Autoimmune cytopenias in patients with chronic lymphocytic leukemia treated with ibrutinib

Autoimmune cytopenias (AIC) are common in chronic lymphocytic leukemia (CLL) and include autoimmune hemolytic anemia, immune thrombocytopenia and, less frequently, pure red blood cell aplasia. It is generally accepted that treatment of CLL-associated AIC should be primarily directed against the autoimmune phenomenon, and CLL-specific therapy is reserved for refractory cases or for patients with additional signs of disease progression.[1]

Ibrutinib, a small-molecule inhibitor of Bruton tyrosine kinase, induces durable remissions in patients with CLL.[2] The activity of ibrutinib in CLL-associated AIC is largely unknown, partly because of the exclusion of patients with uncontrolled AIC from the pivotal clinical trials. Recently, episodes of autoimmune hemolytic anemia during ibrutinib treatment have been described,[3,4] but low incidences of treatment-emergent AIC were found in two different prospective trials (no cases in 195 patients and 6 cases in 301 patients).[5,6] Moreover, the successful management of autoimmune hemolytic anemia with ibrutinib, alone or in association with glucocorticoids, has been described in a few case reports.[5,7-9]

We identified 13 patients with CLL who were treated with ibrutinib and had signs of AIC at the time of treatment initiation, and we studied their clinical presentation, management, and outcome. The patients were treated at The University of Texas MD Anderson Cancer Center (Houston, TX, USA) or at the National Institute of Health (Bethesda, MD, USA). All patients provided informed consent in accordance with the Declaration of Helsinki. Nine patients were enrolled in clinical trials (4 in trial NCT02007044 and 5 in trial NCT01500733), of whom three received ibrutinib in combination with rituximab (weekly infusions during cycle 1 and monthly infusions in cycles 2-6 at a dose of 375 mg/m^2, as previously reported[10]).

Autoimmune hemolytic anemia was defined as anemia without other evident alternative causes, associated with at least one laboratory sign of hemolysis (increased unconjugated bilirubin, elevated lactate dehydrogenase, reduced haptoglobin), and either an increased reticulocyte count or a positive direct antiglobulin test. We defined immune thrombocytopenia as a low platelet count not explained by other clear causes, and with increased or normal megakaryocytes on bone marrow biopsy. Given the difficulty of diagnosing immune thrombocytopenia in the setting of CLL, we considered clinical responses to immune thrombocytopenia-directed treatment as supportive evidence for the diagnosis. Pure red blood cell aplasia was diagnosed in the presence of anemia without signs of hemolysis and with concomitant absence of erythroid precursors in the bone marrow and blood.

AIC was defined active when it was not controlled by the current medical management, controlled when blood counts were maintained stable but not normalized (or, for autoimmune hemolytic anemia, when hemoglobin concentration was normalized but signs of subclinical hemolysis persisted), and resolved when complete normalization of blood counts occurred. We defined a flare of AIC as a sudden reactivation of the autoimmune process after a period of stable peripheral blood counts.

The patients' characteristics at the time of starting ibrutinib treatment are shown in Table 1 and *Online Supplementary Table S1*. The majority of patients had adverse prognostic features (i.e. unmutated immunoglobulin heavy chain variable region, unfavorable cytogenetics, high CD38 expression and/or positive ZAP70 expression), consistent with previous literature reporting an increased incidence of AIC in high-risk CLL.[11,12] Ten patients (77%) had previously received treatment for CLL. The reason for ibrutinib initiation was CLL progression and not AIC in all patients.

Figure 1 and Table 2 summarize the clinical course of the patients. All patients had a previous history of AIC, and eight (62%) had received prior therapy for AIC. At the start of ibrutinib treatment the autoimmune phenomenon was controlled without treatment in seven patients (54%), controlled with a specific treatment in three patients (23%), and active in three patients (23%).

