MPNs, as well as in future changes to dynamic MPN prognostic scoring systems.

Although not considered an equivalent, the absolute number of circulating CD34+ cells is related to PB blasts,[1,6] and its impact on PMF prognosis has recently been explored, with promising results.[25]

Starting from this rationale, we tried to evaluate CD34+ cells levels in a single-centre series of patients with MF at baseline and during ruxolitinib therapy, with the aim of identifying any possible association with spleen response.

PATIENTS AND METHODS

Patients

Between October 2014 and December 2021, circulating CD34+ cells count from 49 consecutive patients with PMF or SMF (32 males and 17 females; median [range] age at diagnosis 66.0 [48.5–82.8] years) were retrospectively assessed at baseline and at 3, 6, 12, 24 and 36 months after starting ruxolitinib.

All patients were reviewed according to the 2016 WHO criteria in the case of PMF,[2] or the International Working Group for Myelofibrosis Research and Treatment

(IWG-MRT) criteria for SMF.[5] Leukaemic evolution was diagnosed according to WHO criteria, with a 20% BM or PB blasts threshold.[26]

Risk categories were assessed at the time patients initiated ruxolitinib treatment according to the DIPSS and MYSEC-PM for PMF and SMF respectively.[8,13]

All patients were treated with ruxolitinib according to current indications, requiring an IPSS risk at least intermediate-1, while in the chronic phase of the disease (i.e. PB and BM blasts <10%).

At the time of each $CD34^+$ cells count, spleen measurement (expressed as the distance from the left costal margin [BCM] in cm) was taken for all patients and a concomitant complete blood cells count was available, along with body weight, body mass index (BMI) and dosage of ruxolitinib.

Follow-up information was updated in April 2022.

Methods

Molecular analyses

The *JAK2*V617F mutation was detected by allele-specific polymerase chain reaction (PCR) according to the protocol of Baxter et al.[27] and confirmed by direct Sanger sequencing. Quantitative analysis of the allele burden of the *JAK2*V617F mutation was performed by real-time quantitative PCR using JAK2 MutaQuant (Ipsogen Inc). The cut-off used for defining a case as negative for *JAK2*V617F mutation was 0.5%.

Myeloproliferative leukaemia (MPL) proto-oncogene, thrombopoietin receptor (*MPL*) mutations, in particular W515L, W515K, W515A, S505N, and G509C, were tested by direct sequencing of exon 10. The primers used were as follows: MPL10F 5' TAGCCTGGATCTCCTTGGTG 3′; MPL10R 5' CCTGTTTACAGGCCTTCGGC 3′.

Mutations in exon 9 of the calreticulin (*CALR*) gene were also assessed using a bidirectional sequencing approach as previously described.[28] All sequencing analyses were performed on an ABI PRISM 310 Genetic Analyser (Applied Biosystems) using the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems).

Cytogenetic analyses

G-banding with trypsin performed on fresh BM aspirates was the standard technique for chromosome analysis with at least 20 metaphases described.[29] Normal karyotype was defined as the absence of any chromosomal abnormality in a minimum of 20 metaphases examined. Chromosomal abnormalities were considered clonal if the same structural or extra-chromosome abnormality appeared in at least two and monosomy in at least three metaphases.

Unfavourable karyotype was categorised as previously described.[9]

Bone marrow biopsy

Histological confirmation of MF diagnosis was performed by an experienced pathologist. Formalin-fixed, paraffin-embedded BM biopsy samples obtained at diagnosis were available for all patients. Sections were stained with haematoxylin–eosin, Giemsa, and Gomori's silver impregnation for the evaluation of morphological features and BM fibrosis grade.

Statistical analysis

We reported medians and ranges for continuous variables, and absolute and relative frequencies for categorical variables. Given the correlated nature of data, to analyse temporal trends over time we fitted time-adjusted (considering time as categorical) random-intercept (to account for variability between subjects at the time of diagnosis) and random slope (to account for possible individual differences in trajectories over time) linear regression models. The same models were used to analyse the relationship between splenomegaly (dependent variable) and circulating log-transformed $CD34^+$ cell counts (independent variable), adjusting for time and ruxolitinib therapy (yes/no). We evaluated the possible effect modification by diagnosis of time trends and of the splenomegaly–$CD34^+$ cell count association by introducing interaction (product) terms between diagnosis (PMF or SMF) and time (categorical) and calculating a global Wald test. When necessary, variables ($CD34^+$ cell counts, lactate dehydrogenase, Hb, WBC and platelet [PLT] counts) were log-transformed to improve the normality of distributions. Statistical analysis was performed with Stata 17 (StataCorp. 2021).

