Experience with ibrutinib for first-line use in patients with chronic lymphocytic leukemia

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***Abstract*:** Ibrutinib is the first in-class, orally administered, Bruton's tyrosine kinase (BTK) inhibitor that abrogates the critical signaling downstream of the B-cell receptor (BCR). This signaling is required for B-cell survival, proliferation and interaction with the microenvironment. Ibrutinib proved active in preclinical models of lymphoproliferative diseases and achieved impressive response rates in heavily pretreated relapsed and refractory (R/R) patients with chronic lymphocytic leukemia (CLL). Ibrutinib prolonged survival compared to standard therapy and mitigated the effect of most poor prognostic factors in CLL, thus becoming the main therapeutic option in high-risk populations. Moreover, compared with standard chemoimmunotherapy (CIT) for adults, ibrutinib causes fewer cytopenias and infections, while having its own unique toxicity profile. Its efficacy in relapsed patients as well as its tolerability have led to its increased use in previously untreated patients, especially in those with poor prognostic markers and/or the elderly. This review elaborates on ibrutinib's unique toxicity profile and the mechanisms of acquired resistance leading to progression on ibrutinib, since both are critical for understanding the obstacles to its first-line use. We will further evaluate the data from ongoing clinical trials in this setting and explore future options for combination therapy.

Keywords: chronic lymphocytic leukemia, ibrutinib, first-line

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Introduction

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is the most common leukemia in Western countries, with an incidence of 4.6 per 100,000 people per year in the US, which in 2016 translated to about 19,000 new patients, mostly older adults, with a median age at diagnosis of 71 years.[1] Since time to first treatment (TTFT) is about 5 years from diagnosis,[2,3] most CLL patients nearing treatment are indeed elderly, have multiple comorbidities and take several prescription drugs, further complicating their management.[4]

CLL has a widely variable course: some patients have indolent disease not requiring treatment for decades, whereas others are affected by aggressive disease demanding immediate treatment.[5] While many prognostic factors affect disease course,[3,6] the most pertinent ones are an unmutated immunoglobulin heavy-chain variable region rearrangement (UM-*IGHV*) and deletion of the short arm of chromosome 17 (del17p) demonstrated by fluorescence in-situ hybridization (FISH) and/or TP53 mutations.[3] These adverse factors influence not only aggressiveness of disease and TTFT, but also response to chemoimmunotherapy (CIT) and progression-free survival (PFS), promoting the concept of risk-stratified treatment.

Current first-line regimens for CLL

The CLL management paradigm has considerably changed over the last decade: from variably effective alkylating agent-based therapy; through potent albeit toxic CIT regimens; to an era of highly effective small, targeted molecules.

The current standard CIT regimen for previously untreated elderly unfit patients, consistent with the German CLL Study Group CLL11 trial, is a combination of chlorambucil with

obinutuzumab, a type II glycoengineered humanized anti-CD20 antibody, resulting in an overall response rate (ORR) of 77%; median PFS of 29 months; and time to next treatment (TTNT) of 51 months. This regimen demonstrated statistically significantly superior ORR, complete response (CR), minimal residual disease (MRD) and PFS compared with either chlorambucil alone or in combination with rituximab, and a trend toward increased overall survival (OS) compared to chlorambucil–rituximab.[7–9] Other common treatment options in elderly patients include bendamustine with rituximab (BR)[10]; and ofatumumab, another human monoclonal anti-CD20 antibody, which is FDA approved for relapsed and refractory (R/W) CLL either alone or in combination with bendamustine, and for untreated CLL in combination with chlorambucil.[11–14] Patients with high-risk CLL features, however, and specifically del17p or mutated TP53, have a lower response rate and shorter PFS and OS with these regimens.

The most efficient CIT for young, fit patients is fludarabine–cyclophosphamide–rituximab (FCR), demonstrating an ORR of 90% and median PFS of 52 months in the CLL8 trial.[15] Long-term follow-up results from this trial (median 5.9 years) established the inferior outcomes of patients with del17p, where PFS was only 15% at 5 years and OS 36%. However, it corroborated the superior results in patients with M-*IGHV*, two-thirds of whom were free from progression at 5 years, compared to only one-third with UM-*IGHV*.[16] In the MD Anderson phase II trial, with an even longer follow up of 12.8 years, the PFS curve had plateaued in patients with M-*IGHV*, 54% of whom were still in remission beyond 10.4 years, suggesting potential cure with FCR in this group of patients. Those with UM-*IGHV*, though, had a median PFS of 4.2 years, and as in other reports, del17p was an independent adverse factor in multivariable analysis.[17] A retrospective Italian analysis of 404 patients after first-line FCR demonstrated patients carrying M-*IGHV*, but not del11q or del17p, had similar 5-year survival as the matched normal population.[18]

Unfortunately, CIT may not only be ineffective in patients with 17p aberration, but is also associated with expansion of subclones with high-risk genetic abnormalities[19–21] and an increased risk of secondary cancers, including treatment-related myelodysplasia and acute myeloid leukemia.[22,23] Furthermore, effective therapeutic options in R/R del17p patients were quite limited, including the anti-CD52 antibody alemtuzumab[24] with high-dose steroids[25]; ofatumumab-based therapy[26]; and allogeneic stem cell transplantation in eligible patients.[27,28] These concerns have driven interest in active non-chemotherapeutic therapies.