In nine patients (69%), including the three patients who were receiving ibrutinib in combination with rituximab, we observed a common pattern, consisting of a flare of the underlying AIC during the first weeks of ibrutinib therapy (median time to AIC flare 3 weeks, range 2-8 weeks). Infectious events did not trigger AIC in these patients. The severity of the flares differed substantially among patients and each patient was managed at the discretion of the treating physician. Four of these nine patients (44%) continued with the original dose of ibrutinib and began additional treatments (glucocorticoids in 2 patients, glucocorticoids plus eltrombopag in 1 patient, glucocorticoids plus intravenous immunoglobulins in 1 patient). In two patients (22%) the dose of ibrutinib was reduced, and tapered administration of glucocorticoids was added (e.g. patient #1, *Online Supplementary Figure S1*). In one patient the dose of ibrutinib was temporarily reduced, and later increased back to 420 mg in association with rituximab and glucocorticoids, and in another patient ibrutinib was withheld and then restarted at a reduced dose (140 mg QD) after the cytopenia had been controlled with glucocorticoids and intravenous immunoglobulins. In one patient ibrutinib was permanently discontinued at the time of the autoimmune flare.

In six out of these nine patients (67%), treatment was able to stabilize and subsequently resolve the autoimmune process. In the remaining three patients, peripheral blood counts stabilized and only mild cytopenia persisted.

Among the cases that followed a different pattern, not manifesting with an AIC flare, patient #2 (*Online Supplementary Figure S2*) is particularly interesting. This patient was diagnosed with transfusion-dependent pure red blood cell aplasia, unresponsive to cyclosporine treatment. Improvement in hemoglobin levels and transfusion independency were achieved with ibrutinib in combination with glucocorticoids, rituximab and intravenous immunoglobulins, and were accompanied by the recovery of erythroid precursors in the bone marrow (*Online Supplementary Figure S3*). Patient #3 also had transfusion-dependent pure red blood cell aplasia with laboratory evidence of autoimmune hemolytic anemia, and started ibrutinib while on cyclosporine. His reticulocyte count and erythroid hypoplasia improved during ibrutinib therapy and the patient became transfusion-independent with persistent laboratory evidence of hemolysis.

In the remaining two patients (17%), no autoimmune flare was noted, and treatment with ibrutinib alone was sufficient to treat and resolve the autoimmune phenomenon with normalization of the peripheral blood cell counts.

Interestingly, in three of the patients with autoimmune hemolytic anemia who responded to treatment with ibrutinib, seroconversion to a negative direct antiglobulin test

Table 1. Patients' characteristics at start of ibrutinib treatment.

#	Age	Gender	Comorbidities	WBC (x10^9/L)	ALC (x10^9/L)	Hb (g/dL)	Plts (x10^9/L)	β_2M (mg/L)	LDH (IU/L)	IgG (mg/dL)	DAT	Rai stage	ZAP70	CD38	IGHV	FISH	TP53	Prior treatment(s)
1	54	m	–	97.2	89	12.9	88	1.8	537#	116	neg	4	neg	neg	U	del13q	WT	FCR + GC, RCD, IVIG, splenectomy, eltrombopag
2	70	m	HTN, upper GI ulcerations, actinic keratosis	10.5	6.06	9	74	2.6	443#	827	neg	4	pos	pos	U	del13q	WT	FCR, PI3Kγ/δ inhibitor, CyA
3	67	m	Seizures, GERD	154	146	8	47	8.7	416*	538	pos (IgG)	4	neg	neg	U	del13q del17p	NA	Fludarabine, GC, CyA, PCR, rituximab, rituximab and GC, R-EPOCH + alloSCT (graft failure), BR, ofatumumab, CyA, auranofin
4	68	m	HTN	48.8	45.9	10.2	87	6.7	1083#	190	pos (IgG)	4	pos	neg	NA	del11q del13q del17p	mut	FCR, CFAR lenalidomide maintenance, rituximab and GC, ofatumumab, rituximab and GC, eltrombopag, RCD, SCR, rituximab and GC, hyperCVAD, OFA
5	60	m	DM, HTN, hypothyroidism, fibromyalgia, sleep apnea	175.6	168	10.3	189	3.5	211*	486	NA	3	NA	neg	U	del11q del13q del17p	NA	None
6	61	m	HTN, HLD, GC induced DM, GERD	50.3	34.68	14.1	129	5.2	552#	345	neg	1	pos	pos	NA	del13q del17p	WT	GC, IVIG, splenectomy, rituximab, cyclophosphamide, rituximab and GC, eltrombopag
7	70	m	HTN, post-traumatic splenectomy	31.7	27.3	14.2	32	11.2	956#	419	NA	4	neg	neg	NA	del13q del17p	mut	FCR, lenalidomide and ofatumumab
8	63	m	HTN	26.1	22.7	10.4	36	NA	758#	471	neg	4	neg	pos	U	tris12	WT	GC, GC and rituximab
9	58	f	hypothyroidism, GERD, hiatal hernia	88.9	83	10.7	164	5	302*	1560	pos (IgG)	3	pos	pos	U	del13q del17p	NA	None
10	70	m	HLD	51.2	50.2	8.2	88	5	679#	854	neg	4	pos	pos	M	del13q	WT	Rituximab
11	68	m	HTN, BPH, GC-induced DM	2.8	2.2	12.2	66	6.5	576#	1077	pos (IgG)	4	pos	pos	U	del11q del13q del17p	mut	FCR, GC, alemtuzumab, IVIG and GC
12	56	m	DLBCL, HTN, ADHD, insomnia, depression	4.57	3	11.4	57	7.1	428*	1380	pos (IgG)	4	NA	pos	M	del13q del17p	NA	Rituximab and GC, CAR T cells, lenalidomide
13	69	m	HTN, HLD, CAD, BPH	13.6	12	12	129	2.2	348*	384	pos (IgG)	2	pos	neg	U	del13q del17p	NA	GC

