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The sympathomimetic agonist mirabegron did not lower *JAK2*-V617F allele burden, but restored nestin-positive cells and reduced reticulin fibrosis in patients with myeloproliferative neoplasms: results of phase II study SAKK 33/14

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ABSTRACT

The β-3 sympathomimetic agonist BRL37344 restored nestin-positive cells within the stem cell niche, and thereby normalized blood counts and improved myelofibrosis in a mouse model of *JAK2*-V617F-positive myeloproliferative neoplasms. We therefore tested the effectiveness of mirabegron, a β-3 sympathomimetic agonist, in a phase II trial including 39 *JAK2*-V617F-positive patients with myeloproliferative neoplasms and a mutant allele burden more than 20%. Treatment consisted of mirabegron 50 mg daily for 24 weeks. The primary end point was reduction of *JAK2*-V617F allele burden of 50% or over, but this was not reached in any of the patients. One patient achieved a 25% reduction in *JAK2*-V617F allele burden by 24 weeks. A small subgroup of patients showed hematologic improvement. As a side study, bone marrow biopsies were evaluated in 20 patients. We found an increase in the nestin$^+$ cells from a median of 1.09 (interquartile range 0.38-3.27)/mm^2 to 3.95 (interquartile range 1.98-8.79)/mm^2 (P<0.0001) and a slight decrease of reticulin fibrosis from a median grade of 1.0 (interquartile range 0-3) to 0.5 (interquartile range 0-2) (P=0.01) between start and end of mirabegron treatment. Despite the fact that the primary end point of reducing *JAK2*-V617F allele burden was not reached, the observed effects on nestin$^+$ mesenchymal stem cells and reticulin fibrosis is encouraging, and shows that mirabegron can modify the microenvironment where the *JAK2*-mutant stem cells are maintained. (Registered at *clinicaltrials.gov identifier: 02311569.*)

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Introduction

Myeloproliferative neoplasms (MPN) are thought to be initiated and maintained from a mutated hematopoietic stem cell (HSC).[1] An acquired mutation in *JAK2* (*JAK2*-V617F) is present in the majority of MPN patients.[2-5] The interplay between