Ibrutinib

Mechanism of action

Bruton's tyrosine kinase (BTK) is a member of the TEC family of kinases that is predominantly expressed in B-lymphocytes, signaling downstream of the B-cell receptor (BCR). Loss of BTK function in humans leads to the disease X-linked agammaglobulinemia (XLA), a primary immunodeficiency characterized by inhibition of B-cell development and immunoglobulin production.[29,30] The survival of many B-cell malignancies, and specifically CLL, is dependent on BTK-mediated signals from the BCR,[31–33] rendering selective BTK inhibition an attractive approach for these diseases. Upon activation of the BCR, Src-family kinases, mainly LYN and SYK, are activated by phosphorylation. This leads to phosphorylation and activation of BTK, which is a critical element in this pathway. Phospholipase-Cγ2 (PLCG2) is a substrate for BTK that, once activated, generates inositol-triphosphate (IP3) and diacylglycerol (DAG), leading to calcium mobilization and activation of downstream effectors such as protein kinase-Cβ (PKCβ), further activating downstream pathways including nuclear factor-kB (NFkB) and mitogen-activated protein kinase (MAPK).[34–36] BTK is also involved in signaling of other cell-surface receptors, such as the CXCR4 and CXCR5 chemokine receptors, and integrin-mediated B-cell trafficking and tissue homing.[37]

Ibrutinib (PCI-32765) is the first-in-class once-daily orally administered potent inhibitor of BTK. It binds covalently to a cysteine residue (Cys-481) in the BTK active site, resulting in sustained inhibition of the enzyme.[38] When added to whole blood, it blocks BCR signaling in human peripheral B-cells. This correlated with occupancy of the BTK active sites, monitored *in vitro* and *in vivo* using a fluorescent affinity probe for BTK.[38] Further preclinical models show that ibrutinib inhibits CLL cell survival and proliferation in primary CLL cells.[33]

Ibrutinib also interferes with the interactions between CLL cells and the microenvironment, as clinically evident by initial lymphocytosis in treated patients, secondary to mobilization of CLL cells to peripheral blood.[39] It decreases CLL cell migration-response toward tissue homing chemokines (CXCL12 and CXCL13) and down-regulates secretion of BCR-dependent chemokines by CLL cells, both in coculture of primary cells with nurse-like cells (NLCs) and as measured in plasma of patients treated with ibrutinib.[40,41] However, ibrutinib does not correct the fundamental dysfunction of NLCs in CLL, allowing them to maintain niches for CLL propagation and facilitate cases of ibrutinib resistance.[42] Gene expression profiling of CLL cells during response to ibrutinib demonstrated downregulation of a plethora of genes of potentially relevant function both 7 and 28 days after initiation of therapy, including the chemokine CCL3; the transcriptional regulators EGR1, 2 and 3; and CD72.[43] In a separate study exploring differential gene expression, ibrutinib significantly turned down multiple gene signatures (e.g. KEGG pathways: cytokine signaling, cell adhesion, p53 response, MAPK signaling and WNT signaling), while no pathways were significantly enriched.

Ibrutinib inhibits 22 kinases other than BTK, sometimes quite potently.[38] This may explain some of ibrutinib's toxicity, which can be partially mitigated by more specific novel BTK-inhibitors.[44,45] Examples of other kinases targeted by ibrutinib include the interleukin-2 inducible kinase (ITK) on T-cells that ibrutinib irreversibly targets, thus specifically causing diminished Th2-based immune responses[46]; epidermal growth factor receptor (EGFR), perhaps contributing to skin and GI toxicity[47]; and other TEC family proteins, which may be the reason for bleeding diathesis,[48,49] as will be discussed below.

Pharmacokinetically, ibrutinib is rapidly absorbed, reaching maximum plasma concentration within 1–2 h, and has a half-life of 4–6 h. Exposure to the drug increases linearly between 420 mg and 840 mg, but since efficacy in CLL was similar between doses, the lower dose was approved.[38,50] Metabolism of ibrutinib occurs primarily through CYP3A4 and it is principally excreted via the feces (10% in urine), mostly in metabolite form; thus no dose modification is necessary for patients with moderate chronic kidney disease. One should avoid co-administration with moderate-to-strong inhibitors or inducers of CYP3A4.[51]

Ibrutinib clinical data in the relapse setting

The FDA first approved ibrutinib for CLL patients previously treated with at least one prior therapy in February 2014, based on preliminary results of a multicenter single-arm trial of 85 patients with R/R disease. Fifty-one patients in this trial received the currently approved dose in CLL of 420 mg/day, which had similar response rates as the higher 840 mg daily dose. The median age in that trial was 66 years; patients had received four prior therapies (range, 1–12, 95% of which included a nucleoside-analogue); 81% had UM-*IGHV* and one-third del17p.[50] With an ORR of 89% (including 18% PR with lymphocytosis) and PFS of 75% at 26 months, including del17p high-risk patients, the FDA granted ibrutinib accelerated approval in relapsed CLL. These results were validated in the phase III RESONATE trial that randomly assigned 391 R/R CLL patients to either ibrutinib or ofatumumab,[52] and had a similar patient population. After only a short median follow up of 9 months, there was a clear PFS benefit to ibrutinib over ofatumumab; and by 12 months there was an OS benefit (90% *versus* 81%).

