

Recombinant interferon- α in myelofibrosis reduces bone marrow fibrosis, improves its morphology and is associated with clinical response

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Recombinant interferon- α represents a well-established therapeutic option for the treatment of polycythemia vera and essential thrombocythemia. Recent studies also suggest a role for recombinant interferon- α in the treatment of 'early stage' primary myelofibrosis, but few studies have reported the bone marrow changes after clinically successful interferon therapy. The aim of the present study is to detail the histological responses to recombinant interferon- α in primary myelofibrosis and post-polycythemia vera/post-essential thrombocythemia myelofibrosis and to correlate these with clinical findings. We retrospectively studied 12 patients with primary myelofibrosis or post-polycythemia vera/post-essential thrombocythemia myelofibrosis, who had been treated with recombinant interferon- α . Six patients had received other prior cytoreductive therapies. Bone marrow biopsy was assessed for the following histological parameters: (i) cellularity; (ii) myeloid-to-erythroid ratio; (iii) megakaryocyte tight clusters; (iv) megakaryocyte and naked nuclei density; (v) megakaryocytic atypia; (vi) fibrosis; and (vii) the percentage of blasts. Clinical and laboratory data were included: (i) constitutional symptoms; (ii) splenomegaly, if present; and (iii) complete cell blood count. The clinical response to therapy was evaluated using the International Working Group for Myelofibrosis Research and Treatment/European LeukemiaNet response criteria. The Dynamic International Prognostic Scoring System (DIPSS) score was calculated before and after recombinant interferon- α administration. Successful interferon therapy for myelofibrosis was associated with a significant reduction of marrow fibrosis, cellularity, megakaryocyte density and naked nuclei density. The presence of JAK2^{V617F} mutation correlated with improved DIPSS score. JAK2^{V617F}-negative cases showed worsening of such score or evolution to acute myeloid leukemia. Cytogenetic analysis documented a normal karyotype in all cases. In conclusion, successful clinical response to interferon- α correlates well with an improvement of bone marrow morphology. The prognostic effect of such therapy may be influenced by the JAK2 mutational status. Additional studies are needed to confirm these preliminary data.

Modern Pathology (2015) 28, 1315–1323; doi:10.1038/modpathol.2015.93; published online 14 August 2015

Chronic myeloproliferative neoplasms are a heterogeneous group of hematological disorders caused by the neoplastic transformation of marrow hematopoietic stem cells. Myeloproliferative neoplasms encompass both *BCR/ABL1*-positive and

BCR/ABL1-negative disorders. The latter group includes primary myelofibrosis, polycythemia vera, essential thrombocythemia and other less common myeloproliferative neoplasms.^{1,2} Approximately 20% of patients with polycythemia vera and essential thrombocythemia experience disease progression to a fibrotic stage, termed post-polycythemia vera/post-essential thrombocythemia myelofibrosis.^{1,3}

Fibrotic-stage primary myelofibrosis, post-polycythemia vera and post-essential thrombocythemia myelofibrosis share several clinical, pathological and molecular features; these include splenomegaly, systemic symptoms (i.e., fatigue, fever, weight loss,

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Received 27 April 2015; revised 19 June 2015; accepted 21 June 2015; published online 14 August 2015

night sweats) and variably abnormal blood cell counts, with teardrop erythrocytes and leukoerythroblastosis.⁴ On a molecular level, myelofibrosis is associated with recurrent mutations of the *JAK2*, *CALR* or *MPL* genes.^{5–8}

Histologically, early myelofibrosis is characterized by increased bone marrow cellularity, with large tight clusters of atypical megakaryocytes and increased myeloid cells, with or without diffuse interstitial fibrosis. End-stage myelofibrosis also includes diffuse osteomyelosclerosis, with reduced cellularity, coarse reticulin fibers, thickened bone trabeculae and overt collagen fibrosis.²

With the exception of allogeneic hematopoietic stem cell transplant, therapies for myelofibrosis have targeted symptoms but have not modified the natural history of the disease.⁹ The recent introduction of the *JAK1/2*-inhibitor ruxolitinib has partially changed the therapeutic landscape, as it has been shown to reduce splenomegaly and myelofibrosis-related constitutional symptoms. Recent evidence may suggest an improved overall survival, but this is questioned by others.¹⁰ Nevertheless, this drug has little effect on neoplastic stem cells and does not significantly reduce the *JAK2*^{V617F} allele burden.^{11,12}

The use of recombinant interferon- α can overcome these limitations by inhibiting and potentially decreasing the proliferation of neoplastic stem cells.¹³ In essential thrombocythemia and polycythemia vera, recombinant interferon- α has been associated with prolonged clinical and molecular responses.¹⁴ Similar results have been recently reported in patients with early-stage primary myelofibrosis, including improvement of marrow morphology in a subset of patients.^{15,16} The effects of recombinant interferon- α in myelofibrosis have been less thoroughly investigated. Recent studies suggest an improvement in clinical symptoms and laboratory values in subsets of interferon-treated myelofibrosis patients,^{17–19} but the effects of this therapy on bone marrow histology have not been reported in detail.