RESULTS

The haematological and clinical characteristics of the MF patients' population studied are summarised in Table 1.

In all, 24 (49.0%) patients were classified as having PMF (10 in the pre-fibrotic and 14 in the overt fibrotic stage) and 25 (51.0%) as having SMF (15 with post-PV and 10 post-ET MF).

*JAK2*V617F mutation was detected in 38 (77.6%) cases, *CALR* mutations in eight (16.3%), including five patients with *CALR* type 1 mutation and three case with *CALR* type 2 mutation, and *MPL*W515L in one (2%). The remaining two patients (4.1%) were defined as 'triple-negative', i.e. without mutations in the *JAK2*, *CALR*, or *MPL* genes. Karyotype was abnormal in 17 (34.7%) patients, being defined as unfavourable in six (12.2%).

At initiation of ruxolitinib treatment (after a median [range] time from MF diagnosis of 33.9 [0.5–272.7] months), the median (range) age was 70.5 (51.6–85.5) years, and 65.3% of patients were men. The DIPSS distribution was as follows: intermediate-1 in 50.0%, intermediate-2 in 45.8%,

TABLE 1 Clinical-laboratory features of the patients

Variable	Value
Number of patients	49
Male/female, *n*	32/17
Age at MPN diagnosis, years, median (range)	66.0 (48.5–82.8)
MPN subtype, *n* (%)	
PMF	24 (49.0)
pre-PMF	10 (20.4)
overt PMF	14 (28.6)
SMF	25 (51.0)
PPV-MF	15 (30.6)
PET-MF	10 (20.4)
Driver gene mutations	
*JAK2*V617F, *n* (%)	38 (77.6)
*JAK2*V617F allele burden, %, median (range)	81.5 (13.9–99.5)
CALR, *n* (%)	8 (16.3)
Type 1	5 (10.2)
Type 2	3 (6.1)
*MPL*W515L, *n* (%)	1 (2.0)
Triple-negative, *n* (%)	2 (4.1)
Cytogenetic abnormalities, *n* (%)	17 (34.7)
Splenomegaly at diagnosis, *n* (%)	35 (71.4)
Age at RUX start, years, median (range)	70.5 (51.6–85.5)
Time from MF diagnosis to RUX start, months, median (range)	33.9 (0.5–272.7)
DIPSS model at RUX start (*N* = 24), *n* (%)	
Intermediate-1	12 (50.0)
Intermediate-2	11 (45.8)
High	1 (4.2)
MYSEC-PM model at RUX start (*N* = 25), *n* (%)	
Intermediate-1	8 (32.0)
Intermediate-2	9 (36.0)
High	8 (32.0)
RUX starting dose (mg BID), *n* (%)	
5	6 (12.2)
10	7 (14.3)
15	7 (14.3)
20	29 (59.2)
Follow-up from RUX start, months, median (range)	32.8 (0.5–108.2)
Death, *n* (%)	19 (38.8)
Leukaemic evolution, *n* (%)	7 (14.3)
Infections, *n* (%)	8 (16.3)
Other MF-unrelated causes, *n* (%)	4 (8.2)

Abbreviations: BID, twice daily (bis in die); DIPSS, Dynamic International Prognostic Scoring System; MF, myelofibrosis; MPN, myeloproliferative neoplasm; MYSEC-PM, Myelofibrosis Secondary to PV and ET Collaboration-Prognostic Model; PET-MF, post-essential thrombocythaemia myelofibrosis; PMF, primary myelofibrosis; PPV-MF, post-polycythaemia vera myelofibrosis; RUX, ruxolitinib; SMF, secondary myelofibrosis.

and high risk in 4.2% of the patients; for the MYSEC-PM, it was intermediate-1 in 32.0%, intermediate-2 in 36.0%, and high risk in 32.0% of the patients. In total, six (12.2%) and four (8.2%) patients had PLT count of $<100 \times 10^9$/L or were RBC transfusion dependent respectively. The spleen was palpable at <5 cm, between 5 and 10 cm, and at >10 cm BCM in 12 (24.5%), 15 (30.6%), and 22 (44.9%) patients respectively.

As reported in Table 2, the median (range) absolute number of circulating CD34$^+$ cells in the overall population at diagnosis was 0.0835 (0.001–1.528) $\times 10^9$/L, with 31 (63.3%) patients showing a CD34$^+$ cells count of $>0.015 \times 10^9$/L. At ruxolitinib start, the median (range) absolute number of CD34$^+$ cells was instead 0.123 (0.002–1.528) $\times 10^9$/L, with 43 (87.8%) cases showing above normal levels.

