Efficacy and safety of ruxolitinib in ineffective erythropoiesis suppression as a pre-transplantation treatment for pediatric patients with beta-thalassemia major

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Abstract

Background: Ineffective erythropoiesis (IE) is the most prominent feature of transfusion-dependent beta-thalassemia (TDT), which leads to extramedullary hemopoiesis. The rejection rate in allogeneic hematopoietic stem cell transplantation (HSCT) is clearly superior in heavily transfused patients (pts) with TDT accompanied by prominent IE. Therefore, a pre-transplantation treatment bridging to HSCT is often used to reduce allosensibilization and IE. Ruxolitinib (RUX) is a JAK-1/JAK-2-inhibitor and has showed its efficacy to suppress IE and the immune system. A previously published study on RUX in adult pts with TDT has revealed that this treatment significantly reduces spleen size and is well tolerated. Procedure: Ten pts (5-14 y.o.) with TDT and an enlarged spleen were enrolled. The dose of RUX was adjusted for age: for pts younger < 11 years: 40 - 100 mg/m2 and for pts >11 years: 20 - 30 mg/m2. HSCT was performed in 8 out of the 10 pts. Results: After the first 3 months of RUX therapy the spleen volume decreased in 9 out of the 10 cases by 9.1 – 67.5% (M = 35.4%) compared to the initial size (p = 0.003). The adverse events of RUX included infectious complications, moderate thrombocytopenia as well as headache and were successfully managed by reducing the dose. The outcomes of HSCT were favorable in 7 out of the 8 cases. Conclusion: RUX is promising as a short-term pre-HSCT treatment for pediatric pts with TDT and pronounced IE.

Introduction

Beta-thalassemia is an inherited hemolytic anemia that is associated with reduced or absent ß-globin synthesis. In severe cases imbalanced accumulation of α-globin chains and the formation of its aggregates impairs terminal erythroid maturation. Beta-thalassemia major (TM) is characterized by early onset, significant changes in the ratio of hemoglobin fractions (HbF > 50%, HbA_2< 4%) and profound anemia.[1-4]

To date allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative therapy. HSCT in TM is associated with increased rate of graft failure and transplant-related complications.[5-8]

Ineffective erythropoiesis (IE) is the most prominent feature of TM, which in severe cases leads to extramedullary hemopoiesis with hepatosplenomegaly and skeletal deformities. Splenomegaly leads to an increase in red blood cell (RBC) transfusion requirement, as a large proportion of the circulating RBC are eliminated by an enlarged spleen. This results in subsequent complications such as accumulation of alloantibodies and iron overload.[9-12]

It is thought that prominent expansion of IE, seen in TM, exerts a negative effect on engraftment and graft function. In view of this fact highly intensive myeloablative conditioning regimens are used in these

patients. On the other hand, most of the patients with TM have a severe iron overload, which is the strong risk factor for transplant-related complications and mortality. Therefore, in heavily transfused patients with TM accompanied by drastic IE, the outcomes of HSCT are often inferior.[7-9] In the recent years several studies aiming to improve outcomes in patients with class 3 TM, including well-tolerated pre-transplantation treatment have been published.[7-9,13,14]

The JAK-STAT signaling pathway is one of the key regulators of normal hematopoiesis which is mediating signals transduction from a variety of cytokines and hematopoietic growth factors in hematopoietic stem cells and precursors.[15] It was shown that inhibition of JAK1 impairs cytokine production and modulate dendritic cell function.[16-18] Ruxolitinib as JAK-1/JAK-2-inhibitor, has shown its efficacy in suppressing IE and immune system.[19-23]

Ruxolitinib treatment effectively reduces spleen size in patients with polycythemia vera, essential thrombocythemia and myelofibrosis.[19-23] The similar effect has been demonstrated in adults with TM and ruxolitinib appeared to be safe and well tolerated.[23] However, a long-term ruxolitinib treatment could lead to severe adverse events (AE) such as infectious complications and hematological and solid tumors.[24-26]

The aim of our study is to evaluate the efficacy and safety of ruxolitinib in IE suppression as a short-term pre-transplantation treatment for pediatric patients with TM.

Methods

Ten pediatric patients with TM (5 males and 5 females, 5 - 14 years old) were enrolled in the study (Table 1). The study was done in accordance with the principles of Good Clinical Practice, the Declaration of Helsinki, and all local regulations (approved by the Independent Ethics Committee of the Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology. All of the parents of the patients signed a written informed consent).

The inclusion criteria were as follows: an established diagnosis of TM, a high blood transfusion-dependency (at least one unit of RBC every 2-3 weeks for at least 1 year) and an enlarged spleen.