This study was aimed to evaluate the histological and clinical response to prolonged recombinant interferon- α administration in patients with primary myelofibrosis and post-polycythemia vera/post-essential thrombocythemia myelofibrosis. Correlations between certain clinical and pathological findings were also investigated.

Materials and methods

Case Selection and Clinical Information

This retrospective study considered a series of recombinant interferon-treated myeloproliferative neoplasms, which fulfilled the 2008 WHO diagnostic criteria for either (i) fibrotic-stage primary myelofibrosis or (ii) secondary myelofibrosis (post-polycythemia vera myelofibrosis; post-essential thrombocythemia myelofibrosis; myeloproliferative neoplasms, not

otherwise specified with myelofibrosis).¹ Bone marrow biopsies were retrieved from the archives of the Hematopathology Division of Weill Cornell Medical College (New York, NY, USA). Selection criteria for the study were: (i) a diagnosis of either primary or secondary myelofibrosis; (ii) a bone marrow cellularity >30%, considered satisfactory for recombinant interferon- α treatment (patients who were end stage or had a severely hypocellular, fibrotic and osteomyelosclerotic myelofibrosis were not considered suitable for interferon- α treatment); (iii) history of interferon- α administration after the diagnosis of myelofibrosis; and (iv) availability of pretreatment and posttreatment bone marrow biopsy samples.

Twelve patients fulfilled the inclusion criteria (fibrotic-stage primary myelofibrosis: 5 cases; post-polycythemia vera myelofibrosis: 3 cases; post-essential thrombocythemia myelofibrosis: 3 cases; myeloproliferative neoplasms, not otherwise specified with myelofibrosis: 1 case). All patients were tested for the *JAK2* mutational status: 7/12 patients carried the *JAK2*^{V617F} mutation, while 5/12 patients were *JAK2*^{V617F}-negative. No information on *MPL* and/or *CALR* status was available for the five *JAK2*^{V617F}-negative cases.

All patients received recombinant interferon- α therapy after the diagnosis of myelofibrosis, according to one of the following regimens: (i) subcutaneous recombinant interferon- α -2b (500,000–1 million units, 3 times weekly, progressively increased to 2–3 million units, 3 times weekly); (ii) pegylated recombinant interferon- α -2a (45–90 μ g weekly). Patients were prescribed either regimen according to preference or insurance coverage. Response to interferon therapy was clinically monitored at 2–3-month intervals, and follow-up bone marrow biopsy was performed annually.

For each patient, the following clinical and laboratory data were assessed: (i) presence of constitutional symptoms (fatigue, fever, weight loss and/or night sweats); (ii) spleen size (as assessed by physical examination in centimeters below the midpoint of the left costal margin); (iii) complete blood and platelet count; and (iv) cytogenetic assessment when aspirate material could be obtained. The Dynamic International Prognostic Scoring System (DIPSS) score was calculated before and after recombinant interferon- α administration. The clinical response to therapy was assessed using the revised International Working Group for Myelofibrosis Research and Treatment/European LeukemiaNet response criteria.²⁰ Appropriate informed consent was obtained from the Institutional Review Board, consistent with the Declaration of Helsinki.

Bone Marrow Histological Evaluation

For each case, the last bone marrow biopsy before recombinant interferon- α therapy was compared

with the last performed in the course of treatment. The following histological parameters were evaluated: (i) cellularity; (ii) myeloid-to-erythroid (M:E) ratio; (iii) large, tight clusters of megakaryocytes (defined as aggregates of ≥ 5 atypical megakaryocytes with no interposed hematopoietic cells); (iv) megakaryocyte density (i.e., number of megakaryocytes/mm² assessed by manual counting of histological sections); (v) megakaryocyte cytology (i.e., percentage of cells with cloud-shaped/bulbous nuclei); (vi) presence and density of naked nuclei; and (vii) degree of bone marrow fibrosis, as assessed by reticulin and trichrome stains. Bone marrow fibrosis was scored according to the WHO four-tiered semi-quantitative grading system.¹

Assessment of the Bone Marrow Blasts

To assess whether treatment with recombinant interferon- α can change the number and distribution of bone marrow blasts, immunohistochemistry for CD34 was performed in eight cases, in which the biopsy was technically adequate. CD34 expression could not be tested in the remaining biopsies, owing to lack of stainable sections (cases sent in consultations) or to exhaustion/poor preservation of tissue cores.

