Ruxolitinib for refractory/relapsed hemophagocytic lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is a clinical syndrome associated with hereditary or acquired immune-dysregulation. HLH is classified into either primary or secondary disease based on whether or not clear genetic defects are present. Patients of primary HLH have clear hereditary or genetic defects and shows functional deficiency of cytotoxicity. Patients of secondary HLH have no familial history or known genetic defects, and etiology of the disease is related to a variety of triggering factors such infection, cancer, and rheumatic disease. Current therapies have improved the survival of HLH patients; however, approximately 30% of patients do not respond to current therapies.[1] At present, there is no unanimously recommended salvage HLH treatment regimen, and there are few case reports or clinical reports with small sample sizes concerning salvage therapy after first line treatment failure.[2] Therefore, alternative therapies for relapsed/refractory HLH is needed.

HLH were the disorders of the immune system characterized by the excessive production of cytokines, including interferon-γ and interleukins 2, 6, and 10 (IL-2, IL-6, and IL-10).[3] The Janus kinases (JAK) transduce signals initiated following engagement of specific receptors that bind a broad array of cytokines, including those overproduced in HLH. JAK1/2 inhibitor ruxolitinib suppresses the harmful consequences of macrophage overactivation characterizing HLH in several murine models.[4,5] Broglie *et al*. used ruxolitinib to treat an 11-year-old male with refractory HLH.[6] Within 24 hours of ruxolitinib treatment, he became afebrile, followed by rapid improvements in respiratory, liver, and hemodynamic function.[6] Sin *et al*. reported the administration of ruxolitinib to a 38-year-old female patient with refractory Epstein-Barr virus (EBV)-related HLH, the patient showed significant improvement in several HLH markers, including serum ferritin, lactate dehydrogenase, fibrinogen, and liver function.[7] The present study was designed to investigate the efficacy of ruxolitinib as a salvage therapy for refractory/relapsed (R/R) HLH.

Thirty-four patients with R/R HLH were enrolled from September 2017 to September 2018, including 18 males and 16 females. The median age was 27.5 years (range: 2–70 years) old. Patients who were enrolled in this study fulfilled the following criteria: (1) Met HLH-2004 diagnostic criteria;[8] (2) were older than 1 year and younger than 75 years of age; (3) were treated with HLH-94 regimen 1 no less than two weeks before enrollment and did not achieve even a partial response (PR) or relapsed after remission with HLH-94 regimen 1; (4) the expected survival time was more than one month. Patients who were excluded from this study fulfilled the following criteria: (1) Uncontrolled infection at the time of enrollment and (2) a history of non-melanoma skin cancer. The study was approved by the Ethics Committee at the Beijing Friendship Hospital, Capital Medical University. All patients provided written informed consent before participating in the study. The main clinical features of the enrolled patients are summarized in Table 1.

In the two HLH patients previously treated with ruxolitinib, the doses given were as follows: 2.5 mg twice daily for an 11-year-old male[6] and 20 mg twice daily for a 38-year-old woman.[7] In adult patients, ruxolitinib at a dose of 10 mg orally twice daily can be tolerated in the treatment of patients with myelofibrosis or corticosteroid-refractory graft-*versus*-host disease.[9,10] Therefore, the dose is 0.3 mg/kg/day according to the average adult weight of 60 kg. There are no recommended doses for children; therefore, the doses were adjusted for age and weight: The dose for adult patients (age ≥14 years) is generally 10 mg twice daily. For children (age <14 years, weight ≥25 kg), the dose was generally 5 mg twice daily. For children (age <14 years, weight <25 kg), the dose was generally 2.5 mg twice daily. In this study, all patients received ruxolitinib at the dose indicated until they received allogeneic hematopoietic stem cell transplants (allo-HSCT) or treatment for underlying disease. Ruxolitinib was used alone for 16 patients, and in combination with glucocorticoids for 18 patients in the following conditions: 17 patients were treated with long-term high-dose glucocorticoids, and the effect was poor. The glucocorticoids could not be stopped suddenly; therefore, ruxolitinib was added together with a small dose of methylprednisolone (4-16 mg/day). One patient with macrophage activation syndrome (MAS) was treated with ruxolitinib, methylprednisolone (32 mg/day) and hydroxychloroquine sulfate (200 mg/day).