In these studies and others, there was a unique pattern of response to kinase-inhibitors. Concomitantly with a rapid reduction in lymph-node size, marked lymphocytosis appeared, peaking at 4 weeks, and declining steadily thereafter over a course of 6–14 months in the majority of patients.[50] This was referred to as 'partial response with lymphocytosis', which was recognized as a new type of response that did not reflect progressive disease (PD). Moreover, while most patients attain partial response gradually over the first 18 months, CR rate remains low at $<10\%$.[50,52,53] In a phase II trial of 144 R/R CLL patients strictly with del17p (RESONATE-17), 92% of whom also had TP53 mutation, the ORR was 83% after a median follow up of 27.6 months; PFS and OS at 24 months were 63% and 75%, respectively – much higher than historical comparators in this population.[54]

Combining ibrutinib with rituximab was next, with the intent to mitigate persistent lymphocyte redistribution and potentially deepen response. A phase II trial from MD Anderson recruited 40 high-risk predominantly relapsed patients, except for four who were previously untreated harboring del17p.[43] Response rate improved gradually and with a median follow up of 47 months, the best ORR was 95%, CR of 23%, of which two patients

were MRD negative.[55] Overall, median PFS was 45 months, but only 23.3 months in patients with del17p. Ten patients came off the study due to disease progression and 14 died, portraying the poor prognosis of high-risk CLL patients even with the advent of ibrutinib. Importantly, from a biologic stand-point, it is possible that ibrutinib actually interferes with the activity of anti-CD20 antibodies by downregulating CD20 expression, hence diminishing complement-dependent-cytotoxicity (CDC).[56,57] Ibrutinib's inhibition of ITK also leads to inhibition of NK-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) *in vitro*, which could also interfere with rituximab activity *in vivo*, although this has not yet been demonstrated.[58] Ongoing trials of the ibrutinib–rituximab combination in previously untreated patients might clarify the clinical relevance of these mechanisms. Clinically, trials utilizing other CD20 antibodies with ibrutinib suggest the combination is beneficial, although these antibodies have different or more potent mechanisms. In a phase Ib/II trial of ibrutinib–ofatumumab in 71 relapsed CLL patients, mostly heavily pretreated and with high-risk features, ORR was 71–100% and estimated 12 month PFS was 75–89%.[59] A trend toward higher efficacy in patients initiating ibrutinib before ofatumumab was observed, and the median time for initial CLL response was only 2.8 months, shorter than expected for ibrutinib alone. A randomized phase III trial compared the combination of ibrutinib with ublituximab (TG-1101), an anti-CD20 antibody that targets a unique epitope and is engineered for enhanced ADCC, to ibrutinib alone in 126 R/R high-risk CLL patients.[60] At a median follow up of 12 months, more patients responded to the combination than to ibrutinib alone (ORR 80% *versus* 44%) in a shorter time (1.97 *versus* 3.8 months) and without added toxicity. Results with longer follow up are awaited. The safety of ibrutinib combination with a more robust CIT was tested in a phase Ib trial using ibrutinib–BR in 30 patients. Most patients finished six cycles of BR without unexpected safety issues, and about half continued ibrutinib after three years. ORR was 97%, with up to 40% CRs in the extension period. Enrollment to an ibrutinib–FCR arm in the same study was slow due to restrictive eligibility, and closed after only three patients, yet all of them attained CR and two were BM-MRD negative.[61] In the phase III HELIOS study, 578 patients were randomized to either ibrutinib or placebo combined with BR. CR and MRD-negative rates were higher in the ibrutinib arm, where the IRC-assessed PFS at 18 months was also significantly better (79% *versus* 24%).[62] However, with the caveat of comparing results between different trials, it has not been established that administration of BR contributes to the long-term benefit of single-agent ibrutinib, given the relatively small increase in CR rates, and the higher rate of neutropenia in particular with CIT.

As more data in CLL and other lymphoproliferative diseases have accumulated, the FDA has approved ibrutinib for both previously untreated and treated CLL patients, including with del17p in any line of therapy; mantle cell lymphoma (MCL), Waldenstrom's macroglobulinemia (WM), R/R marginal zone lymphoma (MZL), and recently for treatment of chronic graft-*versus*-host disease.

Ibrutinib toxicity

As ibrutinib is increasingly widely used in the treatment of CLL and other diseases, it is crucial to understand its unique toxicity profile, which is expected to become increasingly significant in patient management.

The most common adverse events (AEs) reported with ibrutinib are diarrhea, upper GI symptoms, fatigue, arthralgias, hypertension, rash, bruising, neutropenia, infections (mainly upper respiratory and pulmonary), cough and pyrexia. Less frequent, yet important, AEs are atrial fibrillation (AF) and overt bleeding.[43,50,52,63,64] Diarrhea is the most common event (up to 50%) but is usually of low grade, does not require dose reduction or extended drug discontinuation, and seems to diminish over time. Long-term follow up of patients on ibrutinib shows that the frequency of grade 3 AEs occurring during the first and second years generally decreases over time, including infection, cytopenias, diarrhea and pneumonia. In contrast, the rate of grade $\geqslant$3 hypertension is higher in late years of treatment[53,63] and AF continues at a low rate after an early peak.[65] Patients who are treatment-naïve (TN) have fewer grade 3 cytopenias (4% *versus* 16% for neutropenia) and infections (11% *versus* 48%).[53] Neutropenia does not usually require dose modification or discontinuation. With regard to infections, there is evidence of partial immune reconstitution with continuous ibrutinib administration that is associated with fewer infections: although IgG levels remain the same, IgM levels transiently increase, and IgA levels persistently increase.[50,66] CD4 and CD8 T-cells have also been reported to increase during ibrutinib therapy, possibly related to

ITK-inhibition.[67] Major infections requiring either IV antibiotic therapy or an inpatient stay occurred in one-third of patients, and more so in the relapsed setting.[68] Furthermore, opportunistic infections have emerged over the years, such as *Pneumocystis jirovecii* pneumonia (PCP), invasive aspergillosis and disseminated cryptococcal disease.[69–72]