*β_2M: β_2-microglobulin; ADHD: attention deficit hyperactivity disorder; ALC: absolute lymphocyte count; alloSCT: allogeneic stem cell transplant; BPH: benign prostatic hyperplasia; BR: bendamustine and rituximab; CAD: coronary artery disease; CAR T: CD19 chimeric antigen receptor-modified T cells; CFAR: cyclophosphamide, fludarabine, alemtuzumab and rituximab; CyA: cyclosporine A; DAT: direct antiglobulin test; DM: diabetes mellitus; DLBCL: diffuse large B cell lymphoma; f: female; FCR: fludarabine, cyclophosphamide and rituximab; FISH: fluorescent in situ hybridization; GC: glucocorticoids; GERD: gastroesophageal reflux disease; GI: gastrointestinal; Hb: hemoglobin; HLD: hyperlipidemia; HTN: hypertension; hyperCVAD: hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone; IGHV: immunoglobulin heavy chain variable region; IVIG: intravenous immunoglobulin; LDH: lactate dehydrogenase; M: IGHV mutated; m: male; mut: mutated; NA: not available; neg: negative; OFA: oxaliplatin fludarabine, and cytarabine; PCR: pentostatin, cyclophosphamide rituximab; PI3Kγ/δ: phosphatidylinositol 3-kinase γ/δ; Plts: platelets; pos: positive; R-EPOCH: rituximab, etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin; RCD: rituximab, cyclophosphamide and dexamethasone; SCR: sapacitabine, cyclophosphamide and rituximab; U: IGHV unmutated; WBC: white blood cell count; WT wild type. LDH normal range: #313-618 UI/L, *113-226 UI/L.*

was observed after 9-12 months on ibrutinib (patients #9, #12, and #13). These heterogeneous clinical scenarios reflect the variable clinical phenotypes of AIC in CLL and the absence of a standard therapy in the era of kinase inhibitors. Based on our experience, most patients were successfully managed by continuation of ibrutinib and temporary addition of a conventional therapy (i.e., glucocorticoids, intravenous immunoglobulins) to target the autoimmune process. Overall, in nine of the 13 patients (69%), the AIC had been resolved or controlled with ibrutinib treatment only at the time of the patients' last follow-up.

To assess the CLL response to ibrutinib therapy, we used the International Workshop on Chronic Lymphocytic Leukemia-National Cancer Institute guidelines.[1] A persistently elevated lymphocyte count in patients otherwise categorized as in partial response was not considered a sign of

Table 2. Autoimmune cytopenia characteristics, management, and outcome in CLL patients treated with ibrutinib.

