Activity of the Janus kinase inhibitor ruxolitinib in chronic lymphocytic leukemia: results of a phase II trial

B cell receptor (BCR)-signaling inhibitors such as Ibrutinib and Idelalisib do not cure CLL, suggesting the importance of other pathways.[1] Cytokines are also important in CLL biology and mediate transcription of oncogenic genes such as miR-17 by activating signal transducer and activator of transcription (STAT) proteins including STAT5A/B and STAT3 through Janus kinases (JAKs).[2-4] We hypothesized that Ruxolitinib, a selective JAK1/2 inhibitor licensed for myelofibrosis,[5] might have unrecognized activity in CLL and designed a single center phase II trial to evaluate it in patients considered unfit for FCR on the basis of age and comorbidities.[6] The demonstrated safety of Ruxolitinib in myelofibrosis was felt to make it ethical to use as first-line therapy for elderly CLL patients. The primary endpoint was overall response rate (ORR) assessed after 7 treatment cycles.[7] Secondary endpoints were safety and tolerability.[7] The study was approved by the Sunnybrook REB and Health Canada and registered with ClinicalTrials.gov, NCT02015208.

A traditional activity design for phase II trials of single agents was employed[8] at the dose and schedule for Ruxolitinib in myelofibrosis approved by Health Canada. A preliminary phase I design was not considered necessary due to the favorable experience in myelofibrosis.[5,9] Starting doses and modifications were based on platelet and neutrophil counts according to the product label. The maximum daily dose allowed was 100 mg[9] and the average dose was 10 mg twice daily (Table 1). Ruxolitinib was administered on a 28 day cycle to be repeated in the absence of intolerable toxicity or disease progression for a maximum of 7 cycles, based on experience with Ibrutinib that suggested an average time to best response

of 7.4 months.[10,11] The trial planned to enroll 14 patients to target a 20% response rate with a power of 80% and assuming a null response rate of 0%. After enrolling 13 patients between April 23 and October 16, 2014, the trial was stopped due to the uniformity of biological responses, unexpectedly high incidence of anemia, and no examples of decreased lymphocytosis.

The mean age was 74 with 8/13 patients older than 70 years (Table 1). Prior treatment with cytotoxic chemotherapy was not allowed but JAK 3, 5, 7, 8, 9, and 12 were treated with 2-5 cycles of high-dose glucocorticoids for evidence of active disease prior to enrollment.[7,12] JAK 2 and JAK 10 had been treated previously with conventional steroid doses for autoimmune hemolytic anemia, followed by splenectomy. JAK7 also had a prior splenectomy to relieve splenomegaly-associated pain. Five patients had positive direct antiglobulin tests without evidence of active hemolysis at study entry (Table 2).

Ruxolitinib exhibited marked biological activity in all patients. The sum of the products of bidirectional measurements of the largest palpable lymph nodes in the neck, axilla, and inguinal regions[7] plus the spleen-tip distance from the costal margin was obtained by physical examination at each clinic visit. In most cases, palpable lymphadenopathy, although not splenomegaly greater than 3 cm, decreased within 1-2 cycles of therapy (Figure 1A).

Based on CT scans performed before and at end of treatment (EOT) after 7 cycles, marker nodes decreased by 50% in 8 patients, consistent with partial responses (PRs).[7] Radiologic responses were not as marked as changes on physical exam. All patients exhibited increases in white blood cell (WBC) counts, reflecting circulating lymphocytes (Figure 1A), and most patients were anemic on study entry (Table 2), which worsened on Ruxolitinib and obviated classifying the responses as PRs with lymphocytosis.[10,11] Accordingly, 2 patients developed progressive disease on Ruxolitinib, 10 had stable disease, and

Table 1. Patient information.