Ibrutinib is associated with an increased incidence of AF. The mechanism is unclear, although it might involve PI3K-Akt inhibition by BTK and TEC in cardiomyocytes.[73] This excess risk was most evident in the RESONATE study where 10 (5%) patients in the ibrutinib arm had AF *versus* only 1 with ofatumumab.[52] Other trials reported an AF rate of 6–10% with a median follow up of 18 months,[43,74] which was as high as 16% at 28 months, in a different phase II trial of 86 CLL patients.[65] In a large retrospective multicenter study of 56 patients who experienced AF with ibrutinib, 76% developed the arrhythmia within the first year of therapy, yet time to onset was up to 46 months.[75] Notably, according to long-term follow up (median 44 months) of the phase Ib/II PCYC-1102 trial, there were no high-grade AF episodes – that is, symptomatic and incompletely medically controlled (that in practice usually lead to hospitalization) – past two years of ibrutinib initiation.[53,63] In a recently published analysis, AF events were pulled from 1505 patients participating in four randomized controlled trials (RCTs), employing ibrutinib single-agent or with BR, for either CLL or R/R MCL ($n = 193$).[76] As expected, the AF rate was higher in the ibrutinib arm (6.5% *versus* 1.6% in the comparator arm; median follow up of 16.6 months), and few high-grade episodes were reported past 6 months. Although patients on ibrutinib tended to have multiple events (45% *versus* 17%), most patients with AF (86%) did not discontinue ibrutinib. Longer-term follow up in patients on ibrutinib demonstrated that new events of AF occur at a low continuous rate, with an estimated cumulative incidence of 13.8% at 36 months in this clinical trial population.

Real-world studies report a higher rate of AEs leading to discontinuation of ibrutinib. In a large multicenter retrospective study of 616 previously untreated ($n = 80$) and relapsed ($n = 536$) CLL patients, the rate of discontinuation due to intolerance was 24% and 43%, respectively. In this study, 25% of front-line ibrutinib discontinuation was attributed to AF.[77] In order to provide context to help interpret AF risk in CLL patients in general, Shanafelt and colleagues conducted a large retrospective study of 2444 CLL patients seen at the Mayo Clinic that demonstrated AF incidence of 1%/year, and suggested a risk model that includes older age, male sex, valvular heart disease and hypertension, stratifying patients to a 10-year incidence rate of AF ranging from 4% to 33%.[78] Interestingly, in the pooled-RCT analysis mentioned above,[76] the rate of AF ranged between 3.5% and 15.4%, according to Shanafelt's risk categories. This may assist with decision-making in CLL patients prior to ibrutinib administration.

Recently, a study underscoring the occurrence of ventricular arrhythmias and sudden death in ibrutinib-treated patients was published by our group. It was driven by four cases of polymorphic ventricular tachycardia (VT)/ventricular fibrillation (VF), and detected six additional reported cases in the FDA Adverse Event Reporting System (FAERS). The median age of all these patients was 61 years (range 49–85), and median time to event was 65 days (range 6–698) from ibrutinib initiation.[79] By collecting events of sudden death or cardiac arrest from published clinical trials with ibrutinib, the incidence rate was 788 events per 100,000 person-years, which is higher than reported rates of sudden cardiac death for 65-year-olds, which are in the range of 200–400 events per 100,000 person-years.

Bleeding diathesis is another disturbing and potentially serious AE, which may pose a practical predicament in patients on double antiplatelet therapy or anticoagulants, including those who develop AF secondary to ibrutinib itself. While minor bleeding events (ecchymosis, bruising, epistaxis) have been commonly reported in prospective trials (33–55%),[52,54,80] grade 3–4 events or major bleeding usually occur in $<5\%$ of patients, who often have concomitant risk factors, specifically use of other anti-aggregant/coagulant therapy.[54,80] Ibrutinib does not disrupt coagulation, but rather causes platelet dysfunction, as shown by acquired defects in aggregation responses to collagen, ADP and ristocetin.[48,81–83] These are consistent with an on-target inhibition of BTK signaling downstream from both platelet collagen receptor GPVI and the von-Willebrand factor receptor, GPIb-IX.[81,84] Although some have shown that certain aggregation tests may predict bleeding,[85] the utility of platelet function testing for patients on ibrutinib has not been established.

The current recommendation is to discontinue ibrutinib for 3–7 days prior to and after any planned surgical procedures. The combination of ibrutinib with oral vitamin K antagonists is contraindicated, since it has been associated with unacceptable bleeding rates,[86] and these patients were mostly excluded from trials. Other anticoagulants were permitted, including direct oral anticoagulants (DOACs) that are probably safe, albeit experience is still quite limited.[87]

Clinical trials: first-line ibrutinib

Single agent

The trial that led to approval of ibrutinib for previously untreated CLL patients was the phase III RESONATE-2 trial, randomizing 269 patients of age 65 years or older to ibrutinib or chlorambucil (see Table 1 for a summary of results from first-line trials). Patients with del17p were excluded, since chlorambucil was not considered an appropriate alternative in those. Crossover was allowed at progression.[74] At a recently presented update with a median follow up of 28.6 months, 55 patients (41%) initially treated with chlorambucil have crossed over to ibrutinib, while 79% of patients initially assigned to ibrutinib persisted on that regimen. The main reason for ibrutinib discontinuation was AEs (12%), while progression was the cause in only four patients (3%), one of whom had Richter's transformation (RT). Given that chlorambucil was the comparator, ORR was expectedly significantly higher in the ibrutinib arm (92% *versus* 36%), with 18% of CRs seen only there. At 24 months, 89% of patients assigned to ibrutinib were free from progression, as opposed to only 34% in the chlorambucil group. Within ibrutinib-treated patients, those with high-risk features (21% with del11q; 43% with UM-*IGHV*) had similar PFS as the rest with current follow up. The ITT estimate of 2-year survival was 95% *versus* 84%, with ibrutinib and chlorambucil, respectively.[88]

