Autoimmune cytopenias in patients with chronic lymphocytic leukemia treated with ibrutinib

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Supplemental Information

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Contents

Supplemental Table 2
Supplemental Table 1. 2

Supplemental Methods 3
Cytokine/chemokine plasma level quantification. 3

Supplemental Figures 4
Supplemental Figure 1. 4

Supplemental Figure 2. 5

Supplemental Figure 3. 6

Supplemental Figure 4. 7

Supplemental Table

Supplemental Table 1. Summary of patient characteristics at start of ibrutinib treatment.

Characteristic	n=13
Age, years, median (range)	67 (54-71)
Gender, M:F	12:1
WBC, $x10^9$/L median (range)	48.8 (2.8-175.7)
ALC, $x10^9$/L median (range)	34.7 (2.2-168.3)
Hgb, g/dL, median (range)	10.7 (8-14.2)
Plts, $x10^9$/L median (range)	87 (32-189)
β2M, mg/L, median (range)	5.1 (1.8-11.2)
LDH >UNL, no. (%)	8 (61%)
IgG, mg/dL, median (range)	538 (116-1560)
DAT positive, no. (% of available)	6 (54%)
Rai stage, no. (%)	
0-II	2 (15%)
III-IV	11 (85%)
ZAP70 positive, no (% of available)	7 (64%)
CD38 positive, no. (%)	8 (61%)
IGHV unmutated, no. (% of available)	8 (80%)
FISH abnormalities, no. (%)	
Deletion 13q	12 (92%)
Trisomy 12	1 (8%)
Deletion 11q	3 (23%)
Deletion 17p	9 (69%)
TP53 mutated, no. (% of available)	3 (37%)
No. of prior treatments, median (range)	
CLL-directed	2 (0-7)
Autoimmunity-directed	1 (0-8)

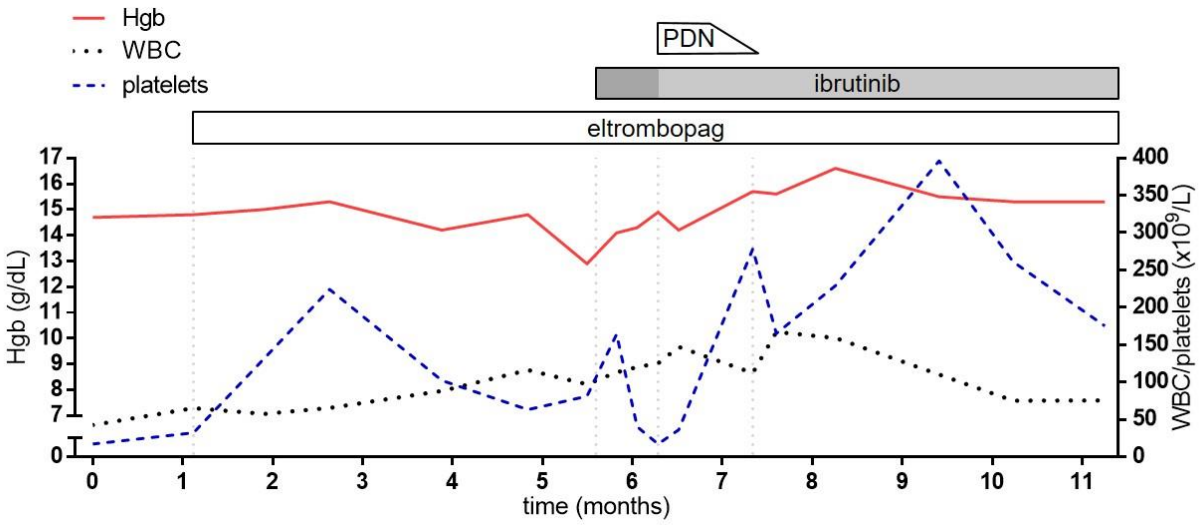
Abbreviations: β2M β2-microglobulin, ALC absolute lymphocyte count, DAT direct antiglobulin test, F female, FISH fluorescent in situ hybridization, Hgb hemoglobin, IGHV immunoglobulin heavy chain variable region, LDH lactate dehydrogenase, M male, Plts platelets, WBC white blood cell count

Supplemental Methods

Cytokine/chemokine plasma level quantification

Plasma levels of 21 T-cell related cytokines and chemokines were assessed in the plasma of four patients for whom sequential samples before and during ibrutinib treatment were available. Cytokines/chemokines were quantified by Milliplex human high sensitivity T cell assay and subsequently read out using a Luminex 100 plate reader, accordingly to the manufacturer's instructions (EMD Millipore, Billerica, MA, USA). The following cytokines/chemokines were tested: IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-17A, IL-21, IL-23, ITAC, GM-CSF, fractalkine, IFNγ, MIP-1b, MIP-1a, MIP-3a, TNFα. Cytokines with plasma concentrations lower than the detection thresholds of the assay were excluded from the analysis.