Immunohistochemistry was performed on 4- μ m-thick formalin-fixed paraffin-embedded sections, using an anti-CD34 monoclonal antibody (Clone: QBEnd10, Leica-Novocastra; prediluted antibody). Heat-based antigen retrieval methods were applied. Antigen detection was performed in an automated immunostainer (Bond III, Leica-Novocastra, Buffalo Grove, IL, USA). Bone marrow endothelial cells were used as positive internal controls. The number of CD34-positive cells was given as the percentage of the overall marrow cellularity.

The immunohistochemical results were confirmed by re-evaluation of paired (pretreatment/posttreatment) bone marrow aspirates (5 cases) and flow cytometry data (8 cases).

Cytological evaluation was performed on methanol-fixed, Wright-Giemsa-stained bone marrow smears. The percentage of myeloblasts was assessed by counting at least 500 nucleated cells. On flow cytometry, the number of blasts was calculated as the percentage of CD45^{dim} CD34^{pos} events over the total population of viable cells (FACSCanto cytometer and FACSDiva software; BD Biosciences, San Jose, CA, USA).

Statistical Analysis

Differences in pretreatment and posttreatment findings were statistically assessed by Student's *t*-test (paired and unpaired quantitative variables), McNemar's test (paired nominal variables) and Fisher's exact test (unpaired nominal variables). Results were considered statistically significant for values of $P < 0.05$.

Results

Effects of Recombinant Interferon- α Therapy on Bone Marrow Histology

Therapy with recombinant interferon- α was associated with significant changes in several histological parameters (Tables 1 and 2). Pretreatment bone marrow cellularity was markedly increased in 11 cases (mean value: 89%) and slightly decreased in 1 case (overall cellularity, 30%). Recombinant interferon- α administration was associated with decreased or equal cellularity in the hypercellular marrows, and the mean posttreatment cellularity (74%) was significantly lower than the mean pretreatment one (*t*-test, $P < 0.05$). In contrast, interferon therapy was associated with increased cellularity in the originally hypocellular marrow (posttreatment value, 50%; Figures 1a and b).

Treatment with recombinant interferon- α was also associated with a marked reduction of megakaryocyte density (mean pretreatment and posttreatment values: 25.4 and 14.6/mm², respectively) and naked nuclei density (mean pretreatment and posttreatment values: 2.2 and 1.0/mm², respectively; Table 2; Figures 1c and d). Differences between pretreatment and posttreatment values were statistically significant (*t*-test, $P < 0.05$). No significant changes were observed in the number of tight clusters, the percentage and topography of atypical megakaryocytes and the M:E ratio.

Recombinant interferon- α administration induced a significant improvement of bone marrow fibrosis. The majority of pretreatment biopsies displayed grade 3 fibrosis (10/12 cases), whereas posttreatment samples were characterized by more variable scores (grade 1: 3 cases; grade 2: 5 cases; grade 3: 4 cases). The median pretreatment and posttreatment scores were 3 and 2, respectively. Differences in pretreatment and posttreatment scores were statistically significant ($P < 0.05$; Table 2; Figures 2a and b).

Interferon therapy was not associated with changes in the percentage of bone marrow blasts, as there was only a very small population of CD34-positive precursor cells in both pretreatment and posttreatment biopsy samples ($\leq 1\%$ of the overall cellularity; Figures 2c and d). These results were confirmed by flow cytometry and bone marrow aspirate counts.

Clinical Features of the Study Population

The study population consisted of 5 women and 7 men whose mean age at first biopsy was 59.3 years (range, 14–83 years). The single pediatric case was affected by post-essential thrombocythemia myelofibrosis and did not have any familial history of myeloproliferative neoplasm or other hematological disorder. Six patients received prior cytoreductive therapy (hydroxyurea: 3 patients; anagrelide: 2 patients; hydroxyurea and anagrelide:

Table 1 Histological features of pretreatment and posttreatment bone marrow biopsies