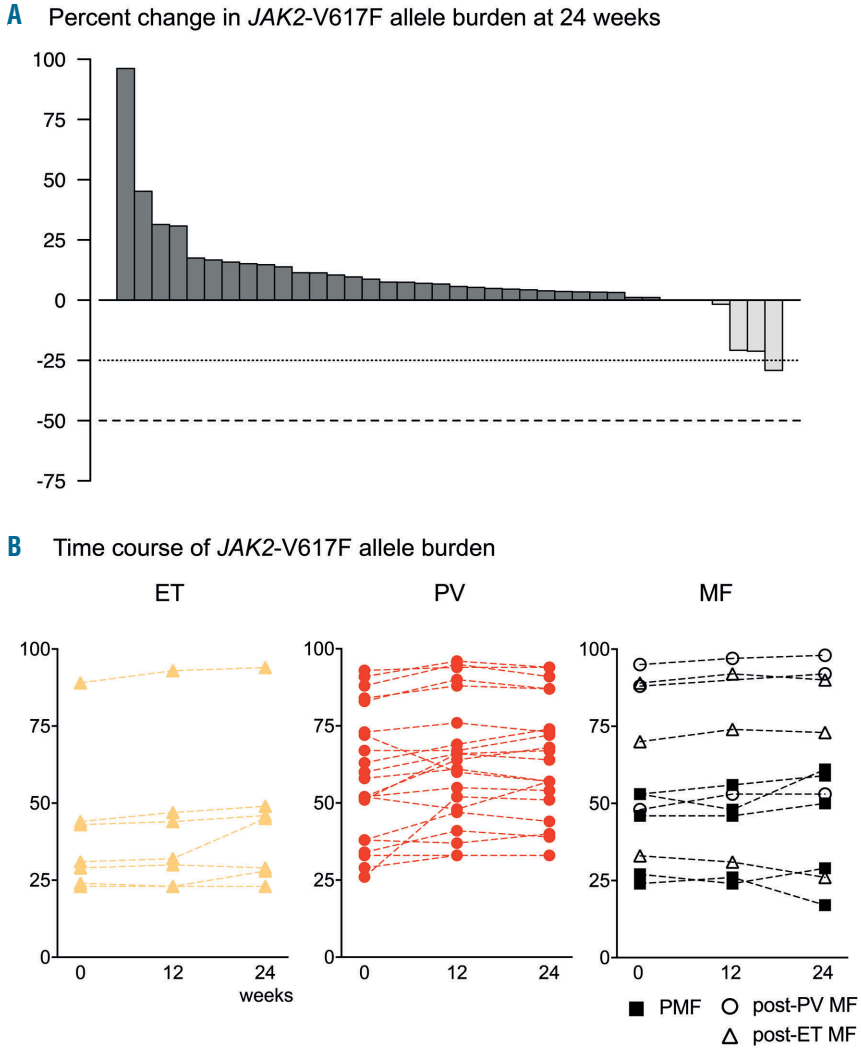

Figure 1. Changes in *JAK2*-V617F allele burden during the treatment period. (A) Waterfall plot representing the percent changes in *JAK2*-V617F allele burden at 24 weeks. (B) Time course of *JAK2*-V617F allele burden in patients treated with mirabegron. The values measured before treatment (week 0), and at week 12 and week 24 of mirabegron treatment are shown for each individual patient, connected by dashed lines. Filled triangles, circles and squares are used for essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) patients, respectively. Within the group of myelofibrosis (MF) patients, post-ET and post-PV myelofibrosis are indicated by open triangles and circles, respectively.

the MPN HSCs and the stem cell niche is being increasingly recognized as crucial for the biology of the disease. Nestin-positive mesenchymal stem cells (nestin$^+$ MSCs) within the bone marrow (BM) niche are innervated by sympathetic nerve fibers and are important in regulating normal HSCs.[6,7] These nestin$^+$ MSCs are strongly reduced in BM from patients with MPN.[8] In a mouse model of MPN expressing human *JAK2*-V617F, this effect was found to be caused by early glial and sympathetic nerve damage and subsequent apoptosis of nestin$^+$ MSCs triggered by the mutant hematopoietic cells. *In vivo* depletion of nestin$^+$ cells accelerated MPN progression. Conversely, MPN phenotype could be reversed by compensating for the sympathetic neuropathy by systemic administration of a β-3-sympathomimetic agonist. Mice with *JAK2*-V617F-driven MPN treated with the β-3-sympathomimetic drug BRL37344 not only restored nestin$^+$ MSCs numbers, but also showed correction of thrombocytosis, neutrophilia, and BM fibrosis, and efficiently reduced mutant hematopoietic progenitor numbers in BM and peripheral blood (PB).[8] Treatment with BRL37344 also corrected the damage inflicted by the MPN clone on the stem cell niche and led to an increase in nestin$^+$ cells.[8] Thus, β-3 sympathomimetic agonists represent a promising novel therapeutic approach to MPN by targeting the stem cell niche rather than the MPN clone itself.

Recently, mirabegron, a β3-adrenoceptor agonist, was approved in North America, Europe, Japan and Australia for the treatment of an overactive bladder.[9] Here, we report the results of a phase II study that tested the efficacy of mirabegron in patients with *JAK2*-V617F-positive MPN.

Methods

Study population

Overall, 39 patients with MPN, including 7 patients with essential thrombocythemia (ET) (18%), 21 with polycythemia vera (PV) (54%), and 11 with myelofibrosis (MF) [28%; of whom 5 were primary myelofibrosis (PMF), 3 post-ET MF and 3 post-PV MF] have been accrued in 10 institutions across Switzerland between May 2015 and February 2016. The patients fulfilled the 2008 World Health Organization (WHO) diagnostic criteria for MPN.[10] All patients were *JAK2*-V617F-positive with a mutant allele burden at study entry more than 20% in granulocyte DNA. The trial was planned and conducted in accordance with the Declaration of Helsinki, the Guidelines for Good Clinical Practice (GCP) issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and the requirements of the respective national regulatory authorities. The local ethics committees of all participating centers have given approval to the trial and written informed consent was obtained