The longest follow-up data we have for ibrutinib stems from the phase Ib/II trial (PCYC-1102 and its extension PCYC-1103), which included 31 TN patients, age 65 years or older (median 71 years, range 65–84), about half of whom had UM-*IGVH* and 6% had del17p abnormality.[63] In a recent update, the median time on study for this cohort was 60 months, when 77% of patients had been treated with ibrutinib for over 4 years.[92] Considering only the 27 patients who were treated with 420 mg ibrutinib (others were treated with 840 mg in the phase Ib part of the trial), 81% remained on ibrutinib with a median time of 30 months on treatment; 3 discontinued therapy due to AEs and 1 secondary to disease progression. Estimated PFS and OS at 30 months were both 96%.[53]

Farooqui and colleagues investigated ibrutinib utility specifically in CLL patients with TP53 aberration.[64] This phase II trial reported response data in 33 of 35 TN patients; ORR was 97% at 24 weeks, and 12 achieved CR as their best response at a median of 48 weeks.[64] The cumulative incidence of progression and OS at 24 months was 9% and 84%, respectively. All patients who progressed had UM-*IGHV*, and manifested with transformation to either RT or PLL. Several ongoing phase III trials compare the use of ibrutinib with CIT in previously untreated patients. Two of those – the ECOG E1912 [ClinicalTrials.gov identifier: NCT02048813], now fully accrued, and the UK FLAIR trial (ISRCTN01844152) – have randomized fit, young patients without del17p to ibrutinib–rituximab combination *versus* FCR. These studies will, hopefully, more comprehensively depict to what extent response depth and duration are driven by prognostic factors, mainly *IGHV*-mutational status, which could guide the choice of first-line treatment in this population. Recently, the FLAIR trial has been amended to include treatment arms with either ibrutinib alone or in combination with venetoclax. Another fully accrued study [the ALLIANCE A041202 (ClinicalTrials.gov identifier: NCT01886872)] comparing first-line ibrutinib alone or with rituximab to BR will try to answer similar questions in the elderly (del17p patients are not excluded).

With availability of effective and safe oral therapy, the traditional watch and wait (W&W) approach has been recently challenged. CLL12 is a randomized double-blind trial allocating patients with intermediate- to high-risk early CLL to either W&W or daily ibrutinib; the primary endpoint is event-free survival (EFS). In a first safety report, 46 randomized patients have stopped the study-drug due to refusal to continue on study. Notable AEs were one case each of non-ST elevation MI; QT-prolongation with VT; and a subdural hematoma in an elderly patient treated with rivaroxaban, promoting a protocol amendment to exclude patients with any anticoagulation treatment from the trial.[91] Although such an approach is interesting, lack of deep response and concerns about

Table 1. Results from trials of first-line ibrutinib in CLL.

Reference	Phase treatment	*n*	Age	ORR	CR/CRi	PFS	OS	AF	HTN (G3–4)	Major bleeding
RESONATE2 Burger[74]	Phase III Ibrutinib *versus* chlorambucil	269	73 (65–90)	86% *versus* 35%	4% *versus* 2%	Median NR *versus* 18.9 mo	3 *versus* 17 pts died = Sudden death in 2 pts treated with ibrutinib	6% (all grades)	4%	4%
Update Barr[88]				92% *versus* 36%	18% *versus* 0%	89% *versus* 34% at 24 mo	95% *versus* 84% at 24 mo	10%	5%	7%
Farooqui[64]	Phase II Single agent	33 first line (15 R/R)	62 (33–82)	97%	34% at 48 wks	82% for all at 24 mo	84% at 24 mo = Sudden death in 1 pt	2%	NA	0%
Byrd[63] (PCYC-1102, PCYC-1103)	Phase Ib/II Single agent	31 first line	71 (65–84)	84%	23%	96% Estimated at 30 mo	97% Estimated at 30 mo	6% (G3–4)	20%	8%
Update Coutre[53]		27 first line (treated with 420 mg/day)		85%	26%		96% at 30 mo	4% (G3)	26%	5%
Davids[89]	Phase II iFCR	35	55 (38–65)	100%	47% (85% BM-MRD negative)	None relapsed	None died	*n* = 1 (G3)	*n* = 0	*n* = 0
Jain[90]	Phase II iFC-obinutuzumab	23 (still recruiting)	59 (25–71)	18/18 pts	9/18 (7/18 BM-MRD negative)	None progressed	None died	*n* = 1	NA	NA
Langerbeins[91]	Phase III Single agent for early disease	170 (randomized 1:1)				*n* = 6 progressive disease		6 cases of 'cardiac disorders', at least one VT		*n* = 1 (subdural hematoma)

*Only M-*IGHV*, non-del17p patients; FC given for three cycles.
AF, atrial fibrillation; BM, bone marrow; CR, complete remission; CRi, CR with incomplete bone marrow recovery; G3–4, grade 3–4; HTN, hypertension; iFCR, ibrutinib–fludarabine–cyclophosphamide–rituximab; mo, months; MRD, minimal residual disease; *n*, number of patients; NA, not available; NR, not-reached; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; pts, patients; VT, ventricular tachycardia; wks, weeks.

resistant relapse will determine its future utility, particularly as treatment is given indefinitely, according to the current protocol. Moreover, OS and not EFS is the appropriate primary endpoint to assess the benefit of early treatment, albeit less practical.