Patient #	Diagnosis	Before rINF α administration						After rINF α administration					
		Fibrosis (MF score)	Cellularity	Meg density (no./mm ²)	Naked nuclei (no./mm ²)	Tight clusters	Atypical Meg	Fibrosis (MF score)	Cellularity	Meg density (n°/mm ²)	Naked nuclei (n°/mm ²)	Tight clusters	Atypical Meg
1	PMF	3	70%	37.1	3.1	Present	50%	1	29.1	1	Absent	20%	
2	PMF	3	90%	14.7	1.0	Present	60%	3	14.3	0.7	Present	70%	
3	Post-ET MF	2	70%	27.9	3.3	Present	20%	1	12.3	0.8	Absent	20%	
4	Post-ET MF	3	90%	17.1	1.8	Present	30%	2	8.0	0.4	Absent	20%	
5	Post-PV MF	3	95%	18.7	6.0	Absent	20%	3	6.0	0.4	Absent	25%	
6	PMF	2	95%	18.1	1.5	Absent	10%	2	11.8	0.9	Present	10%	
7	Post-PV MF	3	100%	26.1	0.5	Absent	30%	3	11.4	2.3	Present	30%	
8	MPN with MF	3	90%	13.8	1.3	Present	60%	3	7.2	0.2	Absent	30%	
9	PMF	3	85%	21.5	0.5	Present	20%	2	18.6	0.7	Present	20%	
10	Post-ET MF	3	95%	39.6	3.5	Present	20%	2	16.6	1.3	Present	10%	
11	Post-PV MF	3	100%	62.3	2.9	Present	5%	2	34.9	1.9	Present	10%	
12	PMF	3	30%	8.7	1.2	Present	20%	1	7.3	0.9	Absent	20%	

Abbreviations: Meg, megakaryocyte; MF, myelofibrosis; MPN, myeloproliferative neoplasm; PMF, primary myelofibrosis; post-ET, post-essential thrombocythemia; post-PV, post-polycythemia vera; rINF α , recombinant interferon- α .

1 patient), whereas 6 patients had not received any treatment for the disease. Four (33%) patients were administered subcutaneous recombinant interferon- α -2b, while 8 (67%) patients received pegylated recombinant interferon- α -2a. The median duration of therapy was 4 years (range, 1–10 years). As for the DIPSS index, 6/12 (50%) patients belonged to the Low, 4/12 (33%) to the Int-1 and 2/12 (17%) to the Int-2 prognostic groups (Table 3).

Before interferon administration, splenomegaly was present in 7/12 (58%) cases. The spleen edge was palpable 0.5–28 cm below the left costal margin on the mid-clavicular line. Over the course of interferon treatment, 11/12 (92%) patients exhibited stable or decreased spleen size. Specifically, reduction of spleen size was observed in 3/12 (25%) cases, and complete resolution of splenomegaly in 2/12 (17%) cases (Table 3).

Systemic symptoms were reported by 2/12 (17%) patients prior to interferon administration and by 4/12 (33%) patients after therapy (Table 3). In treated cases, clinical symptoms of myelofibrosis could not be easily distinguished from those related to interferon administration.

The patients exhibited a decrease of the white blood cell count, which was statistically significant (mean pretreatment and posttreatment values: $12.1 \times 10^9/l$ and $7.2 \times 10^9/l$) (*t*-test, *P* < 0.05). The mean platelet count was also reduced (mean pretreatment and posttreatment values: $382 \times 10^9/l$ and $247 \times 10^9/l$), but this difference showed only a trend toward statistical significance (*t*-test, *P* = 0.07). Differences between pretreatment and posttreatment hemoglobin levels were not statistically significant (Table 3).

The administration of recombinant interferon- α was associated with good clinical response in 10/12 (83%) patients (revised International Working Group for Myelofibrosis Research and Treatment/European LeukemiaNet response criteria). In particular, clinical improvement was observed in 2/12 (17%) and stable disease in 8/12 (67%) cases. The DIPSS score improved or remained stable in 7/12 (58%) patients. A worsening of such score was observed in 4/12 (33%) patients; progression to acute myeloid leukemia was reported in 1 case (Table 3). Subcutaneous recombinant interferon- α -2b and pegylated interferon- α -2a regimens did not result in statistically significantly different clinical and laboratory responses.

Matched pretreatment and posttreatment cytogenetic data were available for 7/12 patients. A normal karyotype (46,XX or 46,XY) was documented in all cases both before and after therapy. *In situ* hybridization for *BCR-ABL1* gene rearrangement studies was negative in all patients.

Impact of Clinicopathological and Molecular Features on the Response to Interferon Therapy

The response to recombinant interferon- α may be affected by several clinicopathological and

Table 2 Mean values of the histological parameters before and after treatment with rIFN α

Parameter	Before rIFN α administration	After rIFN α administration	P-value
Cellularity ^a (%)	89 \pm 10 (70–100)	74 \pm 22 (20–100)	0.02
Megakaryocyte density (no./mm ²)	25.4 \pm 14.5 (8.7–62.3)	14.6 \pm 8.9 (6–34.9)	< 0.01
Naked nuclei density (no./mm ²)	2.2 \pm 1.6 (0.5–6)	1.0 \pm 0.6 (0.2–2.3)	0.03
Megakaryocytes with bulbous nuclei (%)	29 \pm 1 (5–60)	24 \pm 16.0 (10–70)	0.21
Fibrosis (MF score)	2.8 \pm 0.4 (2–3)	2.1 \pm 0.8 (1–3)	< 0.01
Bone marrow blasts ^b (percentage of hematopoietic cells)	1 \pm 1 (0–3)	2 \pm 1 (1–4)	0.41

Abbreviations: MF, myelofibrosis; rIFN α , recombinant interferon- α .