Table 1. Patients' and disease characteristics	Value N=39
Gender	
Female	12 (31%)
Male	27 (69%)
Age at registration [years]	62 (53–72)
Disease	
ET	7 (18%)
PV	21 (54%)
MF	11 (28%)
PMF	5 (13%)
Post-ET MF	3 (8%)
Post-PV MF	3 (8%)
WHO status	
0	32 (82%)
1	7 (18%)
Blood counts	
Hemoglobin [g/L]	134 (127–143)
Neutrophils [x10^9/L]	6 (4–8)
Platelets [x10^9/L]	392 (310–564)
White blood cells [x10^9/L]	8 (6–12)
Burden of mutated alleles [%]	52 (33–73)
Organomegaly	
Liver palpable	4 (11%)
Spleen palpable	11 (29%)
Liver longitudinal diameter (ultrasound) [cm]	15 (13–16)
Spleen longitudinal diameter (ultrasound) [cm]	14 (12–18)
Clinical signs of diseases other than MPN	28 (72%)
Medical history prior to inclusion in study	39 (100%)
Venous complications	30 (77%)
Deep vein thrombosis	3 (8%)
Pulmonary embolism	0
Splanchnic veins	1 (3%)
Retinal vein	0
Unknown/Missing	26 (67%)
Arterial complications	36 (92%)
Cerebral	7 (18%)
Extremity	2 (5%)
Cardiac	4 (10%)
Raynaud's phenomenon	0
Erythromelalgia	0
Unknown/Missing	24 (62%)
Hemorrhagic complications	28 (72%)
Gastrointestinal	1 (3%)
Mucocutaneous	3 (8%)
Intraocular	0
Unknown/Missing	24 (62%)
Previous therapies	39 (100%)
Cytoreductive	28 (72%)
Alkylating agents	0
Hydroxyurea	23 (59%)
Pipobroman	0
Thioguanin	0
Anagrelide	1 (3%)
Antiaggregation	33 (87%)
Anticoagulation	7 (19%)
Interferon	2 (5%)
Phlebotomy	21 (55%)

MF: myelofibrosis; PMF: primary myelofibrosis; ET: essential thrombocythemia; PV: polycythemia vera; WHO: World Health Organization; MPN: myeloproliferative neoplasms. Data are presented as number (N) of patients (%) or median (interquartile range).

from all patients prior to enrollment. Details of the inclusion and exclusion criteria are specified in the *Online Supplementary Appendix*.

Study design and treatment

We performed a multicenter, prospective, single-arm, single-stage and open phase II trial (SAKK 33/14; *clinicaltrials.gov identifier: 02311569*) with the β-3-sympathomimetic agonist mirabegron (Betmiga®). Before the study began, the drug had already been approved in the US, EU and Switzerland for the treatment of patients with an overactive bladder with a maximal recommended dose of 50 mg daily. The trial consisted of mirabegron treatment for at least 24 weeks with an initial dose of 25 mg daily during the first week followed upon good tolerance by 50 mg mirabegron daily during the remaining treatment period. The following treatments were not allowed during the trial treatment phase: other anticancer treatments, drugs known to influence *JAK2*-V617F allele level (e.g. interferon-α), ruxolitinib, or investigational treatments. Established cytoreductive treatment for MPN (e.g. hydroxyurea, pipobroman, or thioguanin) could be continued as previously prescribed. For further details on the study design see the *Online Supplementary Methods.*

Primary end point

The primary end point was defined as reduction in the *JAK2*-V617F allele burden of 50% or more at 24 weeks after registration. Secondary end points and response criteria are described in the *Online Supplementary Methods*).[11,12]

