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Lymphoma and Chronic Lymphocytic Leukemias

A New Era is Coming up in the Treatment of Chronic Lymphocytic Leukemia

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ABSTRACT: The survival of patients with chronic lymphocytic leukemia (CLL) has significantly improved in the last 30 years. The introduction of purine analogs in the treatment armamentarium followed by the combination of these compounds with alkylating agents first improved response rates and progression-free survival (PFS) when compared with alkylating agent-based therapy only. However, the great advance arrived with the development of a chemoimmunotherapeutic approach comparing this with chemotherapy alone demonstrating an improvement not only in terms of response rate and PFS but also, for the first time, in the rate of overall survival (OS). The last decade brought significant achievements in the understanding of CLL pathogenesis leading to the development of new agents targeting cell surface, intracellular pathways, and tumor microenvironment. As traditional chemotherapy is associated with acute and long-term toxicity, interest in these non-chemotherapeutic treatments has been constantly growing. The challenge will be to develop a rationale for non-chemotherapeutic approaches using these new agents as monotherapy, or in combination, with the aim of obtaining an individualized strategy based on disease characteristics and even patient basis. Hopefully, an increasing participation of ultra high-risk CLL patients in clinical trials evaluating the efficacy of these new treatments may lead to reasonable success, if not cure, in this unfavorable setting.

KEYWORDS: chronic lymphocytic leukemia, chemoimmunotherapy, monoclonal antibodies, target therapy

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Introduction

There have been tremendous advances in the treatment of chronic lymphocytic leukemia (CLL) over the past two decades, with the treatment goal shifting from symptom palliation to achieve complete remission (CR) and improve survival.

CLL has traditionally been regarded as an incurable disease of the elderly, where the typical patient was expected to die "with CLL" rather than "of CLL." Chemotherapy was with single-agent alkylators and was purely palliative in intent. The pursuit of maximal disease eradication was not regarded as a worthwhile goal in the majority of patients.

Recent evidence, however, contradicts this traditional view and shows that the majority of patients diagnosed with CLL will die of complications relating to CLL.[1]

Unlike other types of leukemia, once a diagnosis of CLL is made, treatment may not necessarily be initiated. This current consensus by the international community, initially published in 1988,[2] is informed by the observation that single-agent chlorambucil when compared to delayed treatment had no significant impact on overall survival (OS). Findings of a meta-analysis on seven trials conducted in CLL showed that there was no statistically significant difference in survival between those patients who were treated early versus those in whom therapy was deferred until there was a clinical indication for treatment.[3]

However, these trials were performed by using alkylating agents. Since then, the recommendations were revised but not changed in 1996[4] and 2008.[5]

At the present time, treatment should be initiated if the disease is active. Basically, treatment should be started in the presence of cytopenias (anemia and/or thrombocytopenia) because of bone marrow failure, if bulky (>10 cm) or rapidly

progressing lymphoadenopathy occurs, or if a rapid increase (doubling within 6 months) in the lymphocyte counts or severe constitutional symptoms (night sweats, fever, weight loss, fatigue) occur.[5]

Over the last 20 years, there have also been major advances in our understanding of molecular factors associated with increased risk of progression. The clinical utility of these factors is being explored to determine whether we can identify groups of patients who should be treated earlier in their disease course and whether we can tailor therapy for groups of patients with specific molecular markers of disease.

Purine Analogs Era

Fludarabine was the first effective new agent to be extensively evaluated in CLL, achieving response rates of 50–60% in patients who failed traditional alkylating-agent therapy.[6] Fludarabine was soon studied in the frontline setting,[7] where its activity was confirmed in three randomized comparisons.[8–10] All of these trials confirmed higher response rates and longer progression-free survival (PFS) in patients randomly assigned to fludarabine compared to alkylating agents; however, no increase in OS was observed. However, the better overall response rate (ORR) and CR rate observed in the elderly with fludarabine compared to chlorambucil in the German CLL Study Group (GCLLSG) CLL5 trial did not translate into longer PFS.[11]