^aMean values refer to hypercellular cases only.

^bAs assessed by bone marrow smear counts.

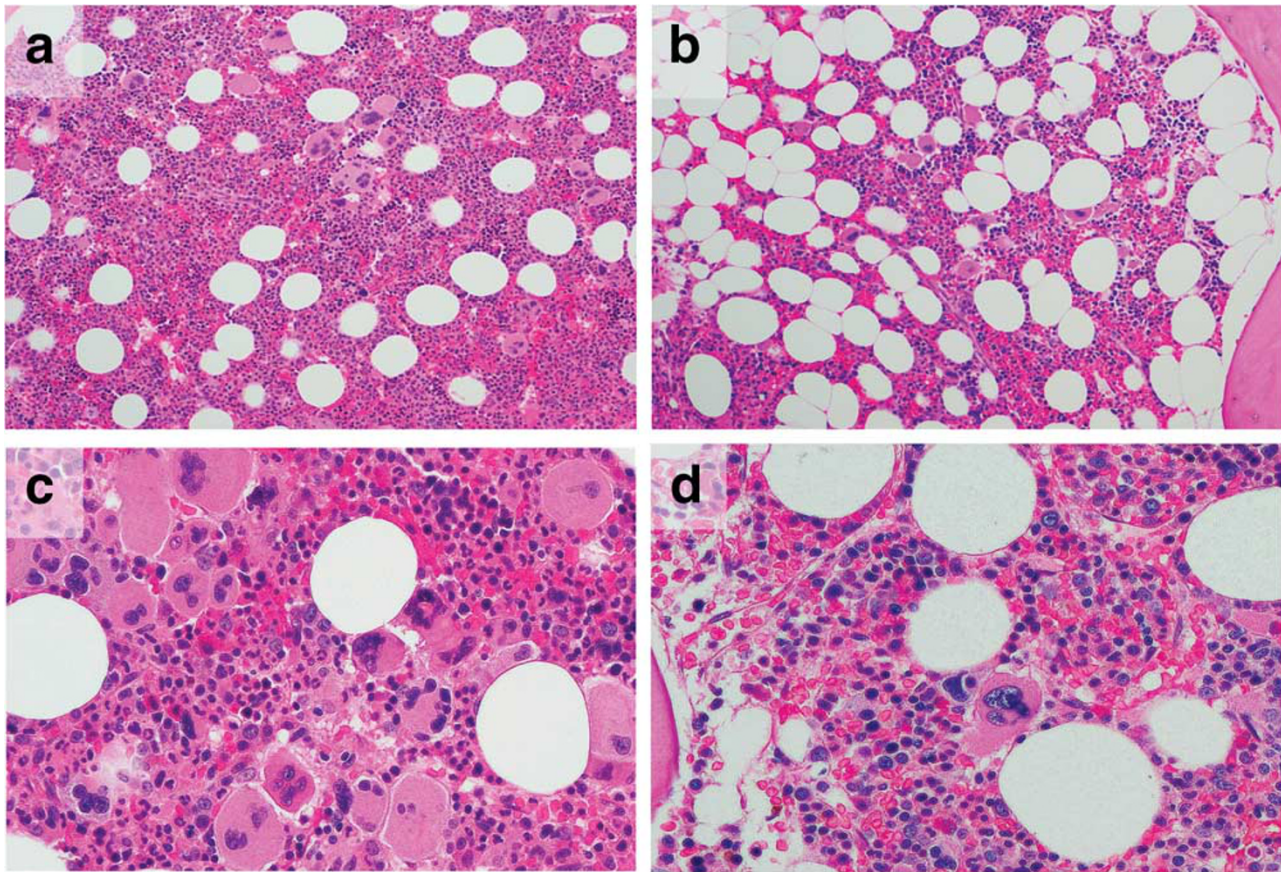


Figure 1 Histological response to recombinant interferon- α in primary myelofibrosis and post-polycythemia vera/post-essential thrombocythemia myelofibrosis. Comparison of pretreatment (a and c) and posttreatment (b and d) biopsies (patient #3). The posttreatment biopsy showed a significant improvement of bone marrow histology with normalization of marrow cellularity (a *versus* b) and decreased megakaryocyte density (c *versus* d) (H&E stain; original magnification, $\times 10$ and $\times 20$).

molecular variables. To test this hypothesis, we compared the effects of interferon- α in specific subgroups of patients, distinguished according to the JAK2 mutational status (JAK2^{V617F}-positive *versus* JAK2^{V617F}-negative), the prior administration of cytoreductive therapies, the myelofibrosis subtype and the duration of interferon therapy (< 5 *versus* > 5 years).