Molecular analyses

The *JAK2*-V617F allele burden was determined on DNA from purified granulocytes isolated from PB sampled in EDTA-containing tubes. The allele-specific PCR of *JAK2* genotyping was performed as previously described.[13] The *JAK2*-V617F allele burden was validated by retesting. Capture-based next-generation sequencing with a panel of 94 genes to detect somatic mutations in granulocyte DNA was performed in patients who consented to this subproject on a voluntary basis. For details see the *Online Supplementary Methods*.[14]

Assessment of myelofibrosis and nestin$^+$ mesenchymal stem cells

Patients who entered the study could also participate on a voluntary basis in a subproject with the goal to test whether mirabegron can restore the nestin$^+$ niche and may have a beneficial effect on BM morphology and the degree of myelofibrosis. BM trephine biopsies were performed at study entry and at week 24. Reticulin and collagen fibrosis was evaluated following established criteria.[15-18]

Statistical analysis

Statistical methods are defined in the *Online Supplementary Methods.*

Results

Patients and study treatment

The characteristics of the 39 MPN patients enrolled in the study are summarized in Table 1. None of the patients was newly diagnosed. The median time between MPN diagnosis and trial registration was 3.6 years (range 1.6-8.6 years). Prior to inclusion, 30 patients (77%) had received cytoreductive therapy and 21 (55%) were treated by phlebotomy. Treatment with mirabegron was completed as per protocol in 32 out of 39 patients (82%). In 2 patients (5%), treatment was stopped due to toxicity, in 2 patients (5%) due to patients' preference, and in one patient (3%) due to breast cancer diagnosis (Table 2). Treatment deviation was described in 16 patients (41%) and was due to patient's decision (n=6; 15%), doctor's decision (n=1; 3%), toxicity (n=2; 5%) or other reasons (n=12; 31%). Thirty-six patients (92%) received concomitant medication (Table 2).

Mutational profiles

In 33 out of 39 patients (84%) who consented to this subproject, granulocyte DNA was sequenced at study entry using a next-generation sequencing (NGS) panel of 94 genes (Table 3). In 10 out of 33 patients (30%), additional somatic mutations were detected (Table 3) and in 3 of these patients (9%) two concomitant mutations were present (*TET2* and *DNMT3A, PIAS2* and *TYK2, TP53* and *PRPF40B*). The presence or absence of additional mutations was not associated with clinical or laboratory parameters.

Response

None of the patients reached the primary end point of a 50% or more reduction of *JAK2*-V617F allele burden at 24 weeks (Figure 1 and *Online Supplementary Appendix*). The median percent change from baseline to week 24 was an increase of 6.1% [interquartile range (IQR) 3.2-13.8%]. One patient reached the secondary end point with a reduction of *JAK2*-V617F allele burden of 25% or more after 24 weeks. Hematologic response according to European LeukemiaNet (ELN) and International Working Group Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria was observed in 5 out of 21 patients with PV (24%): 2 of them had a complete response (CR) (10%) and 3 a partial response (PR) (14%) (Table 4). Two of 7 ET patients (29%) showed a PR, i.e. reduction in platelet count. One patient with MF became transfusion-independent (9%), all other MF patients (n=10) showed no response (91%). There was no difference in spleen size between baseline and 24 weeks by ultrasound [median spleen longitudinal diameter 15 cm (IQR 13-21) *vs.* 16 cm (IQR 14-19)]. All parameters measured are listed in *Online Supplementary Table S1*.

Adverse events

Overall, 33 patients (85%) had at least one adverse event, 3 (8%) of them with Common Terminology Criteria for Adverse Events (CTCAE) 4.0 grade 3, 12 (31%) with worst grade 2, 18 (46%) with worst grade 1, and no patient with grade 4 or 5 event. Five adverse events

Table 2. Treatment.

