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

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
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. These nestin+ MSCs are strongly reduced in BM from patients with MPN. 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 \(\beta\)-3-sympathomimetic agonist. Mice with \(JAK2\)-V617F-driven MPN treated with the \(\beta\)-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). 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. Thus, \(\beta\)-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 \(\beta\)-3-adrenoceptor agonist, was approved in North America, Europe, Japan and Australia for the treatment of an overactive bladder. 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. 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.

![Figure 1. Changes in JAK2-V617F allele burden during the treatment period.](image)
**Table 1. Patients’ and disease characteristics**

| Characteristic | Value |
|----------------|-------|
| 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 | 25 (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 | 25 (72%) |
| Gastrointestinal | 1 (3%) |
| Mucocutaneous | 3 (8%) |
| Intraocular | 0 |
| Unknown/Missing | 24 (62%) |
| Previous therapies | 39 (100%) |
| Cytoreductive | 28 (72%) |
| Alkylation agents | 0 |
| Hydroxyurea | 23 (59%) |
| Pipobroman | 0 |
| Thioguanin | 0 |

**Study design and treatment**

We performed a multicenter, prospective, single-arm, single-stage and open phase II trial (NCT02311569) 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.

**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. 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.

**Assessment of myelofibrosis and nestin+ mesenchymal stem cells**

Patients who entered the study could also participate in 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.

**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 time interval 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; 5%), 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 (9%) of them with Common Terminology Criteria for Adverse Events (CTCAE) 4.0 grade 3, 12 (36%) 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.

| Parameter | Value |
|-----------|-------|
| Number (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 4. Response to mirabegron therapy.

| Outcome | Value |
|---------|-------|
| 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 (70%) |
| 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 of (N) of patients (%) or median (IQR). PV: polycythemia vera; CR: complete response; PR: partial response; ET: essential thrombocythemia; MF: myelofibrosis.

Table 3. Additional somatic mutations in myeloproliferative neoplasm patients.

| Gene          | Mutation | Patients (n=33) |
|---------------|----------|----------------|
| TET2          | M695fs, K1155E, 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 3. Additional somatic mutations in myeloproliferative neoplasm patients.
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 (Gömörí). (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 from a median of 1.09/mm² (IQR 0.38-3.27) to 3.95/mm² (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/mm² (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/mm² without hydroxyurea; decrease in reticulin fibrosis was limited to patients with hydroxyurea treatment without 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/mm²; IQR 16.75-34.25 vs. 22/mm², 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 β3-sympathomimetic agonist BRL37344 lowered platelet and neutrophil counts, and decreased mutant hematopoietic progenitor numbers and spleen size. 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, 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. Selecting patients who have not previously received hydroxyurea, a longer trial duration and a 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 β3-sympathomimetic agonist can modify the microenvironment where the JAK2-mutant stem cells are maintained. These results generate an interest in evaluating β3-sympathomimetic agonists specifically in patients with myelofibrosis not pre-treated with hydroxyurea, and possibly in combination with other substances.

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