As expected, spleen measurements progressively decreased during the first 6 months of ruxolitinib therapy (Figure 1A): specifically, the spleen was palpable at a median of 9.9 cm BCM at ruxolitinib start, and 6 and 5.5 cm after 3 and 6 months of therapy respectively. Conversely, it increased to 8.2 cm after 36 months, with only a more modest increase to ~6 cm after 12 and 24 months.

Interestingly, with the exception of a transient increase after 3 months of ruxolitinib therapy, a progressive reduction in the absolute number of PB CD34$^+$ cells was documented in the whole cohort, with only a slight increase after 24 months, up to a minimum of 0.063×10^9/L after 36 months of therapy (Figure 1B).

A possible association between log(CD34$^+$) cells count and spleen diameter was therefore sought during ruxolitinib therapy: after adjusting for time and ruxolitinib dosage, a positive association was found between the two variables, with a 0.7 cm (95% confidence interval [CI] 0.2–1.2; $p = 0.003$) increase in spleen length for each unit increase in log(CD34$^+$) cells count. The association was slightly lower (+0.4 cm) and statistically less precise (95% CI −0.3 to 1.0; $p = 0.25$) when excluding the first two time points (diagnosis and initiation of ruxolitinib therapy). There was no statistical evidence (p interaction: 0.65) of effect modification by diagnosis (PMF vs. SMF).

The temporal behaviour of other clinical-laboratory features during ruxolitinib therapy is reported in Table 3: in particular, as expected, both Hb levels and PLT counts progressively decreased during the first 3–6 months of therapy, eventually reaching a new steady state for both parameters. In parallel, all patients showed a gradual increase in body weight and BMI for up to 24 months. We found no evidence of effect modification by diagnosis (p interaction: 0.33–0.87).

After a median (range) follow-up from ruxolitinib start of 32.8 (0.5–108.2) months, 21 (42.9%) patients discontinued treatment (with a median [range] treatment duration of 33.5 [0.9–96.0] months), 9 (18.4%) evolved into AML, and 19 (38.8%) died; the causes of death are summarised in Table 1.

TABLE 2 $CD34^+$ cells count and spleen diameter during ruxolitinib therapy

Characteristic	MF study cohort ($N = 49$)	PMF ($N = 24$)	SMF ($N = 25$)
$CD34^+$ cells, $\times 10^9$/L, median (range)			
At MF diagnosis ($N = 49$)	0.0835 (0.001–1.528)	0.0835 (0.001–0.206)	0.073 (0.007–1.528)
At RUX start ($N = 49$)	0.123 (0.002–1.528)	0.1325 (0.002–1.046)	0.094 (0.005–1.528)
After 3 months ($N = 46$)	0.1915 (0.029–2.306)	0.213 (0.031–0.823)	0.170 (0.029–2.306)
After 6 months ($N = 42$)	0.124 (0.003–1.815)	0.146 (0.003–1.235)	0.079 (0.017–1.815)
After 12 months ($N = 35$)	0.1135 (0.004–1.002)	0.100 (0.004–0.832)	0.127 (0.019–1.002)
After 24 months ($N = 30$)	0.113 (0.004–2.100)	0.071 (0.004–0.448)	0.122 (0.008–2.100)
After 36 months ($N = 18$)	0.063 (0.004–0.766)	0.0215 (0.004–0.513)	0.127 (0.034–0.766)
Spleen diameter (cm BCM), median (range)			
At MF diagnosis ($N = 49$)	4.1 (0.0–15.0)	4.4 (0.0–15.0)	3.8 (0.0–12.0)
At RUX start ($N = 49$)	9.9 (0.0–27.0)	9.5 (0.0–18.0)	10.3 (0.0–27.0)
After 3 months ($N = 46$)	6.0 (0.0–20.0)	5.7 (0.0–12.0)	6.4 (0.0–20.0)
After 6 months ($N = 42$)	5.5 (0.0–20.0)	5.4 (0.0–12.0)	5.7 (0.0–20.0)
After 12 months ($N = 35$)	6.3 (0.0–19.0)	6.1 (0.0–17.0)	6.4 (0.0–19.0)
After 24 months ($N = 30$)	6.2 (0.0–19.0)	4.8 (0.0–12.0)	7.2 (0.0–19.0)
After 36 months ($N = 18$)	8.2 (0.0–19.0)	7.3 (0.0–14.0)	8.6 (0.0–19.0)

Abbreviations: BCM, below left costal margin; MF, myelofibrosis; PMF, primary myelofibrosis; RUX, ruxolitinib; SMF, secondary myelofibrosis.