Despite a good clinical response to interferon therapy was reported in both JAK2^{V617F}-positive and

JAK2^{V617F}-negative patients, significant differences in terms of DIPSS score were observed between the two groups. In particular, all patients with the JAK2^{V617F} mutation had a stable or improved score after interferon- α therapy. Conversely, JAK2^{V617F}-negative patients were invariably associated with a worsening of the score or evolution to acute myeloid leukemia (Table 3). No differences in other clinical, laboratory or histological parameters were noted between these molecular subgroups.

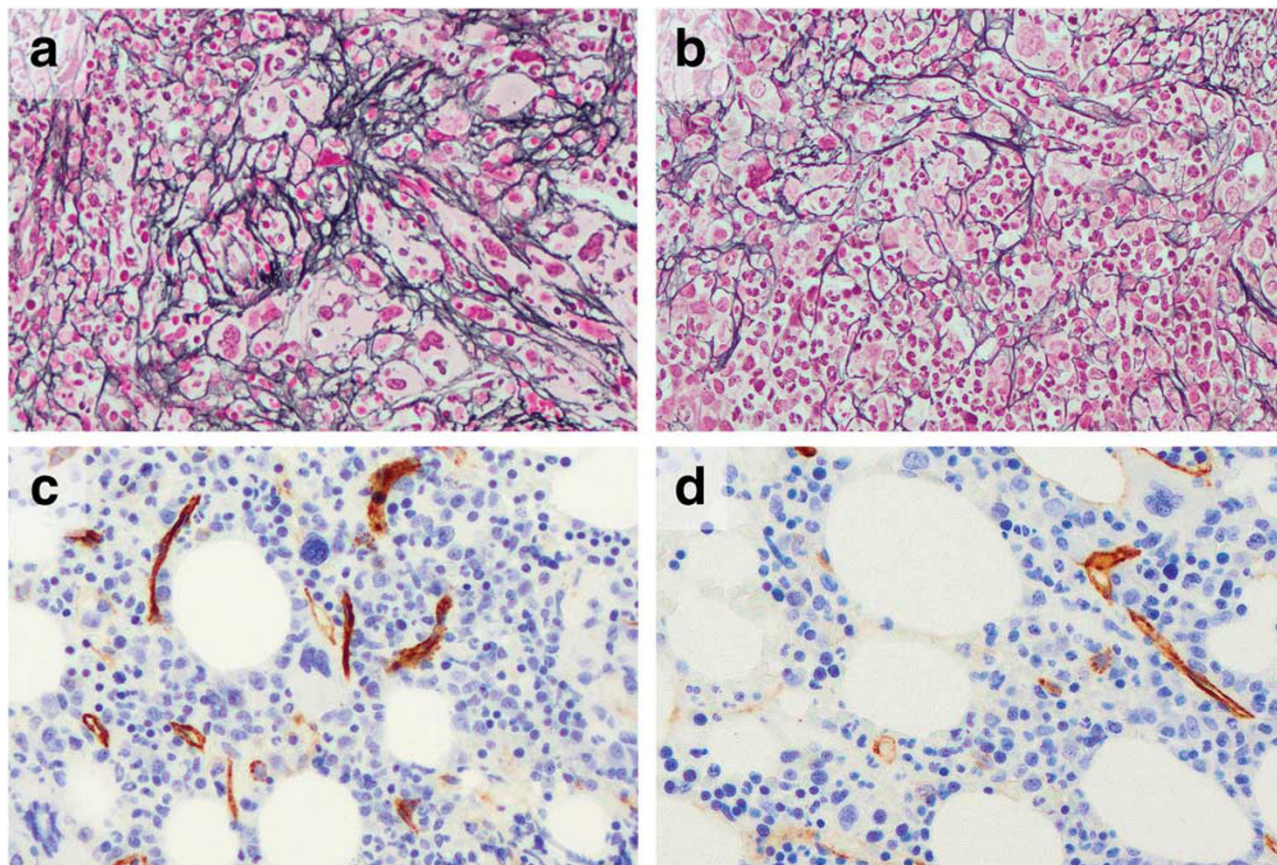


Figure 2 Effects of recombinant interferon- α on bone marrow fibrosis and blast count in primary myelofibrosis and post-polycythemia vera/post-essential thrombocythemia myelofibrosis. Comparison of pretreatment (a and c) and posttreatment (b and d) biopsies (patient #1). The posttreatment biopsy showed a significantly decreased amount of bone marrow fibrosis (a versus b). Immunohistochemistry for CD34 showed a similar low frequency of marrow blasts in both pretreatment (c) and posttreatment (d) samples. (Reticulin and CD34 immunoperoxidase stains; original magnification, $\times 10$ and $\times 20$).