	Value N=39
Total dose of mirabegron [mg]	9275 (8875–9775)
Total treatment duration [weeks]	27.0 (25.9–28.3)
Total dose per week [mg]	345 (344–345)
Main reason for stopping treatment	
Treatment was completed as per protocol	32 (82%)
Unacceptable toxicity	2 (5%)
Other*	3 (8%)
Missing	2 (5%)
Patients receiving any concomitant medication	36 (92%)
Concomitant medication	
Hydroxyurea	20 (51%)
Thioguanin	0
Pipobroman	0
Anagrelide	0
Phlebotomy	11 (28%)
Other cytoreductive drugs	3 (8%)
Other treatment	35 (90%)

Data are presented as number of (N) of patients (%) or median (interquartile range). *The reasons categorized as "Other" are patients' wish and a breast cancer diagnosis.

Table 3. Additional somatic mutations in myeloproliferative neoplasm patients.

Gene	Mutation	Patients (n=33) N	%
TET2	M695fs, K1125E, E1339D, T1393I, Y1345C	5	15
DNMT3A	V468M	1	3
GSN	R687Q	1	3
JAK2	E282G	1	3
NFE	E45G	1	3
PIAS2	S533delinsWS	1	3
PRPF40B	A597T	1	3
TP53	H179R	1	3
TYK2	V673L	1	3

Table 4. Response to mirabegron therapy.

Outcome	Value N=39
Percent change of allele burden at 24 weeks	6.1 (3.2–13.8)
Allele burden reduction of 50% at 24 weeks	0
Allele burden reduction of 25% at 24 weeks	1 (3%)
Overall hematologic response in PV, n = 21	
CR	2 (10%)
PR	3 (14%)
No response	16 (76%)
Overall hematologic response in ET, n = 7	
PR	2 (29%)
No response	5 (71%)
Overall hematologic response in MF, n = 11	
Improvement of anemia	1 (9%)
No response	10 (91%)

Data are presented as number (N/n) of patients (%) or median (IQR). PV: polycythemia vera; CR: complete response; PR: partial response; ET: essential thrombocythemia; MF: myelofibrosis.

were of grade 3, including gastrointestinal disorders (nausea, vomiting), nervous system disorders (headache, paresthesia) and secondary malignancy (one case of breast cancer). The latter was reported as a serious adverse event and assessed as unrelated to the trial treatment. Nine adverse events were considered to be possibly related to the trial treatment by the investigators (nausea, vomiting, headache, paresthesia, insomnia, pruritus, prostatic obstruction, mucositis, vestibular disorder). No death was observed. The observed adverse events reflect the known profile of mirabegron.

Disease-related symptoms

During the trial, 20 patients (51%) suffered from at least one disease-related symptom (DRS). Considering the highest CTCAE 4.0 grade DRS per patient, only one (3%) DRS was grade 3 (abdominal distension), 6 (15%) were grade 2, and 13 (33%) grade 1. Most DRS were gastrointestinal (abdominal distension, early satiety), general (fatigue, fever), microvascular (erythromelalgia, acroparesthesia, digital ischemia), headache, and pruritus.