Supplemental Figures

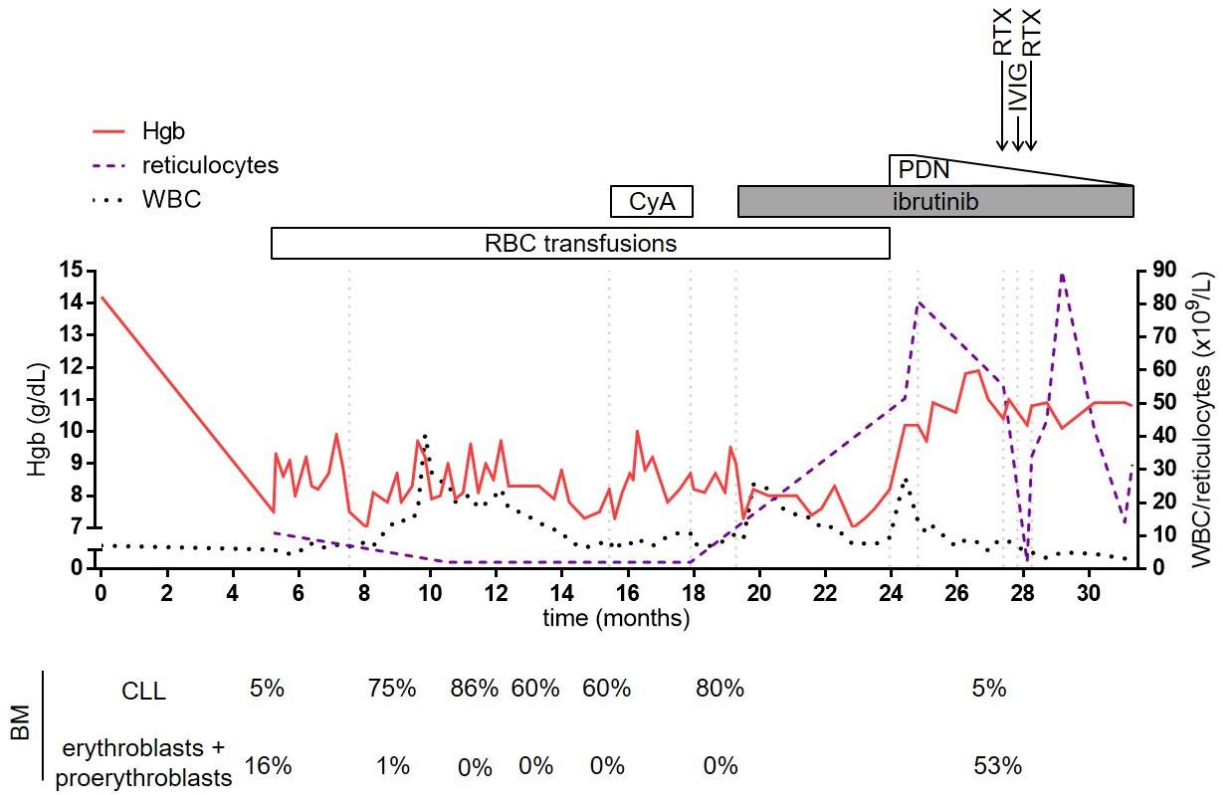


Supplemental Figure 1. Graphic representation of the clinical course of patient #1.

Patient #1 had known ITP which was controlled on eltrombopag at the time of ibrutinib initiation and flared during the first month of ibrutinib. Thrombocytopenia resolved after the dose reduction of ibrutinib and an addition of GC taper.

Lines represent the trend of hemoglobin (red), platelets (blue), and WBC (black) over time. The treatments administered are displayed in the upper section of the figure. The two different shades of gray in the ibrutinib field represent a dose reduction of the drug.

Abbreviations: Hgb: hemoglobin, WBC: white blood cells, PDN: prednisone.