#	History of AIC	AIC status at ibrutinib start	Treatment	AIC flare (weeks on ibrutinib)	Clinical course and management	AIC outcome	CLL response	Follow-up (months)
1	ITP	Controlled with eltrombopag	Ibrutinib 420 mg	3-4	At ITP flare (plts 17x10⁹/L) ibrutinib reduced to 140 mg and GC taper added. Continued eltrombopag.	Resolved	PRL	8
2	PRCA	Active, RBC transfusion dependent	Ibrutinib 420 mg	–	CLL responded but patient remained transfusion dependent. Treated with GC taper, rituximab, and IVIG.	Controlled without transfusions	PR	12
3	AIHA, ITP, PRCA	Active, on cyclosporine, transfusion dependent	Ibrutinib 420 mg	–	Gradual PRCA improvement (increased reticulocytes, decreased erythroid hypoplasia). Continued cyclosporine. Ibrutinib finally withheld due to multiple complications, including Richter transformation.	Controlled without transfusions	PR	8
4	AIHA, ITP	Controlled without treatment	Ibrutinib 420 mg + rituximab	3-4	At AIHA and ITP flare (nadir Hb 5.1 g/dL, plts 37x10⁹/L) treatment with GC taper and IVIG.	Resolved	SD	16
5	AIHA	Controlled without treatment	Ibrutinib 420 mg	4-8	Hb stable, but worsening of hemolytic parameters (haptoglobin undetectable, increased bilirubin, DAT positive). Treated with GC taper.	Resolved	PR	21
6	ITP	Controlled with IVIG and eltrombopag (discontinued at ibrutinib initiation)	Ibrutinib 420 mg + rituximab	4	ITP flare (plts 45x10⁹/L) treated with GC taper.	Resolved	PRL	14
7	ITP	Active	Ibrutinib 140 mg (due to drug interaction)	4	Initial improvement of platelet count followed by ITP flare (plts 18x10⁹/L). Treated with GC taper and eltrombopag.	Controlled on eltrombopag	SD	7
8	AIHA, ITP	Controlled without treatment	Ibrutinib 420 mg	3	Platelet count improved but developed hyporegenerative anemia (Hb 5 g/dL). Ibrutinib temporarily reduced to 140 mg QD. Diagnosis of PRCA. Ibrutinib full dose restored and treated with rituximab and GC taper.	Resolved	CR	6
9	AIHA	Controlled without treatment	Ibrutinib 420 mg	5	At AIHA flare (nadir Hb 7.7 g/dL) ibrutinib reduced to 280 mg and GC taper added.	Controlled	PR	36
10	Possible ITP	Controlled without treatment	Ibrutinib 420 mg + rituximab	2	ITP and AIHA flare (Hb 6.1 g/dL, plts 4 x10⁹/L). Ibrutinib held, treated with GC taper and IVIG. Ibrutinib resumed at reduced dose (140 mg).	AIHA resolved, ITP controlled	SD	4
11	AIHA, ITP	Controlled without treatment	Ibrutinib 420 mg	3	At AIHA flare (Hb 3.3 g/dL) ibrutinib held and never resumed. AIHA treated with GC taper, cyclophosphamide and rituximab, splenectomy.	Resolved	–	12
12	AIHA, ITP	Controlled without treatment	Ibrutinib 420 mg	–	Gradual recovery of anemia and thrombocytopenia.	Resolved	PRL	33
13	AIHA	Controlled with GC	Ibrutinib 420 mg	–	Gradual improvement of Hb, GC independence obtained.	Resolved	PR	18

The follow-up duration is calculated from the start of ibrutinib treatment. AIC: autoimmune cytopenia; AIHA: autoimmune hemolytic anemia; BM: bone marrow; CR: complete response: GC: glucocorticoids; Hb: hemoglobin; IVIG: intravenous immunoglobulin; ITP: immune thrombocytopenia; Plts: platelets; PR: partial response; PRCA: pure red cell aplasia; PRL: partial response with lymphocytosis; RBC: red blood cells; SD: stable disease.