Ibrutinib resistance

As stipulated above, patients do relapse continuously on ibrutinib. Of 85 R/R patients in the initial phase II trial, after a median follow up of 21 months, 11 patients (10 with del17p) had CLL progression and seven had RT,[50] probably reflecting the heavily pretreated high-risk population on that trial. At an update with 101 patients and longer follow up of 44 months, CLL progression was at 17%, but with no additional RT.[63] The incidence of progression and RT was also high in the RESONATE-17 trial that specifically depicted patients with 17p aberration. At a median follow up of 27 months, 27% of patients had progressed, 44% of whom had RT mostly occurring within 6 months (11/17 patients) and up to 25 months of treatment initiation.[54] In another study of patients with 17p aberration that included both TN and relapsed patients, 5 of 51 progressed within 2 years, all transformed (3 DLBCL, 2 PLL).[63,64]

A much lower incidence of RT was encountered on the RESONATE trial, where only 4 of 391 patients developed RT, 2 in each arm, ibrutinib *versus* ofatumumab.[52] Similar estimates were reported in 40 patients with high-risk CLL treated with ibrutinib–rituximab, where 3 progressed, 1 with RT, albeit follow up was short.[43] Of 31 TN patients in the PCYC-1102 trial, only 1 patient harboring del17p progressed with RT. Similarly, rate of progression was 3% at 2 years in the RESONATE-2 trial, which enrolled TN patients without del17p. There was one case of RT in each arm (ibrutinib or chlorambucil).[74,88]

There are several mechanisms for ibrutinib resistance in patients with CLL; the most common is a newly acquired cysteine-to-serine (C481S) mutation in BTK at the binding site of ibrutinib, which results in a protein that is only reversibly inhibited by ibrutinib. This was first demonstrated in six patients by whole exome sequencing (WES) of samples from baseline and time of relapse, when five acquired the BTK-C481S mutation, and three had distinct gain-of-function mutations in PLCG2, which is downstream from BTK, allowing for BTK-independent BCR signaling.[93] Similarly, BTK-C481S or PLCG2 mutations were detected in 8 of 10 patients with progressive CLL on ibrutinib and one with PLL transformation in an NIH phase II trial enrolling 84 patients who were either elderly or had TP53 aberration. Mutations were detected up to 15 months prior to progression, and multiple subclones carrying different mutations arose in five patients.[94]

In order to further investigate the importance of BTK/PLCG2 mutations in predicting relapse, a large-scale effort of deep sequencing for these mutations was undertaken in relapsed cases from among 308 patients enrolled in four prospective studies at Ohio State University (OSU). The estimated cumulative incidence of progression at 4 years was 19%. Of 46 relapsed patients who were sequenced, 40 (85%) had acquired mutations of BTK and/or PLCG2. In 20 patients who had serial samples, mutations were detected in 18 at a median of 9.3 months (range 3–18) prior to clinical relapse. In a cohort of 112 patients that were sequenced prospectively, all 8 who eventually relapsed had an acquired BTK-C481S mutation before relapse, and similar mutations were detected in an additional 8 patients who do not meet criteria for progression yet. Importantly, no early signs of progression were noted in patients without these resistance mutations.[95] Another trial confirms that no BTK or PLCG2 mutations were evident prior to ibrutinib initiation in 44 patients, using a high-sensitivity wild-type blocking-PCR assay.[96]

Burger and colleagues also used WES and targeted deep sequencing in serial samples from five heavily pretreated ibrutinib-resistant patients to investigate the role of clonal evolution in CLL progression. BTK-C481S and PLCG2 mutations were detected in two, while the other three exhibited an expansion of clones harboring deletion of the short arm of chromosome 8 (del8p). The TNF-related apoptosis-inducing ligand receptor (TRAIL-R) is located on 8p, and its deletion has been shown to confer resistance to TRAIL-induced apoptosis in primary cell lines. Additional putative driver mutations were detected in EP300 (a histone acetyltransferase), MLL2 (a chromatin regulator) and EIF2A (a translation initiation factor).[97] Infrequently, other mutations in C481 have been reported.[98,99] A novel mutation (T316A) in the BTK Src-homology-2 (SH2) domain was recently described in a patient who relapsed on ibrutinib.[100] This mutation conferred functional

resistance to ibrutinib in a transfected lymphoma cell line (TMD8) together with decreased pBTK, pAKT and pERK.

The prognosis of patients who discontinue ibrutinib largely depends on the reason for stopping. A real-world analysis of 178 patients treated with kinase-inhibitors (KI, mostly ibrutinib) and followed for a median of 14 months demonstrated a longer PFS in KI-intolerant patients than in those with CLL progression or RT, the latter doing the worst.[101] Similarly, of 308 patients in the four OSU prospective trials with a median follow up of 3.4 years, those who stopped ibrutinib due to RT (n = 28) had a median survival of only 3.9 months, while those who stopped for CLL progression (n = 55) had a median OS of 22.7 months.[95] In the MD Anderson experience, 28% of 290 patients discontinued ibrutinib after a median of 3 years; the median survival was only 2 months in patients with RT (10%), 16 months in CLL progression, and 33 months for ibrutinib intolerance or other causes.[102] Among the 19 patients who had progressive CLL, only 8 (42%) responded to subsequent therapy, 5 of whom achieved a partial response on venetoclax-based therapy.[102]

A prospective phase II trial of venetoclax specifically for R/R CLL patients after KI enrolled 43 patients previously treated with ibrutinib for a median of 17 months, 91% of whom were ibrutinib-refractory. The ORR was 70%, and median PFS and OS have not been reached, yet 16 patients have discontinued venetoclax at a median of 9 months, mostly for disease progression.[103] In a large multicenter retrospective analysis of 683 patients treated with KI, sequential treatment with another KI or venetoclax was superior to CIT, and there was a marginally better PFS associated with venetoclax as opposed to idelalisib.[104] Indeed, venetoclax has become the favored therapeutic option for CLL patients who progress on ibrutinib, in the absence of a clinical trial.