Fludarabine inhibits excision repair of DNA interstrand cross-links induced by cyclophosphamide, thereby enhancing treatment activity and providing a rationale for combining these agents.[12,13] Three randomized trials[14–16] have compared fludarabine to fludarabine and cyclophosphamide (FC) as first-line therapy; all have shown significantly greater CR rate and ORR and longer PFS with FC. In all these three trials, no survival advantage for frontline treatment using FC was shown. However, an analysis of the subgroup of patients without high-risk genetic deletions in the CLL4 trial of the GCLLSG showed that FC did prolong the OS time in these patients when compared with fludarabine monotherapy.[17]

The Arrival of Monoclonal Antibodies

The management of patients with non-Hodgkin's lymphoma (NHL) and CLL has dramatically changed with the introduction of monoclonal antibodies (MoAbs) directed against specific proteins expressed by the neoplastic B-cells. Rituximab is a chimeric anti-CD20 monoclonal antibody with anti-leukemia action, including complement-dependent lysis (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and direct induction of apoptosis.[18] Rituximab when given as a single agent has more limited clinical activity in CLL than in follicular lymphoma, unless very high doses are used.[19,20] There are several compelling arguments for combining chemotherapy with monoclonal antibodies. First, chemotherapy and monoclonal antibodies cause cell death by different mechanisms, and CLL-cells that are resistant to one mechanism of cell killing may be susceptible to the other. Second, overlapping toxicity is negligible. Third, there is preclinical evidence to suggest that chemotherapy and monoclonal antibodies may act in a synergistic manner.[21,22]

Consistent with these observations, the addition of a relatively small dose of rituximab (500 mg/m^2, repeated every 4 weeks) to fludarabine, cyclophosphamide, and rituximab (FCR) resulted in the most active frontline CLL regimen developed to date.[23] The GCLLSG conducted a prospective randomized phase III trial including 817 previously untreated physically fit CLL patients randomly assigned to receive FCR versus FC alone (CLL8 trial).[24] The results of this trial showed for the first time a survival advantage among fit CLL patients. Treatment strategies including monoclonal antibodies like GCLLSG CLL8 trial not only determined an amelioration of response rates but also allowed to achieve better quality of responses with a significant proportion of patients achieving the eradication of minimal residual disease (MRD).[25] The achievement of MRD, by polymerase chain reaction (PCR)-based amplification of the IGVH rearrangement, correlates with the improvement of time to retreatment (TTR) and PFS.[26]

Moreover, there are data suggesting that an MRD-negative CR state is associated with improved survival.[27] MRD-negative CR is not a reasonable goal in all patients; nevertheless, in a young, fit patient with CLL, the reasonable goal of treatment should be CR—possibly MRD-negative CR. However, the vast majority of CLL patients are old, 71 years being the median age at diagnosis. Such elderly patients are frequently compromised by concurrent pathological conditions and/or physiological decline of organ function. Major comorbidities are present in 46% of unselected patients with newly diagnosed CLL and advanced age.[28] Therefore, many patients will have one or more significant comorbidities when they require therapy, meaning that relatively intensive chemoimmunotherapeutic approaches, such as FCR, may be considered too toxic. Notwithstanding the demonstrated efficacy of FCR to a treatment-naïve patient with CLL, this cannot currently be considered the "standard" care for all patients. Selection of the most appropriate initial therapy in CLL must be based primarily on patient characteristics such as presence and number of comorbidities that pose specific restrictions on treatment choice.

A dose-modified FCR-Lite regimen was designed to maintain the efficacy but decrease the toxicity of the FCR regimen.[29] This regimen reduced the dose of FC and increased the dose of rituximab, and used a maintenance regimen for rituximab given every 3 months until progression. In a recent update of results, the authors confirmed the activity of this chemotherapy attenuate combination leading to a not inferior number of responses, historically compared to the standard FCR, with long duration of response and favorable toxicity profile.[30]

Among purine analogs active in CLL, the degree of myelosuppression of pentostatin seems to be favorable when associated with cyclophosphamide and compared to fludarabine-based combinations.[31] The efficacy of pentostatin, cyclophosphamide, and rituximab (PCR), with pentostatin administered at the dose of 2 mg/m^2, has been documented in 64 untreated patients.[32] In the subsequent study, the authors outlined that PCR can be safely administered to older patients (≥70 years) and those with modestly decreased creatinine clearance.[33] The results appear to contrast with the tolerability of FCR regimen; in the PCR study, older patients were as likely as younger patients to complete the intended 6 cycles and to achieve response without an excess of grades 3–4 toxicity.