The exclusion criteria were as follows: platelet count $< 75 \times 10^9$/L, an absolute neutrophil count $< 1.5 \times 10^9$/L; a cardiac ejection fraction of $< 50\%$ or impaired renal, hepatic or gastrointestinal functions.

All patients were heavily transfused and had moderately severe iron overload. Liver iron concentration was measured by MRI (GE Signa 1.5). Chelation therapy continued during the study. All patients had very low pre-transfusion Hb level (<80 g/L) before enrolling in this study, heralding severe IE.

The dose of ruxolitinib was age-adjusted and based on previously published reports on ruxolitinib pharmacokinetics in children: for patients younger than 11 years it was 40-100 mg/m^{2}total dose three times daily; for patients older than 12 years it was 20 - 30 mg/m^2 total dose twice daily. Toxicities were graded according to the Common Terminology Criteria for Adverse Events version 4.0 (http://ctep.cancer.gov).

Eight patients received ruxolitinib for 6 months, while patient 1 and 9 received ruxolitinib for 1 and 1.5 years, respectively.

HSCT was performed in 8 out of 10 patients. The source of stem cells was bone marrow from matched sibling donor (MSD; n = 2), bone marrow from matched unrelated donor (MUD; n = 1) and peripheral blood from MUD (n=1) or haploidentical related donor (MMRD; n = 2). Two of the patients (2 and 4) have not received HSCT.

Conditioning regimen for the first transplant was treosulfan (42 g/m^2 total dose; days -4, -3 and -2), thiotepa and (300 mg/m^2 total dose; day - 5) and fludarabine (150 mg/m^2 total dose; days -5, -4, -3, and -2). In addition patients received serotherapy with rabbit antithymocyte globulin (ATG;) (5 mg/kg total dose); days -5 and -4). On day -7 and - 1 patients received 375 mg/m^2 rituximab for reducing the risk of Epstein-Barr virus related post-transplant lymphoproliferative disease. Grafts from MUD and MMRD (n=5) were TCRab$^+$/CD19$^+$ depleted by using an immunomagnetic method in accordance with the manufacturer's

instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). Donor-specific anti-HLA antibodies were not detected in any of patients.

A decrease in spleen volume as assessed by MRI was chosen as the main criterion of ruxolitinib suppression of IE. Additional efficacy criteria were as follows: a decrease of soluble transferrin receptor (sTfR) concentration, a decline in proerythroblast (CD45-/CD71+/CD117+) and erythroblast (CD45-/CD71+/CD235+) count in the bone marrow, assessed by flow cytometry. Data obtained from previously published studies were used for interpreting the results.[27-29] Also percentage changes of RBC transfusion volume (ml/kg) which was required to maintain Hb at 11.0 – 12.0 g/L compared to baseline and graft failure/rejection rate were used for efficacy evaluation.

Statistical analysis

Statistical analysis was done in MS Excel and XLSTAT 2020.5.1. Statistical significance of reduction was tested using one-sample Student's t-test and Wilcoxon's signed-rank test applied to all ten patients' data. Statistical significance of reduction was tested using one-sample Student's t-test. The null-hypothesis was rejected if a corresponding p-value was less than the significance level of 0.05. M - average value (mean); +/- SD - standard deviation; p – *p* value. All values are listed in the supplements.

Results

After the first 3 months of ruxolitinib therapy the spleen volume decreased in 9 out of the 10 cases by 9.1 – 67.5% (M = 35.4%) compared to the initial size (p = 0.003). The difference in size was 180 cm^3 (SD +/- 146 cm^3) on average. In the interval from 3 to 6 months after therapy start the spleen volume was reduced in 7 out of the 10 patients by 2.5 – 44.9% (M = 17.6%, p = 0.811). In 2 cases (Pt 2 and 3) the spleen volume increased by 43.9% and 18.3% respectively after reducing the dose of ruxolitinib due to infection. In patient 4 ruxolitinib was discontinued 5 months after therapy start and the spleen volume increased by 90% in one month virtually returning to the initial size. Therefore, the maximum effect in the spleen volume reduction was achieved after the first 3 months of ruxolitinib therapy. Differences in the spleen volume at the interval from 0 to 3 months and from 3 to 6 months after therapy start are shown on Fig. 1.

The sTfR concentration decreased in 9 out of the 10 patients by 15.6 – 77.7% (M = 49%, p = 0.011) after the first 3 months of ruxolitinib therapy and continued to decrease thereafter in between 3 to 6 months of therapy start in 6 of them by 3.9 – 68.9% (M = 37.3%, p = 0.411) reflecting IE suppression (Fig. 2).

The RBC transfusion volume (ml/kg) required to maintain Hb at 110 - 120 g/L compared to baseline decreased in line with reduction of the spleen volume. After first 3 months of ruxolitinib therapy the RBC transfusion volume was reduced by 16.7 – 54.8% (M=37.5%) compared to the initial volume (p = < 0.001), and 3 months later it decreased in 9 out of the 10 patients by 8.3 – 36.0 % (M = 20.4%, p = 0.025) (Fig. 3).