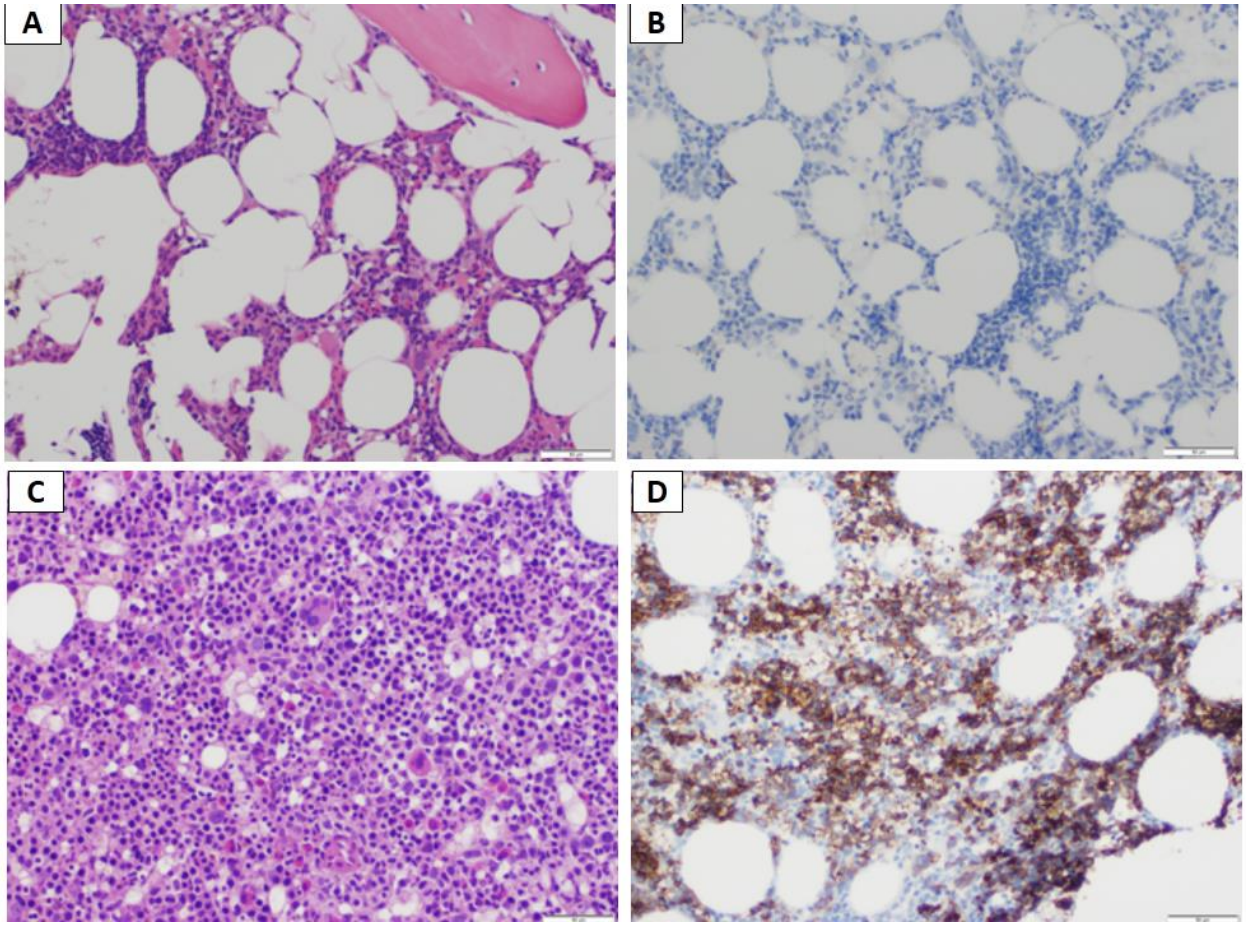


Supplemental Figure 2. Graphic representation of the clinical course of patient #2.