Combination therapy

Given the response pattern with ibrutinib and arising resistance, it has become clear that, although KIs are absolutely essential in CLL therapy, as single agents, they do not constitute a panacea for the disease. The long-term data from the MD Anderson and the German CLL8 trials strongly imply that MRD negativity may be a crucial endpoint in our pursuit of long-term remission. Combinations of the potent drug ibrutinib with standard CIT and/or other novel agents may result in deeper remission, longer PFS and, possibly, cure. Bringing this treatment modality to the front line, before CLL resistance mechanisms evolve, seems highly sensible.

Following trials of rituximab–ibrutinib combinations in R/R CLL, a phase Ib/II study utilized the more potent anti-CD20 antibody, obinutuzumab, in conjunction with ibrutinib, in 32 previously untreated patients over the age of 65 or with comorbidities, none of whom had del17p.[105] Obinutuzumab is given for six cycles and ibrutinib for up to 3 years. In an early safety report, generally mild AEs were noted, and one case of grade 3 pneumonia. Interestingly, there were only mild infusion-related reactions (IRR), suggesting ibrutinib might mitigate the incidence and severity of IRR. A phase III trial comparing ibrutinib–obinutuzumab to the standard chlorambucil–obinutuzumab in elderly/unfit patients or those with del17p/TP53 mutation has recently finished recruitment [PCYC1130 (ClinicalTrials.gov identifier: NCT02264574)].

Promising preliminary results from the phase II study of ibrutinib–FCR (iFCR) as front-line therapy in 35 fit CLL patients were reported recently. In the original scheme, ibrutinib is given for a week, followed by up to six cycles of FCR with continuous use of ibrutinib until progression. Patients' age was 38–65 years (median 55), about half had UM-*IGHV* and 11% (four patients) del17p. There were no unexpected safety signals and all but two patients remained on ibrutinib: 20% had grade 3–4 neutropenia; 9% had grade 3 or above infections; there were two cases of grade 1 epistaxis; and one case of grade 3 AF. All patients responded to therapy; median best time to response was 95 days; 47% achieved CR/CRi and 84% became BM-MRD negative in a median of 104 days (100% for M-*IGVH*).[89] Following these encouraging results, an amendment was added allowing for MRD-negative patients to stop ibrutinib after 2 years.

In another attempt to achieve high MRD negativity rates and restrict the use of ibrutinib, a trial of ibrutinib–FC–obinutuzumab was designed specifically for M-*IGHV*, young, fit, previously untreated patients, who do not harbor del17p.[90] Only three cycles of FC are given; the extent of obinutuzumab administration is stratified according to response, yet all patients who achieve

BM-MRD negativity are planned to stop all treatment. Even as soon as 3 months, all 18 evaluable patients had responded to therapy, and 14 were MRD negative. Best response assessment demonstrated a CR/CRi rate of 50% and 89% MRD negativity. Interestingly, FC was dose-reduced in 10 of 23 accrued patients due to grade 3–4 neutropenia and thrombocytopenia in 11 and five patients, respectively, and neutropenic fever in 4.

Regardless of the potential efficacy of iFCR, most patients with CLL are ineligible for fludarabine-based treatment. The German CLL Study Group, in their CLL2-BIG trial, present a more suitable regimen for less fit patients. It follows a scheme of debulking with mild chemotherapy, induction using a combination of novel agents, and then MRD-driven maintenance. In this trial specifically, bendamustine is used for two cycles for debulking; obinutuzumab and ibrutinib for six cycles as induction; and ibrutinib and obinutuzumab as maintenance according to MRD negativity. It accrued 30 TN and 31 R/R patients, ages 36–83 years (median 66) with the expected distribution of risk factors. Similar to iFCR, all patients responded to therapy; 43% of TN patients attained CR/CRi; and 53% were MRD negative in the peripheral blood at the end of induction. Toxicity was manageable.[106]

Naturally, chemotherapy-free regimens leading to MRD negativity and off-drug long-term remissions are highly appealing. Venetoclax (ABT-199) is a BCL2 inhibitor that has shown efficacy in CLL patients,[107,108] even after ibrutinib failure,[100,101] and is approved for treatment of R/R CLL patients with del17p. Ample preclinical data support the synergism between ibrutinib and venetoclax in CLL[109,110] as well as in other lymphoproliferative disorders.[111–114] Consequently, several ongoing trials in the relapsed and first-line settings utilize this combination alone or with other drugs. A favorable first safety report of the obinutuzumab–ibrutinib–venetoclax (GIVe) combination in 12 R/R CLL patients was recently presented [ClinicalTrials.gov identifier: NCT02427451], and established 400 mg as the venetoclax dose for the corresponding phase II trial.[115] Other trials using ibrutinib–venetoclax alone (IVe) in previously untreated high-risk patients are also available at MD Anderson [ClinicalTrials.gov identifier: NCT02756897] and in Britain (CLARITY). Lastly, two important trials are being conducted in Germany: the CLL13 that randomizes TN patients without 17p aberrancies to either FCR/BR, rituximab–venetoclax (RVe), obinutuzumab–venetoclax (GVe) or obinutuzumab–ibrutinib–venetoclax (GIVe) [ClinicalTrials.gov identifier: NCT02950051]; and the CLL2-GIVe trial [ClinicalTrials.gov identifier: NCT02758665] that follows the logic of their CLL2-BIG trial discussed earlier. The concurrent CLL14 phase III trial [ClinicalTrials.gov identifier: NCT02242942] compares venetoclax–obinutuzumab with the standard chlorambucil–obinutuzumab and can provide further insight into the utility of novel-agent combination. Importantly, treatment cessation is built into the protocol of all these trials, reflecting the next paradigm in CLL management.