Pt.#	Sex	Age (years)	Disease Length (years)	Stage*	CD38 (%)	FISH	Initial WBC (x10⁹/L)	Initial β2M (mg/L)[1]	Tx[2]	Initial LDH (U/L)[3]	Avg. Ruxo Dose (mg)[4]	Response	Status
JAK2	M	66	8	4	1	del13q	147	3.3	2	373	25	PD	FR/I
JAK3	F	82	10	3	4	del13q	210	4.4	1	173	12	SD	W
JAK4	F	76	7	4	1	N	223	5.6	0	224	10	SD	Sp
JAK5	F	78	2	4	1	N	121	6.6	1	175	5	SD	W
JAK6	F	90	5	4	11	del13q	202	5	0	196	15	SD	died
JAK7	F	68	10	3	1	del13q	850	4	2	275	20	SD	W
JAK8	F	78	3	3	1	del17p	202	5	1	466	10	PD	I
JAK9	M	69	1	4	3	T12⁵	38	4	1	360	5	NE	died
JAK10	M	68	12	4	1	del13q	30	3	2	168	20	SD	W
JAK11	M	66	3	3	1	N	122	5	0	226	18.6	SD	W
JAK12	F	70	7	3	1	del13q	60	5	1	195	17	SD	W
JAK13	M	80	1	4	14	del11q, T12, del13q	48	6	0	258	18.6	SD	W
JAK14	F	72	9	3	1	del13q	282	4.9	0	197	11	SD	W

*IgVH status was not available; [1]normal range=0.6-2.3 mg/L; [2]number of prior treatments including glucocorticoids and/or splenectomy; [3]normal range=100-250 U/L; [4]mg BID; [5]trisomy 12. PD: progressive disease; SD: stable disease; W: watch and wait; I: Ibrutinib; FR: Fludarabine plus Rituximab; N: normal FISH; Sp: splenectomy; NE: not evaluable.

Table 2. Anemia in trial patients.

Pt.	Hb pre (g/L)[†]	Lowest Hb (cycle)*	Retic Pre (x10⁹/L)**	Retic Post (x10⁹/L)	Retic on Darbo (x10⁹/L)[#]	Transfusions (Units)	Darbo	DAT[b]
JAK2	113	94 (C3)	36	23	–	0	No	+
JAK3	90	67 (C5)	38	19	NA	6	Yes	–
JAK4	115	78 (C5)	46	57	NA	2	Yes	+
JAK5	93	76 (C2)	34	35	38	2	Yes	–
JAK6	117	51 (C6)	NA	11	21	6	Yes	+
JAK7	122	66 (C5)	73	21	65	2	Yes	+
JAK8	88	62 (C3)	59	39	NA	4	No	–
JAK9	87	66 (C3)	193	129	NA	4	No	–
JAK10	120	79 (C6)	62	29	NA	–	No	–
JAK11	94	74 (C4)	70	18	44	2	Yes	–
JAK12	108	62 (C2)	NA	9	NA	6	Yes	–
JAK13	115	76 (C6)	NA	30	88	0	Yes	+[c]
JAK14	95	74 (C4)	48	21	85	4	Yes	–
Summary	104.4±3.6	71±2.9 (C4)	51.8±4.9[a]	26±3.8	56.8±11.0	10/13 required transfusion	9/13 started on Darbo	5/13 DAT+

[†]Anemia defined as hemoglobin (Hb)<120 g/L; *cycle of ruxolitinib in which lowest Hb occurred; **normal range 30-110x10⁹/L. [#]Darbopoetin. [a]Excluding results for JAK9. [b]Direct Coombs or antiglobulin test. [c]Converted to normal at cycle 5 of ruxolitinib. NA: not available.

JAK9 was not evaluable due to early death.

Disease control appeared somewhat transient. Beta-2 microglobulin levels (Table 1) decreased initially but began to increase towards EOT and accelerated following discontinuation of Ruxolitinib (Figure 1A). Lymphadenopathy also began to increase towards EOT (Figure 1A). Three patients progressed within several months of completing Ruxolitinib and went on to other treatments (Table 1). Since all patients warranted treatment at study entry, it can be concluded that Ruxolitinib delayed the need for another treatment by almost a year.

Profound lymphocytosis accompanied decreased lymphadenopathy, suggesting a change in compartmentalization of CLL cells. The lymphocytosis was sustained throughout treatment and associated with increased plasma lactate dehydrogenase (LDH) (Figure 1A). Increased LDH might reflect tumor lysis or hemolysis but no changes were noted in other markers of these conditions, including serum phosphate, uric acid, bilirubin, and haptoglobin. Blood lymphocyte and LDH levels declined at EOT, or when treatment was interrupted for thrombocytopenia or infections (Figure 1A, B), suggesting they were caused by Ruxolitinib and did not represent disease progression.

The major toxicities were infections and anemia. Only 3 patients did not require antibiotics for suspected mild infections. Extrapulmonary *Blastomycosis dermatiditis* developed in JAK10. JAK6 died of viral pneumonia after 7 cycles and JAK9 died of septic shock following an accidental laceration after cycle 3. JAK6 and JAK9 had not been splenectomized or treated with steroids.