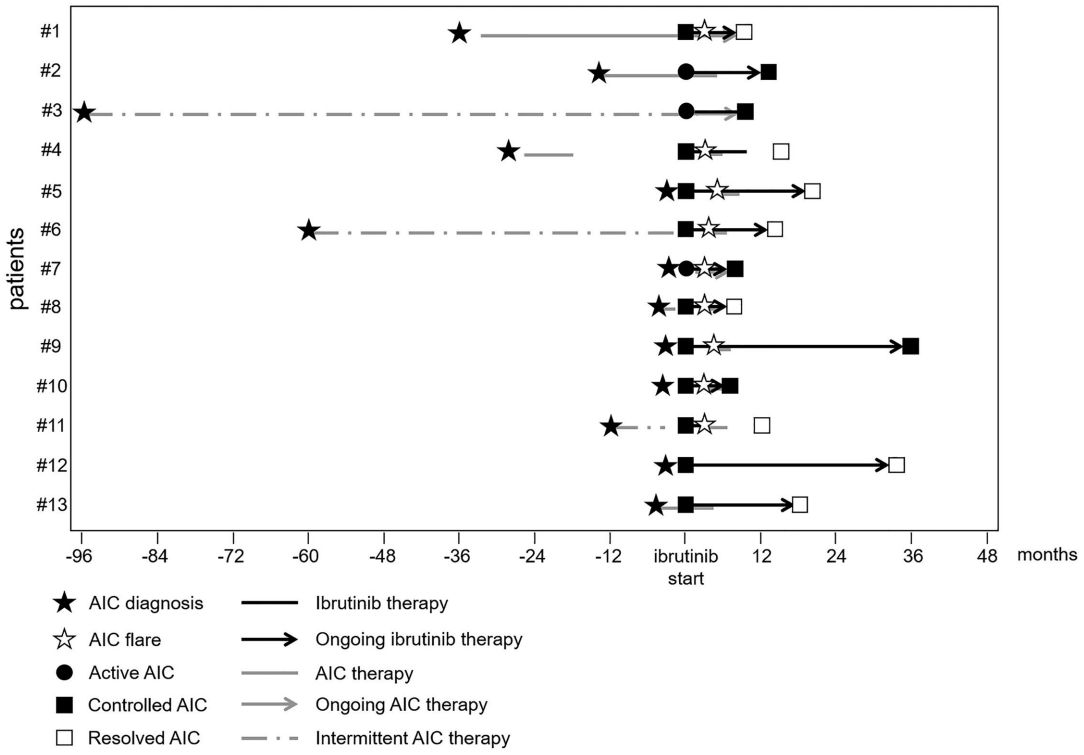

Figure 1. Graphic representation of ibrutinib and AIC therapy for each patient of the cohort.

disease progression.[13] Nine of 13 patients (69%) responded to the treatment: one patient (8%) had a complete response unconfirmed by bone marrow biopsy, five patients (38%) had a partial response, and three patients (23%) achieved a partial response with persistent lymphocytosis. Three patients had stable disease: one of these three discontinued ibrutinib after 9 months due to lack of response (patient #4), while the other two continued treatment after 4 and 7 months, respectively. One patient was not evaluable for response due to early discontinuation (patient #11). Overall, ten patients (77%) were taking ibrutinib at the time of the data analysis (median follow-up since starting ibrutinib therapy: 13 months, range 4-36). These data are in line with the notion that the treatment of the underlying CLL is critical for the long-term control of CLL-associated AIC.

The impact of ibrutinib on AIC in CLL is of particular interest because inhibition of Bruton tyrosine kinase can have pleiotropic effects on different components of the immune system.[14] Ibrutinib also functions as an inhibitor of interleukin-2-inducible kinase and can promote a shift from Th2- towards Th1-polarized immunity.[15] To gain more insights into plasma cytokine changes during autoimmune flares, we analyzed the plasma concentrations of 21 different cytokines in four of the patients from our series (*Online Supplementary Methods*). Overall, the results were heterogeneous (*Online Supplementary Figure S4*), but two patients (patients #4 and #11) had peaks of plasma concentrations of different cytokines, such as intereferon-γ, interleukin-21, and interleukin-23, at the time of the autoimmune flares. However, there was no significant bias towards Th1- over Th2-specific cytokines. Studies in larger populations of patients are needed to better define the modulatory activity of ibrutinib on inflammatory cytokines, and their potential role in triggering AIC.

In conclusion, AIC remain a relevant complication of CLL in the era of targeted therapies, such as ibrutinib, and we anticipate that a wider use of ibrutinib and other kinase inhibitors in CLL therapy will change the overall incidence of AIC and influence our therapeutic strategies.

Because of the observed pattern of AIC flares after the start of ibrutinib in our series, we recommend close monitoring of peripheral blood counts and parameters of hemolysis in patients with a history of AIC during the first weeks of treatment, in order to promptly detect and treat any AIC reactivation. It is important to note that most patients in our series continued to take ibrutinib with or without the transient addition of other therapies targeted towards the autoimmune component of the disease. This strategy ultimately resulted in control and/or resolution of autoimmunity, and generally permitted patients to transition to long-term single-agent ibrutinib treatment.

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