Alternative BTK-inhibitors

As ibrutinib targets kinases other than BTK alone and some may be related to its unique toxicity profile, second-generation BTK-inhibitors have been investigated, with acalabrutinib (ACP-196) being the most advanced. It is an irreversible inhibitor with improved pharmacologic features including favorable plasma exposure, rapid oral absorption and reduced targeting of alternative kinases. Acalabrutinib repressed the proliferation of a canine lymphoma cell line (CBL1) through inhibition of BTK activity and its downstream effectors, and it was effective and well-tolerated in a canine lymphoma model.[116] Furthermore, acalabrutinib specifically and effectively inhibits BTK in primary CLL cells, and abrogates downstream signaling from the receptor.[44] Consistent with acalabrutinib selectivity, it has less off-target effects on Src-family kinases in healthy T-lymphocytes in comparison with ibrutinib, although the effect on primary CLL cells was similar with both agents.[117]

The most prevalent AEs of acalabrutinib, albeit mostly mild, were diarrhea, headache and increased weight in a phase I/II trial of 61 R/R CLL patients with no prior exposure to ibrutinib. Although most other AEs were quite similar to ibrutinib, there were no incidents of major hemorrhage or AF. ORR was 95%; only one high-risk patient (harboring del17p) progressed, after developing C481S-BTK and PLCG2 mutations.[44] A preliminary report of first-line acalabrutinib treatment in 74 patients found it was tolerable in 97% at a median of 11 months on study.[118] Most common mild AEs were headache (42%), diarrhea (35%) and arthralgia (22%); grade 3 syncope and hypertension (two patients each) were reported and no AF. There was one case of grade 3 upper

GI bleeding and one fatal pneumonia. Best ORR was 96% with a median time to response of 2 months; no CRs; no PD. As expected, acalabrutinib is being investigated in several clinical trials in CLL and other NHLs. Other highly selective BTK/TEC-inhibitors have also entered clinical trials, namely CC-292[119]; ONO/GS-4059[120]; and BGB-3111.[121] SNS-062 is a novel non-covalent BTK inhibitor that can inhibit C481S-mutated BTK, and will start trials in ibrutinib-resistant patients soon. A detailed discussion of these agents is beyond the scope of this review.

Cost-effectiveness

Ibrutinib, similar to other novel targeted agents, is priced at approximately $130,000 per year, which is more than twice the average US household income,[122] and is currently recommended until progression. A simulation model evaluating the economic burden of CLL treatment with CIT or targeted agents as the standard of care projected the annual cost of CLL management to increase by 590% between 2011 and 2025, reaching over $5 billion in 2025. This model considered targeted therapy in second line and beyond or in first line for patients with del17p. Health-related quality of life was assessed according to age and disease response. Compared with the CIT scenario, oral targeted therapies resulted in an incremental cost-effectiveness ratio of $189,000 per quality-adjusted life-year.[123] Another study estimated 10-year pharmaceutical costs for 100 patients with newly diagnosed CLL. The 10-year cost per patient would rise from about $46,000 with CIT to $164,000 with ibrutinib first line, while the cost of treating a patient throughout this period would rise from $157,000 to $566,000. This also drives out-of-pocket cost, which under certain conditions is expected to increase from $325 (in patients receiving FCR or BR) to $36,000 per treated patient, with CIT *versus* first-line ibrutinib.[122] Treatment patterns in the first-line setting are already changing, employing regimens that include obinutuzumab and ibrutinib more frequently, with less CIT use.[124] First-line ibrutinib would be expected to increase these costs yet further, with higher costs per quality-adjusted life-year, making it crucial to consider the cost of treatment as a potential hurdle for optimal care in the near future.

Conclusion

Ibrutinib altered the therapeutic landscape in CLL. It begins to address the gargantuan unmet need of effective therapy for the majority of the CLL population needing treatment – that is, elderly and/or frail patients; those carrying high-risk markers with reduced benefit from CIT; and patients with relapsed disease after CIT. Ibrutinib, however, does usually only lead to partial remissions, and needs to be given indefinitely in most patients. Long-term exposure requires the continued endurance of cumbersome AEs, such as diarrhea, ecchymosis, arthralgias and fatigue. While many AEs diminish over time, they do not abate completely, and the risk of AF and bleeding remain substantial. Moreover, acquired mutations in CLL cells, among other putative mechanisms, cause gradual relapse of disease, which frequently portends poor prognosis and limited therapeutic options. Even though treatment with ibrutinib in previously untreated patients achieves somewhat higher rates of complete remission and generally has a better toxicity profile, long-term follow up of a large cohort of patients is still lacking, as is a greater understanding of how we salvage those who relapse. In addition, the accumulating financial burden is significant. Nonetheless, it is essential to explore the utilization of BTK-inhibitors in the first-line setting in combination with other agents, as part of a novel time-limited, MRD-driven paradigm of CLL care that will hopefully allow for long-term remissions and potentially cure.

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Conflict of interest statement

Jennifer R. Brown is a consultant for Janssen, Pharmacyclics, Abbvie, AstraZeneca, Sun, Redx, Gilead, and TG Therapeutics.

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