Table 1. The main clinical features of the patients.

Clinical features	Before ruxolitinib number (percentage [%])
Fever ≥38.5°C	34 (100.0)
Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood)	17 (50.0)
Splenomegaly	27 (79.4)
Ferritin >500	28 (82.4)
Hypofibrinogenemia	14 (41.2)
Hypertriglyceridemia	15 (44.1)
Hemophagocytosis in the bone marrow	23 (67.6)
Low or absent NK-cell activity	22 (64.7)
Elevated sCD25	31 (91.2)
Underlying disease	
FHL-2	1 (2.9)
EBV-HLH	25 (73.5)
MAS	2 (5.9)
Unclear	6 (17.6)
Prior therapy	
Only HLH-94	14 (41.2)
HLH-94 and DEP/L-DEP	16 (47.1)
HLH-94 and IVIG	3 (8.8)
HLH-94 and splenectomy	1 (2.9)

HLH: hemophagocytic lymphohistiocytosis; FHL-2: familial HLH-2; MAS: macrophage activation syndrome; EBV-HLH: Epstein-Barr virus-associated HLH; L: PEG-aspargase; IVIG: intravenous immunoglobulin; DEP: doxorubicin-etoposide-methylprednisolone; NK-cell: natural killer cell.

The efficacy of ruxolitinib for the treatment of HLH was assessed every two weeks according to the evaluation criteria proposed by Marsh *et al*.[11] The best overall response were defined as the highest degree of remission during eight weeks. The overall response rate (OR) of the 34 patients was 73.5% (25 of 34 patients), with 14.7% (5 of 34 patients) in complete response (CR) and 58.8% (20 of 34 patients) in PR. The median time to achieve response (CR+PR) for patients was two weeks (range: 1-8 weeks). Most patients achieved PR, whereas only a few patients achieved CR, suggesting the remission depth was not sufficient. This may be due to the low dose used in the present study. Of the 25 patients with R/R EBV-

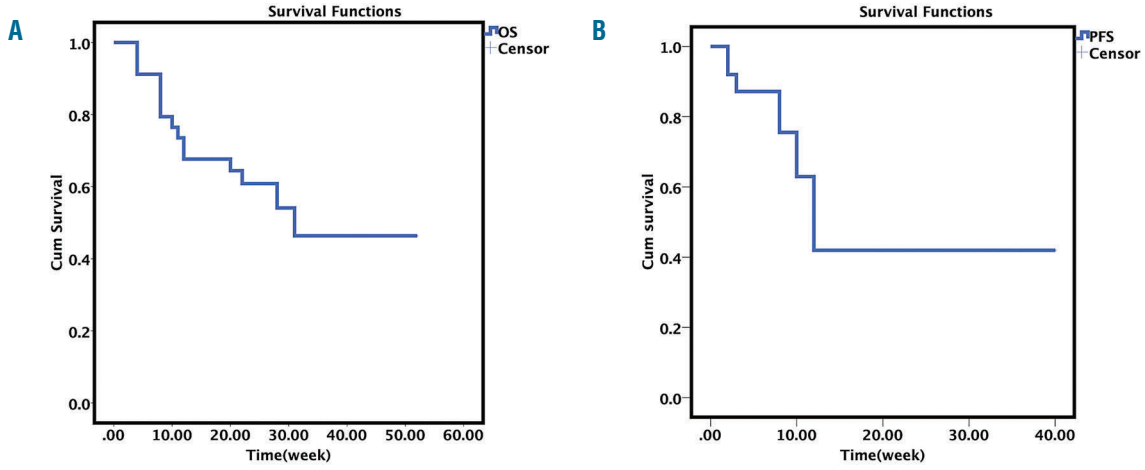

Figure 1. Survival of patients with refractory /relapsed hemophagocytic lymphohistiocytosis treated with ruxolitinib. (A) Overall survival (OS). (B) Progression-free survival (PFS). The median survival time was 22 weeks (range: 4-52 weeks). The median PFS time was eight weeks (range: 2-40 weeks). Cum Survival: cumulative survival.

Table 2. Changes in selected indicators before and after ruxolitinib.