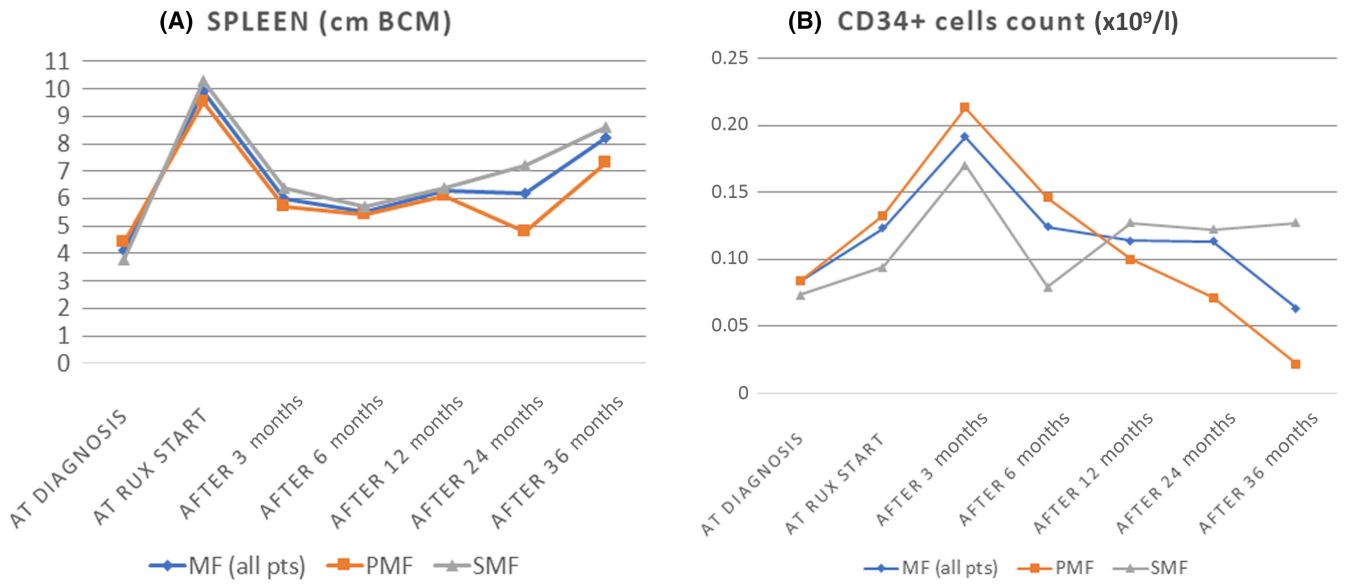

FIGURE 1 Trend in spleen measurements (**A**) and circulating $CD34^+$ cells count (**B**) in patients with myelofibrosis (MF) during treatment with ruxolitinib (RUX). BCM, below left costal margin; PMF, primary myelofibrosis; SMF, secondary myelofibrosis [Colour figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

The functional imbalance of the malignant haematopoietic clone in patients with *BCR::ABL1*-negative MPNs results in an increase in progenitor cells, including pluripotent and committed progenitors, in both the PB and BM.[30–34] Particularly in MF the number of circulating haematopoietic precursors, typically measured by flow cytometry, has always been reported as constantly high, with average levels from eight- to 167-times higher than those found in control subjects. Consequently, it has been hypothesised that the circulating pool of $CD34^+$ cells in these patients may increase along with the proliferative capacity of the individual disease, and this pool may have the potential to represent the proliferative patterns of such malignancies.

As they can potentially represent a diagnostic and prognostic tool for these diseases, in particular for MF, in 2001 Barosi et al.[1] first systematically assessed the absolute count of PB $CD34^+$ cells in 84 patients with MF and in 23 with other *BCR::ABL1*-negative MPNs. In addition to showing that a