Immunosuppressive effects on T and dendritic cells have been attributed to Ruxolitinb but were not studied formally in this trial.[13] Immunoglobulin levels were transiently suppressed in 7 patients (whose IgA, IgG, and IgM levels (reported as mean±standard error) decreased from 0.54±.18, 5.7±1.88, and 0.82±.69 to 0.46±.12, 4.8±.85, and 0.69±.52 but increased to 0.60±.16, 5.52±1.02, and 1.27±.82 g/L, respectively, after 30 days off Ruxolitinib). JAK2, 7, 10, and 12 were on immunoglobulin replacement prior to the study while JAK6 and JAK9 were unavailable for this analysis.

Neutropenia was not seen but platelets often decreased temporarily early in the course of treatment and necessitated dose reductions or treatment delays but not platelet transfusions in 7 patients. In contrast, anemia was a significant problem. The average decline in Hb was 30 g/L by cycle 4 (Table 2). All patients were transfusion-independent at study entry but 10/13 were transfused on Ruxolitinib. Positive direct Coombs tests pre-existed in 5/13 patients but there was no evidence for development of autoimmune hemolytic anemia. Coombs positivity in JAK13 even disappeared after several months of Ruxolitinib (Table 2). Reticulocytes decreased below 30x10⁹/L in many patients, suggesting the anemia resulted from impaired erythrocyte production. Red cell aplasia was not seen on bone marrow biopsies performed at study entry and Darbopoietin improved the anemia in 9/13 patients (Table 2).

Despite variations in baseline measurements and magnitude of change, expression of phospho-STAT5, -STAT3, and its signature gene product miR-17 were consistently decreased by cycle 3 (Figure 1C), suggesting JAK1/2 had been inhibited *in vivo*.[2,3] Plasma levels of a number of chemokines and cytokines were changed by Ruxolitinib, including GCSF, which increased in all patients (Figure 1D).

The results of the trial suggest an important role for JAK/STAT signaling in CLL, particularly in the homing of CLL cells. Although the sample size was small, had an unusual sex distribution and unknown IgVH status, and almost a quarter had been splenectomized, Ruxolitinib

induced a stereotyped response in all patients consisting of a rapid decrease in lymphadenopathy coupled with increased lymphocytosis and plasma LDH levels (Figure 1A). These responses resemble those of Idelalisib and Ibrutinib in some ways. However, the sustained lymphocytosis and increased LDH levels (Figure 1A) are not features of BCR-inhibitors. Response durations with Ruxolitinib were also much shorter (Figure 1A), perhaps because the increase in factors such as GCSF (Figure 1D) counter growth-inhibitory effects of decreased miR-17 (Figure 1C) in the same way they mediate resistance to kinase inhibitors in other cancers.[14] The toxicity profile also differs, with suggestions that infectious risks and anemia are more severe with Ruxolitinib in CLL compared to other conditions.

Results with Ruxolitinib in 21 untreated and 15 previ-

ously treated patients not requiring systemic therapy were recently described.[15] Ruxolitinib was used at 10 mg BID for 3 months and significantly reduced fatigue, disease related symptoms, and β2M levels. WBCs increased and then decreased below baseline, suggesting a reduction in tumor burden. These observations are similar to our results after 3 cycles (Figure 1). However, high rates of anemia requiring transfusion (Table 2) and infections were not reported. Differences between these studies may reflect different patient populations or duration of treatment (3 vs. 7 months) with Ruxolitinib.