Indicators	Before	Two weeks after	*P*
WBC (x10⁹/L)	3.4 (0.05-12.44)	4.13 (0.83-11.88)	0.056
PLT (x10⁹/L)	111.5 (1-543)	107.5 (3-658)	0.443
Ferritin (μg/L)	1,862.5 (43.5-75,000)	1,579 (15.7-68,015)	0.003
Fbg (g/L)	1.9 (0.62-5)	1.57 (0.54-5.89)	0.127
TG (mmol/L)	2.885 (0.66-6.85)	1.87 (0.87-13.38)	0.255
ALT (U/L)	52 (9-789)	63.5 (4-296)	0.607
TBIL (μmol/L)	20.2 (4.06-470.09)	14.82 (5.27-177.19)	0.059
sCD25 (pg/mL)	3,382 (672-35,822)	1,145 (305-34,008)	0.012
Spleen thickness (cm)	4.75 (3.4-6.9)	4.25 (3-6.1)	0.079

WBC: white blood cell; PLT: platelet; Fbg: fasting plasma glucose; TG: triglyceride; ALT: alanine transaminase; TBIL: bilirubin test; sCD25: soluble IL-2 receptor.

HLH, 17 (68%) patients responded to ruxolitinib. This response rate was lower than OR.

Nine variables were assessed before and two weeks after the ruxolitinib. These comprised white cell count, platelet count, ferritin, fibrinogen, triglyceride, alanine aminotransferase (ALT), total bilirubin, soluble IL-2 receptor (sCD25) and ultrasonic spleen thickness (Table 2). Comparisons between multiple samples and groups were performed using the Wilcoxon rank sum test. $P<0.05$ was considered to statistically significant. Within 24 hours of starting ruxolitinib, 88.2% (30 of 34) of our patients became afebrile. Ferritin ($P=0.003$) and sCD25 ($P=0.012$) were significantly lower at two weeks after the ruxolitinib. In addition, EBV-DNA levels of whole blood ($P=0.388$) and plasma ($P=0.064$) in 25 EBV-HLH patients before and after ruxolitinib treatment did not change, indicating that ruxolitinib reduces inflammation without affecting the underlying primary cause of HLH.

Among the 34 patients after ruxolitinib, leukopenia of grade III/IV in two (5.9%) patients, thrombocytopenia of grade III/IV in four (11.8%) patients, elevated transaminases of grade III/IV in six (17.6%) patients, elevated bilirubin of grade III/IV in three (8.8%) patients, hypertriglyceridemia of grade III/IV in one (2.9%) patient. Pulmonary infections in three patients were aggravated and urinary infection in one patient occurred during the treatment of ruxolitinib. No one stopped therapy due to toxicity.

Survival times were calculated from the date of ruxolitinib salvage therapy. All patients were followed up until death or January 1, 2019, whichever occurred first. A total of 15 of 34 patients had died, indicating a mortality rate of 44.1%. The median follow-up was 26.5 weeks (range: 15-52 weeks). The median survival time was 22 weeks (range: 4-52 weeks). The median progression-free survival (PFS) time was eight weeks (range: 2-40 weeks) (Figure 1).

Six of 10 EBV-HLH patients who achieved CR or PR but were not given allo-HSCT relapsed, and EBV-DNA elevation was present in two of these patients prior to relapse. Conversely, 2 of 8 non–EBV-HLH patients who achieved CR or PR relapsed 12 weeks after ruxolitinib treatment. The prognoses and responses of patients receiving ruxolitinib salvage therapy for HLH varied depending on the underlying disease. EBV-HLH patients had the lowest rate of remission and the highest rate of recurrence. For these patients, the median time to recurrence after ruxolitinib treatment was eight weeks (range:

3-12 weeks). Therefore, if a matching donor is available, allo-HSCT should be performed within eight weeks of ruxolitinib treatment. In the present study, the median time for the patient to receive transplantation after ruxolitinib treatment was two weeks (range: 2-10 weeks). For those patients who went on to allo-HSCT, we discontinued ruxolitinib one day before hematopoietic stem cell transfusion.