TABLE 3 Clinical-laboratory features during ruxolitinib therapy

Characteristic	MF study cohort (*N* = 49)	PMF (*N* = 24)	SMF (*N* = 25)
Hb, g/L, median (range)			
At MF diagnosis (*N* = 49)	119 (80–164)	113 (80–145)	125 (91–164)
At RUX start (*N* = 49)	106 (69–149)	104 (69–143)	108 (72–149)
After 3 months (*N* = 46)	92 (64–137)	91 (71–121)	94 (64–137)
After 6 months (*N* = 42)	93 (69–138)	90 (69–132)	96 (73–138)
After 12 months (*N* = 35)	96 (70–123)	97 (78–123)	96 (70–122)
After 24 months (*N* = 30)	98 (76–129)	95 (77–120)	99 (76–129)
After 36 months (*N* = 18)	100 (73–134)	99 (88–119)	101 (73–134)
WBC count, $\times 10^9$/L, median (range)			
At MF diagnosis (*N* = 49)	12.8 (1.02–38.25)	14.0 (5.13–38.25)	11.6 (1.02–29.42)
At RUX start (*N* = 49)	14.1 (2.93–49.56)	14.3 (2.93–49.56)	13.9 (5.12–38.13)
After 3 months (*N* = 46)	11.3 (2.55–37.17)	9.8 (3.08–22.07)	12.9 (2.55–37.17)
After 6 months (*N* = 42)	14.9 (1.94–47.09)	15.1 (1.94–38.79)	14.7 (3.67–47.09)
After 12 months (*N* = 35)	12.0 (3.25–44.26)	10.8 (3.33–36.27)	12.9 (3.25–44.26)
After 24 months (*N* = 30)	11.4 (4.49–43.82)	9.2 (4.49–15.11)	12.9 (4.79–43.82)
After 36 months (*N* = 18)	10.1 (3.47–34.25)	7.4 (4.12–15.77)	11.4 (3.47–34.25)
PLT count, $\times 10^9$/L, median (range)			
At MF diagnosis (*N* = 49)	448 (26–1267)	436 (58–1087)	461 (26–1267)
At RUX start (*N* = 49)	349 (57–1425)	299 (61–935)	400 (57–1425)
After 3 months (*N* = 46)	221 (54–1057)	181 (54–462)	264 (81–1057)
After 6 months (*N* = 42)	204 (55–1061)	162 (59–505)	240 (55–1061)
After 12 months (*N* = 35)	202 (54–658)	178 (54–486)	218 (72–658)
After 24 months (*N* = 30)	202 (51–1146)	157 (85–378)	232 (51–1146)
After 36 months (*N* = 18)	205 (55–842)	178 (71–427)	218 (55–842)
LDH, iu/L, median (range)			
At MF diagnosis (*N* = 49)	758 (195–2639)	764 (195–2639)	752 (212–1808)
At RUX start (*N* = 49)	756 (210–3736)	793 (229–3736)	719 (210–1672)
After 3 months (*N* = 46)	806 (207–3359)	841 (207–3359)	769 (252–1881)
After 6 months (*N* = 42)	812 (197–3294)	912 (197–3294)	725 (283–1590)
After 12 months (*N* = 35)	784 (189–3860)	935 (364–3860)	676 (189–1755)
After 24 months (*N* = 30)	660 (113–2103)	592 (299–1076)	705 (113–2103)
After 36 months (*N* = 18)	616 (163–1351)	602 (319–959)	623 (163–1351)
Weight, kg, median (range)			
At RUX start (*N* = 49)	70.6 (51–103)	73.4 (55–103)	67.9 (51–85)
After 3 months (*N* = 46)	72.3 (51–106)	74.9 (53–106)	69.5 (51–86)
After 6 months (*N* = 42)	73.2 (51–105)	76.3 (55–105)	70.5 (51–87)
After 12 months (*N* = 35)	74.1 (51–105)	77.4 (57–105)	71.8 (51–88)
After 24 months (*N* = 30)	75.7 (57–109)	78.2 (59–109)	74.1 (57–90)
After 36 months (*N* = 18)	73.7 (58–100)	76.5 (58–100)	72.3 (60–80)
BMI, kg/m^2, median (range)			
At RUX start (*N* = 49)	24.1 (19.2–32.2)	25.1 (19.5–32.2)	23.2 (19.2–30.1)
After 3 months (*N* = 46)	24.8 (19.4–33.0)	25.5 (20.8–33.0)	23.9 (19.4–30.5)
After 6 months (*N* = 42)	24.9 (19.4–33.0)	25.8 (20.4–33.0)	24.2 (19.4–30.8)
After 12 months (*N* = 35)	25.5 (19.4–33.0)	26.8 (20.1–33.0)	24.6 (19.4–31.2)
After 24 months (*N* = 30)	26.1 (19.3–34.0)	27.4 (21.0–34.0)	25.3 (19.3–31.9)
After 36 months (*N* = 18)	25.6 (20.3–33.0)	28.0 (24.8–33.0)	24.4 (20.3–28.0)

Abbreviations: BMI, body mass index; Hb, haemoglobin; LDH, lactate dehydrogenase; MF, myelofibrosis; PLT, platelet; PMF, primary myelofibrosis; RUX, ruxolitinib; SMF, secondary myelofibrosis; WBC, white blood cell.