The powerful biologic activity of Ruxolitinib in CLL may be exploited by combining it with BCR-signaling inhibitors and a study is being planned to add Ruxolitinib to CLL patients with persistent disease despite Ibrutinib. Since Ruxolitinib-induced anemia and thrombocytopenia
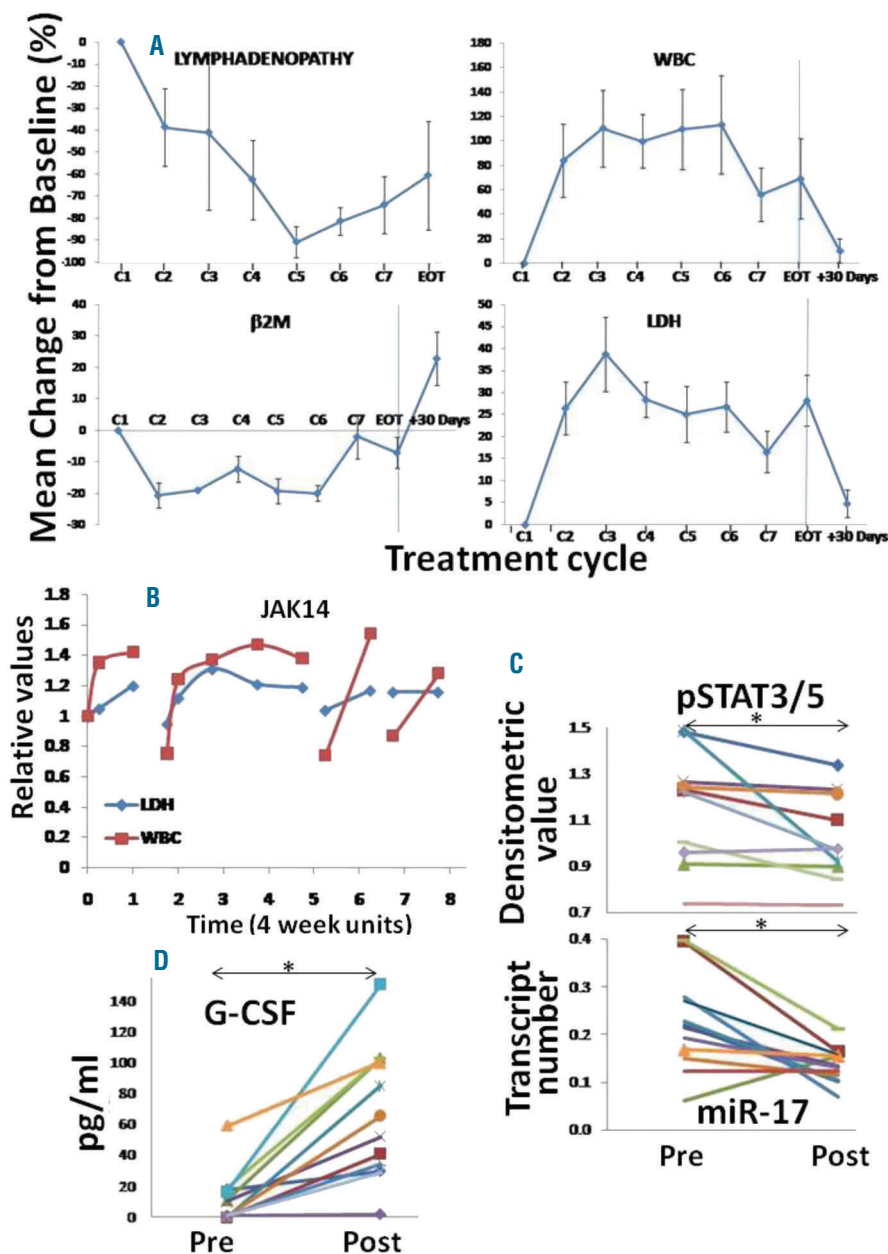

Figure 1. **Ruxolitinib-induced changes in adenopathy, lymphocytosis, LDH, β2M, phospho-STAT3/5, miR-17, and cytokines.** (A) A lymphadenopathy score defined as the sum of the products of bidimensional measurements of the largest palpable lymph nodes plus the length of the spleen in cm below the costal margin was recorded at each clinic visit. WBCs, β2M and LDH at each visit, at the end of treatment (EOT), and 30 days after stopping Ruxolitinib were also recorded. The average and standard error of the percent differences in each of these measurements from the baseline values at cycle 1 are shown for each time-point. (B) Clinical course of JAK14 where Ruxolitinib was held due to low platelet counts and restarted several weeks later. Circulating lymphocytes and LDH levels increased rapidly each time Ruxolitinib was started but returned to baseline levels or lower when Ruxolitinib was held. LDH and WBC counts are normalized to the values at cycle 1. Initial lymphocyte counts, β2M, and LDH values for each patient are shown in Table 1. (C) Upper panel: Phospho-proteins in CLL cells obtained pre-treatment at cycle 1 and post-treatment at cycle 3 were measured with kits (#ARY003B) from R&D (Minneapolis, MN, USA). These times were chosen since lymphadenopathy scores and β2M levels had clearly decreased by cycle 3 (Figure 1A). STAT5A/B phosphorylated at tyrosine residues 694 and Y699 and STAT3 phosphorylated at Y705 were quantified by densitometry and the sums of these values are indicated in the graph. Lower panel: miR-17 expression was measured at these times by quantitative real-time PCR as before.[3] (D) Plasma collected before each treatment cycle was analyzed by multiplex laser-bead technology (Eve Technologies, Calgary, AB). The summary graph indicates GCSF levels before treatment and the greatest change following treatment with Ruxolitinib. *P<0.05.