Before ruxolitinib therapy start the relative erythrocyte progenitor count in bone marrow was on average 4.7-fold higher for proerythroblasts and 1.6-fold higher for erythroblasts compared to normal. The erythrocyte progenitor count tended to decline after the first 3 months of taking ruxolitinib, which was consistent with the reduction of the spleen volume and the sTfR concentration. More specifically the proerythroblast (CD45-/CD71+/CD117+) count was reduced in 5 out of the 9 cases by 17.5 – 67.5% (M = 40.6%, p = 0.910) and the erythroblast (CD45-/CD71+/CD235+) count was reduced in 7 out of the 9 cases by 13.1 – 56.8% (M = 26,4%, p=0.313) (Fig. 4).

AE probably related to ruxolitinib were observed in 9 out of the 10 patients, mainly grade II - III in severity. Stomatitis, herpes labialis, respiratory tract infections or furunculosis were present in the 7 cases; thrombocytopenia (the minimum platelet count was 75 x 10^9/L) – in one case; one patient had a headache. All AE resolved after ruxolitinib dose reduction by 30-50%.

At the time of analysis eight patients have undergone HSCT from allogeneic donors. Primary engraftment occurred in 7 out of the 8 cases. One patient (3) experienced primary graft failure, then rejected second graft from another 9/10 HLA-compatible MUD, and achieved engraftment after third transplant from an HLA-

haploidentical parent. In one patient (Pt 7), long-lasting thrombocytopenia occurred and was successfully managed with romiplostim.

One patient (Pt 5) developed moderately severe veno-occlusive disease of liver, which resolved with defibrotide treatment, one patient (Pt 9) had acute graft-versus-host disease (GVHD) stage IV (gastrointestinal tract and liver - grade IV, skin – grade II). Details of transplant procedure and outcomes are reported in Table 1.

All patients are currently alive. Two out of the 10 patients (Pt 2 and 4), who have not received HSCT due to the lack of a compatible donor, discontinued ruxolitinib therapy after 6 and 5 months, respectively, and the clinical features of IE almost returned to baseline.

Discussion

Our study showed that ruxolitinib therapy in children with TM accompanied by prominent features of IE leads to considerable reduction of the spleen volume and the RBC transfusion requirement. This correlates with significant decrease in sTfR concentration and tendency to declining of erythrocyte progenitor in the bone marrow. The maximum effect of IE suppression was achieved mostly after the first 3 months of ruxolitinib therapy. It appears that therapeutic effect of ruxolitinib was dose-dependent as clinical and laboratory features of IE rapidly returned to initial after ruxolitinib dose reduction.

The outcomes of HSCT were favorable in 7 out of the 8 "difficult-to-transplant" patients with TM. We suggest that IE which is one of the main risk factors potentially leading to primary and secondary severe graft failure and immune suppression may both impact on the favorable engraftment rate. There are two important limitation factors of ruxolitinib therapy, one is rapid loss of response after drug interruption and another is potentially severe AE with long-term use. Thus, short-term application of ruxolitinib in patients with TM and pronounced IE can be used safely and seems to be a promising approach for preparing pediatric patients with TM and pronounced IE for HSCT.

Conclusions

Ruxilitinib therapy is effective in suppressing IE, which is one of the main risk factors potentially leading to primary and secondary severe graft failure. Therefore, this kind of therapy is promising for preparing pediatric patients with TM and pronounced IE for HSCT.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Figure legends

FIGURE 1 The differences in the spleen volume for each patient after the first 3 months of ruxolitinib therapy start and in the interval from 3 to 6 months of ruxolitinib therapy.

FIGURE 2 The differences in StfR concentration for each patient after the first 3 months of ruxolitinib therapy start and in the interval from 3 to 6 months of ruxolitinib therapy.

FIGURE 3 The differences in the RBC transfusion volume which is required to maintain Hb at 11.0-12.0 g/L compared to baseline for each patient after the first 3 months of ruxolitinib therapy start and in the interval from 3 to 6 months of ruxolitinib therapy.

FIGURE 4 The differences in early erythrocyte progenitor count in the bone marrow (for proerythroblasts and for erythroblasts) for each patient after the first 3 months of ruxolitinib therapy start and in the interval from 3 to 6 months of ruxolitinib therapy.

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`Table1.docx` available at https://authorea.com/users/415751/articles/523552-efficacy-and-safety-of-ruxolitinib-in-ineffective-erythropoiesis-suppression-as-a-pre-transplantation-treatment-for-pediatric-patients-with-beta-thalassemia-major