In the 25 patients who achieved PR or CR, the levels of cytokines, specifically IFN-γ (P=0.006), IL-18 (P=0.016), macrophage inflammatory protein (MIP)-1α (P=0.029), and interferon-γ-inducible protein (IP)-10 (P=0.004), were significantly decreased within two to four weeks of ruxolitinib treatment. Mechanistically, ruxolitinib probably decreases a variety of cytokines by regulating the JAK1/2 signaling pathway, which is the downstream pathway of IFN-γ and other inflammatory cytokine receptors. Our results showed that ruxolitinib treatment improves several inflammatory biomarkers, such as body temperature, ferritin, and sCD25, suggesting the role of the JAK/STAT pathway in HLH progression, showing that ruxolitinib reduces inflammation and alleviates the HLH syndrome by decreasing the levels of cytokines, including IFN-γ. This is consistent with the results of earlier murine models.[4,5]

Ruxolitinib is a safe and effective salvage therapy for R/R HLH, and increases the possibility of patients with R/R HLH receiving allo-HSCT or treatment for underlying disease. Because ruxolitinib can improve the inflammatory status well, but the remission depth is not sufficient, we hope to combine ruxolitinib with our previous DEP (doxorubicin-etoposide-methylprednisolone) regimen to treat HLH. Consequently, a prospective multicenter large-scale clinical trial is underway in China and aims to validate the DEP-ruxolitinib (DEP-Ru) regimen for refractory/relapsed HLH (*ClinicalTrails.gov Identifier: NCT03533790*).

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References

1. Swerdlow SH CE, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds). WHO classification of tumours of haematopoietic and lymphoid tissues (revised 4th edition). Lyon. IARC; 2017.
2. Cervantes F, Dupriez B, Pereira A, et al. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. Blood. 2009;113(13):2895-2901.
3. Gangat N, Caramazza D, Vaidya R, et al. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. J Clin Oncol. 2011; 29(4):392-397.
4. Vallapureddy RR, Mudireddy M, Penna D, et al. Leukemic transformation among 1306 patients with primary myelofibrosis: risk factors and development of a predictive model. Blood Cancer J. 2019;9(2):12.
5. Mudireddy M, Gangat N, Hanson CA, Ketterling RP, Pardanani A, Tefferi A. Validation of the WHO-defined 20% circulating blasts threshold for diagnosis of leukemic transformation in primary myelofibrosis. Blood Cancer J. 2018;8(6):57.
6. Tefferi A, Mudireddy M, Mannelli F, et al. Blast phase myeloproliferative neoplasm: Mayo-AGIMM study of 410 patients from two separate cohorts. Leukemia. 2018;32(5):1200-1210.
7. Guglielmelli P, Lasho TL, Rotunno G, et al. MIPSS70: Mutation-Enhanced International Prognostic Score System for Transplantation-Age Patients With Primary Myelofibrosis. J Clin Oncol. 2018; 36(4):310-318.
8. Boiocchi L, Espinal-Witter R, Geyer JT, et al. Development of monocytosis in patients with primary myelofibrosis indicates an accelerated phase of the disease. Mod Pathol. 2013;26(2):204-212.
9. Tefferi A, Shah S, Mudireddy M, et al. Monocytosis is a powerful and independent predictor of inferior survival in primary myelofibrosis. Br J Haematol. 2018;183(5):835-838.
10. Elliott MA, Verstovsek S, Dingli D, et al. Monocytosis is an adverse prognostic factor for survival in younger patients with primary myelofibrosis. Leuk Res. 2007;31(11):1503-1509.
11. Barraco D, Cerquozzi S, Gangat N, et al. Monocytosis in polycythemia vera: Clinical and molecular correlates. Am J Hematol. 2017;92(7):640-645.
12. Boiocchi L, Gianelli U, Iurlo A, et al. Neutrophilic leukocytosis in advanced stage polycythemia vera: hematopathologic features and prognostic implications. Mod Pathol. 2015;28(11):1448-1457.
13. Bonicelli G, Abdulkarim K, Mounier M, et al. Leucocytosis and thrombosis at diagnosis are associated with poor survival in polycythaemia vera: a population-based study of 327 patients. Br J Haematol. 2013;160(2):251-254.
14. Tefferi A, Rumi E, Finazzi G, et al. Survival and prognosis among 1545 patients with contemporary polycythemia vera: an international study. Leukemia. 2013;27(9):1874-1881.
15. Iurlo A, Cattaneo D, Gianelli U. Blast Transformation in Myeloproliferative Neoplasms: Risk Factors, Biological Findings, and Targeted Therapeutic Options. Int J Mol Sci. 2019;20(8):1839-1852.