The prior administration of other cytoreductive treatments, the duration of interferon therapy and the specific subtype of myelofibrosis did not significantly affect clinical and/or histological responses.

Discussion

BCR/ABL1-negative myeloproliferative neoplasms are a heterogeneous group of hematological disorders, characterized by variable clinical, pathological and molecular features. The management of these diseases remains a clinical challenge and most therapies target disease-related symptoms, with little (if any) effect on the underlying disease pathobiology.²¹

The introduction of recombinant interferon- α for the treatment of myeloproliferative neoplasms has partially changed this therapeutic landscape. Recombinant interferon- α has been shown to induce complete hematological remission in $>70\%$ of patients with polycythemia vera or essential thrombocythemia, with a decrease of *JAK2*^{V617F} allele burden.¹⁴ Preliminary reports document a role

for recombinant interferon- α also in early primary and fibrotic-stage myelofibrosis.^{15–19}

In early primary myelofibrosis, interferon administration is associated with clinical response or disease stability in $>80\%$ of patients. Complete resolution of splenomegaly is observed in the majority of cases, while a significant improvement of bone marrow histology (i.e., reduction of bone marrow fibrosis and megakaryocyte atypia) is documented in 26.7% of patients.¹⁵ In markedly fibrotic-stage primary myelofibrosis, recombinant interferon- α therapy has produced inconsistent or no significant clinical response, despite a reduction of platelet and white blood cell counts,^{17–19} and some occasional improvement of splenomegaly.^{18,19}

This study assessed the histological and clinical effects of recombinant interferon- α therapy in 12 patients with fibrotic-stage myelofibrosis and with residual hemopoietic foci in $>30\%$ of the marrow specimen. Comparison of pretreatment and posttreatment clinical data revealed a stable or reduced spleen size in the majority of patients, with a statistically significant reduction of the white blood cell count (Table 3). Mean platelet levels were also

Table 3 Clinical and laboratory features of the study population

Patient no.	Gender	Before rIFN α administration					After rIFN α administration								
		Age at first biopsy (years)	Systemic symptoms	Hb (g/l)	WBC ($\times 10^9/l$)	Platelets ($\times 10^9/l$)	Splenomegaly	DIPSS class of risk	Systemic symptoms	Hb (g/l)	WBC ($\times 10^9/l$)	Platelets ($\times 10^9/l$)	Splenomegaly	DIPSS class of risk	Therapy duration (years)
1	F	55	Absent	108	7.1	276	Absent	Low	Present	117	4.1	517	Absent	Int-1	6 ^a
2	F	56	Absent	110	5.8	168	Present	Int-1	Present	78	2.6	13	Present (unchanged)	Evolved to AML	6
3	M	62	Absent	142	5.9	658	Absent	Low	Absent	130	2.5	161	Absent	Int-1	4 ^a
4	M	62	Absent	104	7.7	570	Absent	Low	Present	117	5.6	415	Absent	Int-1	2 ^a
5 ^b	F	72	Absent	156	22.8	236	Present	Int-1	Absent	128	4.4	114	Absent	Int-1	5 ^a
6 ^b	F	58	Absent	116	8.4	204	Present	Low	Absent	128	11.0	306	Present (increased)	Low	6
7 ^b	M	68	Present	83	5.9	90	Present	Int-2	Present	82	4.4	38	Present (unchanged)	Int-2	3
8 ^b	M	66	Present	129	19.4	133	Present	Int-1	Absent	106	16.1	123	Present (unchanged)	Int-1	4
9 ^b	M	83	Present	138	28.6	324	Present	Int-2	Absent	130	17.2	142	Present (reduced)	Int-1	1
10	M	14	Absent	148	10.9	937	Present	Low	Absent	133	7.2	390	Present (reduced)	Int-1	4
11 ^b	M	53	Absent	102	16.0	700	Present	Low	Absent	105	5.2	300	Present (reduced)	Low	2 ^a
12 ^b	F	63	Absent	87	6.8	358	Present	Int-1	Absent	131	5.6	439	Absent	Int-1	10 ^a

Abbreviations: DIPSS, Dynamic International Prognostic Scoring System; Hb, hemoglobin; rIFN α , recombinant interferon- α ; WBC, white blood cells.