There are several therapies for patients who are not candidates to purine analogs treatment. In this patient population, chlorambucil is still accepted as the first-line treatment. Although chlorambucil is generally well tolerated, complete responses are rare and remission durations are usually short. Two phase II trials report the addition of rituximab to chlorambucil and show better ORRs and longer PFS compared to chlorambucil alone in previous trials but CRs remain low, and patients rarely achieve eradication of detectable MRD.[34,35]

New Monoclonal Antibodies

Ofatumumab. The success of rituximab in the treatment of B-cell malignancies, and also its recognized limitations, has fueled the development of several MoAbs that target different epitopes of the CD20 surface antigen with high specificity. Among these agents, the furthest (ahead in terms of clinical development in CLL) is ofatumumab. This second-generation fully human anti-CD20 monoclonal antibody, binding to the small extracellular loop of the target, demonstrated a superior CDC compared to rituximab. The use of ofatumumab is at the moment licensed for patients refractory to fludarabine and alemtuzumab.[36] A recently concluded randomized phase III trial demonstrated increased efficacy of ofatumumab in combination with chlorambucil in front-line treatment of CLL patients who are poor candidates for fludarabine-based therapy. Furthermore, results compared favorably with those of the combination of chlorambucil and rituximab, side effects were manageable, and treatment was generally well tolerated.[37]

The results of this trial allow the registration of this MoAb in first-line setting (Table 1).

Using the same pentostatin and cyclophosphamide platform, the Mayo Clinic group recently published the results of a phase II trial that enrolled 48 previously untreated CLL patients who received both agents with the addition of ofatumumab instead of rituximab. Compared to their historical experience in a very similar patient population with rituximab-based chemoimmunotherapy (CIT), using the same chemotherapeutic combination, the ORR and CR rate observed with ofatumumab-based CIT compare favorably to that of rituximab-based CIT.[38]

Using the same combination, an Italian cooperative group recently presented preliminary results in 48 previously untreated older patients with CLL. Again in this trial, ofatumumab added to pentostatin and cyclophosphamide demonstrated clinically important results and a more manageable side effect profile in this older population.[39] Overall, data from these two trials suggesting a randomized comparison of rituximab-based CIT to ofatumumab-based CIT in CLL may be warranted.

Obinutuzumab. The different use of the mechanisms of action of rituximab (CDC, ADCC, and direct induction of cell death) represents the basis of the distinction between type I and type II antibodies. Obinutuzumab (GA101) is a unique, glycoengineered type II anti-CD20 monoclonal antibody that more potently induces direct cell death and may provide an advantage when combined with chemotherapy. In in vitro models, obinutuzumab has demonstrated significantly increased ADCC compared with rituximab. In a recently published phase III trial, 781 patients, with previously untreated CLL and a score higher than 6 on the Cumulative Illness Rating Scale (CIRS) or an estimated creatinine clearance of 30–69 mL/minute, were randomly assigned to receive chlorambucil, obinutuzumab and chlorambucil, or rituximab and chlorambucil.[40] The primary end point was investigator-assessed PFS. Treatment with obinutuzumab–chlorambucil, compared with rituximab–chlorambucil, resulted in prolongation of PFS and higher rates of CR and molecular response. Moreover, treatment with obinutuzumab–chlorambucil, compared with chlorambucil alone, prolonged OS. Infusion-related reactions and neutropenia were more common with obinutuzumab–chlorambucil than with rituximab–chlorambucil.

The results of this trial allow the registration of this MoAb in first line in patients with comorbidities (Table 1).

Monoclonal antibodies under development. Transmembrane proteins such as CD23, CD37, CD40, and CD74 are being pursued as targets for MoAbs therapy and are at various phases of development. This is also the case for CD19, a glycoprotein member of the immunoglobulin superfamily, and HLA-DR, a class II antigen of the major histocompatibility complex (MHC). Additional targets being pursued include the immune-suppressive molecule CD200.

Alkylating Agent and Purine Analogs or Both in a Single Agent?