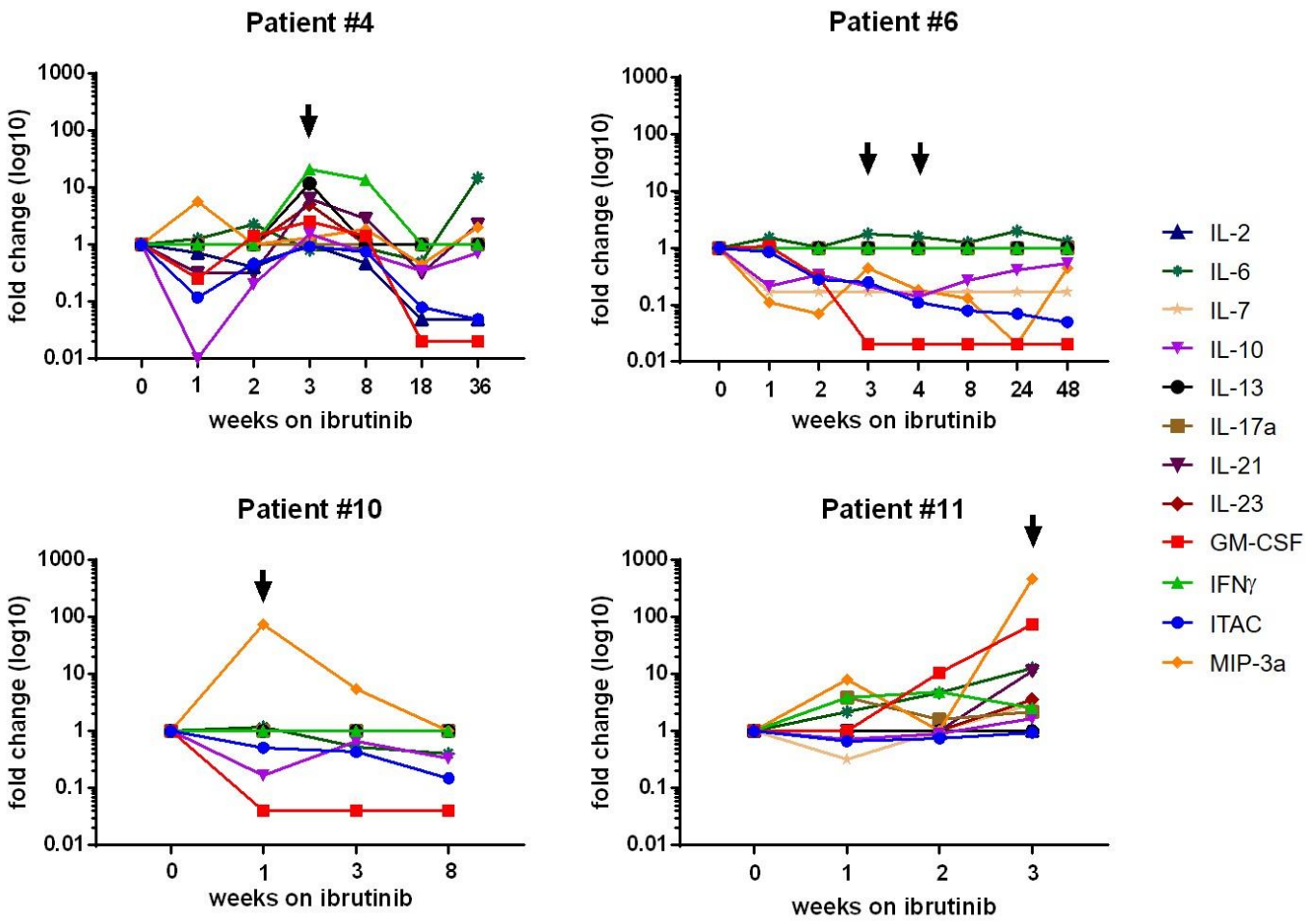
Patient #2 was diagnosed with transfusion-dependent PRCA unresponsive to cyclosporine treatment. Ibrutinib alone did not modify transfusion dependence. Nevertheless, the addition of GC, rituximab and IVIG controlled the autoimmune process, and subsequently transfusion-independence was achieved.

Lines represent the trend of hemoglobin (red), reticulocytes (purple), and WBC (black) over time. The treatments received are represented in the upper section of the figure. The percentage of CLL cells, erythroblasts and pro-erythroblasts detected in bone marrow biopsies over time are depicted in the lower section of the figure.

Abbreviations: Hgb hemoglobin, WBC white blood cells, CyA cyclosporine A, PDN prednisone, RTX rituximab, IVIG intravenous immunoglobulins, RBC red blood cells, BM bone marrow.



Supplemental Figure 3. Representative sections of sequential bone marrow biopsies of patient #2. Bone marrow biopsy performed at the time of PRCA diagnosis demonstrated scattered lymphoid aggregates and virtually no erythroid precursors (A); the lack of erythroid precursors was confirmed by immunohistochemical stain with anti-CD71 antibody (B). Bone marrow biopsy performed approximately 8 months after ibrutinib treatment initiation detected recovery of erythroid elements with erythroid hyperplasia (C); numerous erythroid precursors are highlighted by anti-CD71 antibody (D). (A) and (C)– Hematoxylin and Eosin; (B) and (D) – CD71 immuno-histochemical stain; all photomicrographs were taken at 200X magnification.



Supplemental Figure 4. Cytokine/chemokine quantification in plasma of patients treated with ibrutinib. Cytokines/chemokines were quantified in the plasma samples of four patients at different time points before and during ibrutinib treatment. Two patients (patients #4 and #11) demonstrated peaks of plasma concentrations of different cytokines, such as IFNγ, IL-21, and IL-23, at the timepoints corresponding to the clinical autoimmune flares. Patient #10 showed a clear increase of MIP-3a at the time of autoimmune flare, whereas patient #6 showed no significant cytokine increase. Data are expressed as fold change on a log scale compared to baseline. Arrows indicate the time of the clinically evident autoimmune flare.