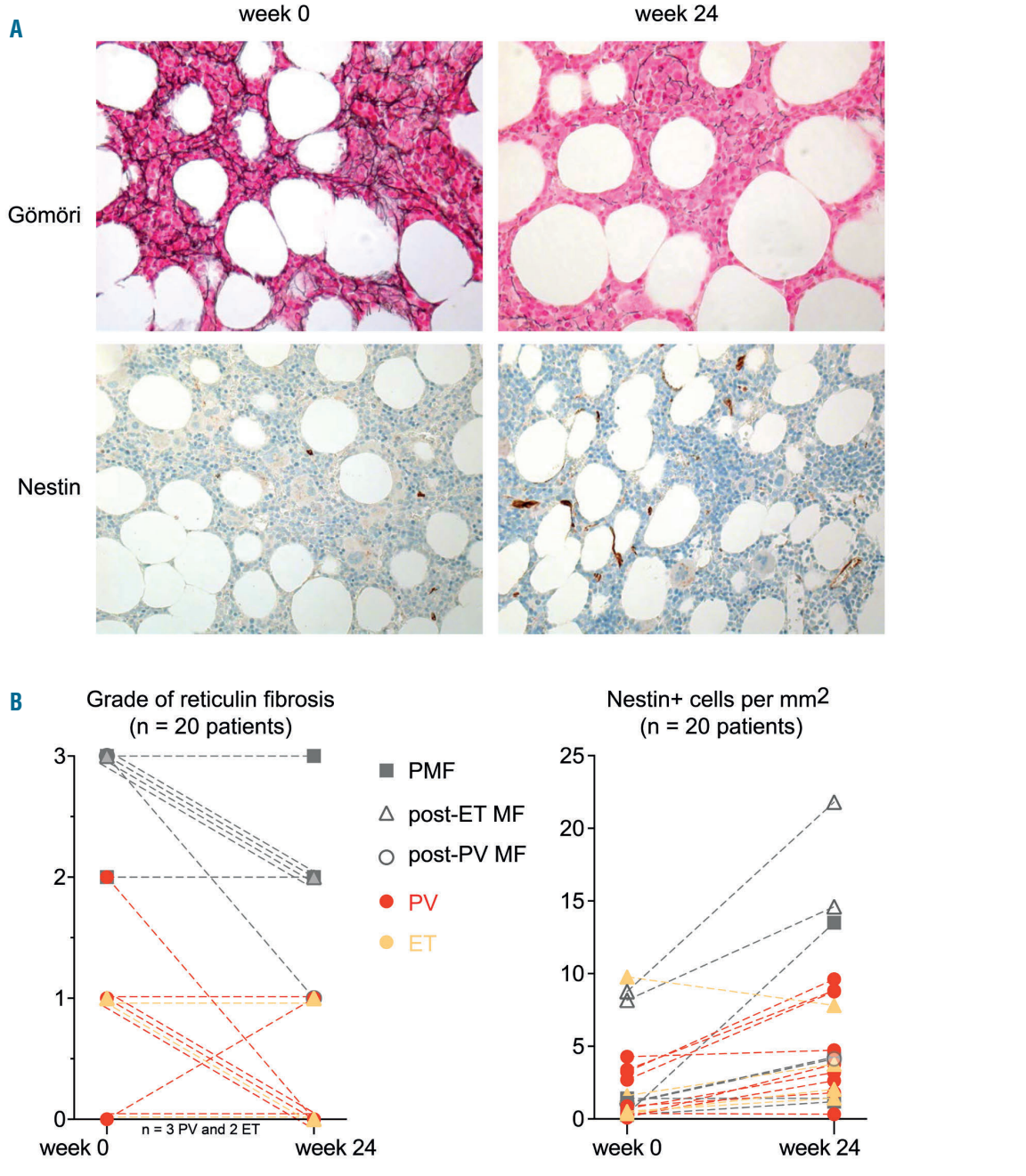

Figure 2. Reticulin fibrosis and nestin positive (nestin⁺) cells before and after treatment with mirabegron. (A) Bone marrow histology of a patient before (week 0) and at the end (week 24) of treatment with mirabegron. (Top) Reticulin fibers are stained black by silver impregnation (Gömöri). (Bottom) Immunohistochemical staining with a monoclonal antibody against human nestin protein. Note decrease of reticulin fibrosis and increase of nestin⁺ cells (brown staining) after 24 weeks of treatment. Magnification: 200x. (B) Single patient evolutional curves of the grade of reticulin fibrosis (left) and nestin⁺ mesenchymal cells/mm² (right) at study inclusion and after 24 weeks of mirabegron. n: number; PMF: primary myelofibrosis; ET: essential thrombocythemia; PV: polycythemia vera; MF: myelofibrosis.

Bone marrow histology

Bone marrow biopsies before and after mirabegron treatment were obtained in 20 patients of the 39 patients who consented to this subproject (51%). These included 9 PV, 4 ET, 4 PMF, 2 post-ET, and 1 post-PV MF patients. The biopsies were evaluated in a blinded fashion.