^aPrior cytoreductive therapy.

^bJAK2^{V617F}-mutated MF.

reduced, but the difference between pretreatment and posttreatment counts showed only a trend toward statistical significance. These results are consistent with those reported in the literature.^{17–19} Histologically, interferon therapy was associated with a significant decrease in mean marrow cellularity, megakaryocyte density, naked nuclei density and marrow fibrosis (Tables 1 and 2; Figures 1 and 2). No significant changes in the percentage of megakaryocytes with atypical/bulbous nuclei, the number of bone marrow blasts, the M:E ratio and the presence of tight clusters of megakaryocytes were observed.

A reduction of marrow fibrosis after interferon therapy has been reported in the literature,^{22–24} but the mechanisms underlying such improvement are still debated. In myelofibrosis, bone marrow fibrosis seems to result from the abnormal interaction between neoplastic megakaryocytes and polymorphonuclear leukocytes. Impaired emperipoiesis of neutrophils may alter the intramegakaryocytic trafficking of α -granules, thus leading to the release of pro-fibrogenic growth factors (i.e., TGF β and PDGF).^{25,26} Our study shows that the administration of recombinant interferon- α can reduce both megakaryopoiesis (decrease megakaryocyte density) and granulopoiesis (decrease of cellularity, which in myelofibrosis is mainly related to the myeloid lineage). These observations may provide an explanation for the interferon-associated decrease of marrow fibrosis.

The reduction of megakaryocytic density may also trigger some speculation over the effect of recombinant interferon- α in the pathobiology of myelofibrosis. The density of megakaryocytes in the bone marrow reflects their proliferation rate because megakaryocytes mature by endomitotic division without cytokinesis.^{27,28} The marked reduction of megakaryocytes after recombinant interferon- α therapy may thus indicate a direct effect of this drug on the proliferation of neoplastic hematopoietic progenitors and/or on their capability of megakaryocytic differentiation.

This hypothesis is corroborated by a recent study using a murine model of JAK2-mutated myeloproliferative neoplasm, which showed a sharp reduction of neoplastic hemopoietic stem cells upon recombinant interferon- α administration.²⁹ Flow cytometry and gene expression data demonstrate that this effect is mediated by the forced differentiation of neoplastic stem cells. The consequent exhaustion of the stem cell pool leads to a decrease of extra-medullary hematopoiesis, with a significant improvement of spleen size. Thus, in myeloproliferative neoplasms, the therapeutic activity of recombinant interferon- α seems to be mediated more by the direct suppressive effects on neoplastic stem cells than by anti-inflammatory and/or immunoregulatory mechanisms.^{29–31} The latter may, however, contribute to the clinical and histological response to recombinant interferon- α therapy, considering that increased levels

of pro-inflammatory cytokines have an important role in the pathobiology of myeloproliferative neoplasms.³² The interferon-mediated decrease of serum cytokines may thus resemble the anti-inflammatory effects of ruxolitinib, which are thought to be responsible for the improvement of spleen size after ruxolitinib administration.³³

Our study evidenced a high percentage of good clinical responses in both JAK2^{V617F}-positive and JAK2^{V617F}-negative myelofibrosis. Nonetheless, an improvement of the DIPSS score after interferon administration was only observed in JAK2^{V617F}-positive cases. The significance of this observation requires further investigation on larger cohorts of patients. It is, however, possible that our series of JAK2^{V617F}-negative cases was enriched by triple-negative or *CALR*-negative/*ASXL1*-positive cases, which are known to bear a worse outcome.³⁴ In the future, a complete molecular characterization of these cases will possibly confirm this hypothesis.

In conclusion, the present study provides the first thorough characterization of the histological response to recombinant interferon- α in myelofibrosis and confirms the data in the literature on the clinical effects of this therapy. Our results also highlight a possible role for JAK2 mutational status in the prognosis of interferon-treated myelofibrosis, with little (if any) contribution of other clinical parameters. The documented reduction of splenomegaly and the parallel improvement of bone marrow morphology confirm the clinical assumption that spleen size is a good surrogate marker for bone marrow response. The morphological evaluation of bone marrow remains, however, of pivotal importance for the diagnosis and follow-up of myelofibrosis. Additional studies on larger cohorts of patients with marrow biopsies performed regularly are needed to confirm these results and to better characterize the biological effects of recombinant interferon- α in primary and post-polycythemia vera/post-essential thrombocythemia myelofibrosis.

Acknowledgments

We are deeply grateful to Dr Fabio Fuligni (SickKids Hospital, Toronto, ON, Canada) for his invaluable contribution to the statistical analysis of our data. This study is aided by a grant from the Cancer Research and Treatment Fund.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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