$CD34^+$ cells count of 0.015×10^9/L produce an almost complete discrimination between patients with off-therapy MF and other MPNs (positive predictive value, 98.4%; negative predictive value, 85.0%), the authors also demonstrated that overall and leukaemia-free survival from the time of $CD34^+$ cell analysis were both significantly shorter in patients with $>0.300 \times 10^9$/L $CD34^+$ cells ($p = 0.005$ and $p = 0.0005$ respectively). Regarding the possible role of $CD34^+$ cells level as a follow-up parameter, the PB count of $CD34^+$ precursors has also been shown to fluctuate with tumour burden in individuals treated with hydroxycarbamide (hydroxyurea), which was the standard cytoreductive treatment for MF at the time; even if the authors considered only a very limited number of patients (with only one case analysed for a long time), they first hypothesised that $CD34^+$ cells could be a candidate in the search for disease activity markers, albeit without drawing any definitive conclusions.[1]

Although subsequent studies have not been able to support an independent prognostic value for PB $CD34^+$ cells count alone in MF,[35] Mannelli et al.[25] have recently proposed to use this parameter determined at diagnosis by multiparameter flow cytometry (MFC) in a series of 363 patients with MF with the aim of improving the performance of existing PMF scores. The integration of two parameters determined by MFC, i.e. absolute $CD34^+$ cells count and granulocytes to lymphocytes side scatter (SSC) ratio, in established prognostic models (in particular MIPSS70+) in place of the morphological count of PB blasts was able to improve their performance compared to the standard counterparts.

Interestingly, in a previous study by our group, we found that $CD34^+$ cells count was positively correlated with the degree of BM fibrosis ($p < 0.001$) in a single-centre series of 108 consecutive patients with PMF, thus potentially representing a reliable and user-friendly marker of this histological parameter, with potential consequences on patients' outcome.[36]

As already reported by other authors, the preliminary results of our study show that PB $CD34^+$ cells are increased in most patients with MF, both at diagnosis and during follow-up.

Above all, to the best of our knowledge, we are the first to report the changes in circulating $CD34^+$ cells count during ruxolitinib treatment, with the most relevant data represented by a progressive decrease up to a minimum of 0.063×10^9/L after 36 months of therapy. As a parallel reduction in spleen measurements was documented during the first 6 months of treatment, we therefore sought a possible association between log($CD34^+$) cells count and spleen diameter: after adjusting for time and ruxolitinib dosage, we were able to demonstrate a positive association between the two variables, with a 0.7 cm (95% CI 0.2–1.2; $p = 0.003$) increase in spleen length for each unit increase in log($CD34^+$) cells count. Interestingly, there was no statistical evidence (p interaction: 0.65) of effect modification by diagnosis (PMF vs. SMF).

We are aware that our study has some recognisable limitations, mainly related to its retrospective design. However, as in daily clinical practice response to ruxolitinib therapy in terms of spleen reduction is commonly assessed on deep palpation as the distance BCM in cm, thus using a strictly operator-dependent method without the possibility of evaluating spleen volume, we believe that our approach may introduce a reproducible and simple tool that could facilitate the assessment of spleen response more objectively in patients with MF treated with ruxolitinib, although this needs to be confirmed by further studies.

AUTHOR CONTRIBUTIONS

Alessandra Iurlo and Daniele Cattaneo were responsible for study concept and design and wrote the paper. Dario Consonni was responsible for statistical analyses. Alessandra Iurlo, Nicole Galli, Cristina Bucelli, Silvia Artuso and Daniele Cattaneo followed the patients and collected data. All authors critically reviewed and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

This study was partially funded by Italian Ministry of Health - Current research IRCCS. Open access funding provided by BIBLIOSAN.

CONFLICTS OF INTEREST

All the authors declare they have no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Iurlo A, Galli N, Bucelli C, Artuso S, Consonni D, Cattaneo D. Trend of circulating CD34+ cells in patients with myelofibrosis: Association with spleen response during ruxolitinib treatment. Br J Haematol. 2023;200(3):315–322. https://doi.org/10.1111/bjh.18526