A slight decrease in reticulin fiber content from a median grade of 1.0 (IQR 0-3) to 0.5 (IQR 0-2) (P=0.01) and an increase in the nestin$^+$ MSCs cells from a median of 1.09/mm^2 (IQR 0.38-3.27) to 3.95/mm^2 (IQR 1.98-8.79) (P<0.0001) were observed (Figure 2). The mean change in the nestin$^+$ cells from baseline to week 24 was 3.52/mm^2 [95% confidence interval (CI): 1.65-5.39]. We found no correlation between reticulin fibrosis or nestin$^+$ cell content with time from diagnosis to study inclusion, blood counts, splenomegaly, and *JAK2*-V617F allele burden. The decrease in reticulin fibrosis was limited to patients without hydroxyurea treatment (-0.85/mm^2 without hydroxyurea *vs.* 0.0/m^2 with hydroxyurea; P=0.042). No statistically significant differences in CD34$^+$ cell numbers were noted on paired samples before and after 24 weeks of mirabegron. Quantitative assessment of megakaryocyte numbers showed no differences between baseline to week 24 (median 25.5/mm^2, IQR 16.75-34.25 *vs.* 22/mm^2, IQR 14.38-29.63; P=0.371), but a trend towards reduction in megakaryocyte cluster formation and decrease in numbers of large megakaryocytes with staghorn-like morphology was noted in some patients.

Discussion

Mirabegron was safe and well tolerated in patients with *JAK2*-mutated MPNs. However, the primary end point of reducing the *JAK2*-V617F allele burden was not reached (Figure 1). A slight overall hematologic improvement was seen in a subset of patients, but was not considered clinically relevant (Table 4). In a *JAK2*-V617F-driven mouse model of MPN, treatment with the β-3-sympathomimetic agonist BRL37344 lowered platelet and neutrophil counts, and decreased mutant hematopoietic progenitor numbers and spleen size.[8] However, we did not observe effects on blood counts, spleen size or CD34$^+$ cells in our phase II study. Species differences in the β3-adrenergic signaling and responsiveness of β3-adrenergic receptors towards different agonists between human and mouse could contribute to the observed discrepancies. Mirabegron is selective for the human β3-adrenergic receptor and was less effective in mice,[8] whereas BRL37344 shows higher affinity for the murine β3-adrenergic receptor.

Nevertheless, some of the effects observed in the preclinical *JAK2*-V617F mouse model treated with BRL37344, i.e. increase in nestin$^+$ bone marrow MSCs and decrease in myelofibrosis, were also seen in our mirabegron study: BM biopsies performed in a subset of 20 patients revealed a significant increase in the nestin$^+$ MSCs and a decrease in reticulin fibrosis (Figure 2). Although the beneficial effect of mirabegron on reticulin fibrosis was moderate, the duration of treatment was also rather short (24 weeks), as it was mainly designed to assess the primary end point of reduction of allele burden. The question of whether a higher dose of mirabegron might have been more effective is difficult to answer. Although doses of 100 mg daily have been tested in earlier clinical studies, no clear dose-dependent effect has been observed, while cardiovascular symptoms and a prolongation of the QT interval were noted.[9,19] The fact that nestin$^+$ cells showed a robust increase at 24 weeks of treatment indicates that mirabegron at 50 mg daily had one of the expected biological effects that had previously been described in mouse experiments.

Surprisingly, the effect on reticulin fibrosis was limited to patients who did not receive hydroxyurea treatment (41% of patients). The mechanism of how hydroxyurea interfered with the effect of mirabegron on reticulin fibrosis is currently unknown. Previous reports suggest that hydroxyurea alone can reduce reticulin fibrosis in some MPN patients.[20,21] Selecting patients who have not previously received hydroxyurea, a longer trial duration and higher dosage of mirabegron will be considered for future studies in MPN.

Despite the fact that the primary end point of reducing *JAK2*-V617F allele burden was not reached in this trial, the observed effects on nestin$^+$ MSCs and reticulin fibrosis is encouraging and shows that a β-3-sympathomimetic agonist can modify the microenvironment where the *JAK2*-mutant stem cells are maintained. These results generate an interest in evaluating β-3-sympathomimetic agonists specifically in patients with myelofibrosis not pretreated with hydroxyurea, and possibly in combination with other substances.

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