generally appeared in the 3rd and 4th weeks of each cycle and reversed within 2-3 weeks off treatment without apparently compromising therapeutic efficacy (Figure 1B), an intermittent dosing-strategy, shortened to 4 cycles, will be incorporated to lessen potential hematologic toxicities. Erythropoietin agonists and prophylactic antibiotics will also be used to limit transfusions (Table 2) and prevent infections.

David E. Spaner,[1,2,3,4,5] Guizhei Wang,[1] Lindsay McCaw,[1] Yanmei Li,[1] Patricia Disperati,[6] Mary-Ann Cussen,[3] and Yonghong Shi[1]

[1]Biology Platform, Sunnybrook Research Institute, Toronto; [2]Dept. of Medical Biophysics, University of Toronto; [3]Sunnybrook Odette Cancer Center, Toronto; [4]Dept. of Medicine, University of Toronto; [5]Dept. of Immunology, University of Toronto; and [6]Toronto East-General Hospital, Canada.

References

1. Woyach JA, Johnson AJ. Targeted therapies in CLL: mechanisms of resistance and strategies for management. Blood. 2015;126(4):471-477.
2. Tomic J, Lichty B, Spaner DE. Aberrant interferon-signaling is associated with aggressive chronic lymphocytic leukemia. Blood. 2011;117(9):2668-2680.
3. Li Y, Shi Y, McCaw L, et al. Microenvironmental interleukin-6 suppresses toll-like receptor signaling in human leukemia cells through miR-17/19A. Blood. 2015;126(6):766-778.
4. Steele AJ, Prentice AG, Cwynarski K, et al. The JAK3-selective inhibitor PF-956980 reverses the resistance to cytotoxic agents induced by interleukin-4 treatment of chronic lymphocytic leukemia cells: potential for reversal of cytoprotection by the microenvironment. Blood. 2010;116(22):4569-4577.
5. Harrison C, Kiladjian JJ, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med. 2012;366(9):787-798.
6. Del G, I, Mauro FR, Foa R. Chronic lymphocytic leukemia in less fit patients: "slow-go". Leuk Lymphoma. 2011;52(12):2207-2216.
7. Hallek M, Cheson BD, Catovsky D, et al. International workshop on chronic lymphocytic leukemia. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111(12):5446-5456.
8. Gehan EA. The determination of the number of patients required in a preliminary and a follow-up trial of a new chemotherapeutic agent. J Chronic Dis. 1961;13:346-353.
9. Eghtedar A, Verstovsek S, Estrov Z, et al. Phase 2 study of the JAK kinase inhibitor ruxolitinib in patients with refractory leukemias, including postmyeloproliferative neoplasm acute myeloid leukemia. Blood. 2012;119(20):4614-4618.
10. Byrd JC, Furman RR, Coutre SE, et al. Three-year follow-up of treatment-naive and previously treated patients with CLL and SLL receiving single-agent ibrutinib. Blood. 2015;125(16):2497-2506.
11. O'Brien S, Furman RR, Coutre SE, et al. Ibrutinib as initial therapy for elderly patients with chronic lymphocytic leukaemia or small lymphocytic lymphoma: an open-label, multicentre, phase 1b/2 trial. Lancet Oncol. 2014;15(1):48-58.
12. Spaner DE. Oral high-dose glucocorticoids and Ofatumumab in fludarabine-resistant Chronic Lymphocytic Leukemia. Leukemia. 2012; 26(5):1144-1145.
13. Heine A, Brossart P, Wolf D. Ruxolitinib is a potent immunosuppressive compound: is it time for anti-infective prophylaxis? Blood. 2013;122(23):3843-3844.
14. Ebos JM, Lee CR, Christensen JG, Mutsaers AJ, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. Proc Natl Acad Sci USA. 2007;104(43):17069-17074.
15. Jain P, Keating M, Cleeland C, et al. Ruxolitinib reduces cytokine/chemokine levels and improves fatigue and chronic lymphocytic leukemia (CLL)-related symptoms in CLL patients not requiring systemic therapy. Blood (ASH annual meeting abstracts). 2015;126(21):abstract#3310.