Bendamustine. The other question that is being addressed in CLL treatment is whether there is a chemotherapeutic approach more active than chlorambucil but less toxic, in terms of risk of myelosuppression and life-threatening infection, than purine analogs and alkylating agents' combination.

Bendamustine, an alkylating agent with concomitant properties of purine analogs, has proven considerable activity in previously treated or untreated CLL as well as in other lymphoproliferative disorders with good safety profile.

Table 1. Results of published clinical trials evaluating Anti CD20 new monoclonal antibodies used alone or in combination.

[REF.] TREATMENT	NEW MONOCLONAL ANTIBODY					
	NO PATIENTS	ORR (%)	CR (%)	MEDIAN PFS (MONTHS)	DEL(17) (p13.1)	MEDIAN OS (MONTHS)
[36] Ofa (300 mg w 1; 2000 mg/w for 8 w → 2000 mg/mo for 4 mo)	138 (FA-ref = 59; BF-ref = 79)	58% FA-ref 47% BF-ref	0% FA-ref 1.3% BF-ref	5.7 FA-ref 5.9 BF-ref	Yes	13.7 FA-ref 15.4 BF-ref
[37] CLB (10 mg/m^2 d 1–7), q28 d (12 cycles)	221	69	1	13.1	Unknown	Not reached at 29 mo
vs						
Ofa (1000 mg d1) + CLB (10 mg/m^2, d 1–7), q28 d (12 cycles)	226	82	12	22.4		Not reached at 29 mo
[38] P (2 mg/m^2, d1), CTX (600 mg/m^2, d1) Ofa (C1: 300 mg, d1, 1000 mg/m^2, d2; C2–6: 1000 mg/m^2 d1), q21 d (6 cycles)	48	96	46	(TTR) not reached	Yes	n.s.
[39] P (2 mg/m^2, d1), CTX (600 mg/m^2, d1) Ofa (C1: 300 mg, d1, 1000 mg/m^2, d2; C2–6: 1000 mg/m^2 d1), q21 d (6 cycles)	47	89.4	51.1	(TTP) Not reached	Yes	Not reached at 20 mo
[40] CLB (0.5 mg/Kg d1; 15 q28 d) (6 cycles)	118	31.4	0	11.1	Yes	Not reached
vs						
CLB (0.5 mg/kg d1; 15 q28 d) + R (375 mg/m^2 d1C1 and 500 mg/m^2 d1 C2–6 q28 d) (6 cycles)	330	65.3	7.3	16.3		Not reached
vs						
CLB (0.5 mg/kg d1; 15 q 28 d) + (Obi 1000 mg d1; 8; 15 c1 and 1000 mg d1 C2–6 q28 d) (6 cycles)	333	77.3	22.3	26.7		Not reached

Abbreviations: Ofa, Oftumumab; CLB, Chlorambucil; P, Pentostatine; CTX, Cyclophosphamide; Obi, Obinotuzumab; ORR, overall response rate; CR, complete response; PFS, progression free survival; OS, overall survival; FA-ref, Fludarabine reractory; BF-ref, bulky-fludarabine refractory; D, day/s; w, week/s; mo, month/s; C, cycle; q, every; n.s., not specified.

Bendamustine demonstrated good single-agent activity in previously untreated CLL. In a randomized phase III trial, bendamustine was compared to chlorambucil in 319 previously untreated patients with advanced (Binet stage B or C) CLL.[41] Patients <75 years of age received a maximum of six cycles of chemotherapy. Treatment was well tolerated, and dose-intensity was similar in both treatment arms. The ORR and CR rate were superior in the bendamustine arm compared with those observed in the chlorambucil arm by blinded independent review. Bendamustine also significantly improved PFS compared to chlorambucil. At a median follow-up of 54 months, median PFS was 21.2 months in the bendamustine arm compared to 8.8 months in the chlorambucil arm ($P = 0.0001$). This updated analysis also showed that bendamustine significantly prolonged median time to next treatment.[42]

But the role of bendamustine in the treatment of CLL continues to evolve.

The regimen including bendamustine and rituximab was investigated as first-line therapy in 117 CLL patients.[43] Bendamustine was administered at a dose of 90 mg/m^2 on days 1 and 2 combined with 375 mg/m^2 rituximab on day 0 of the first course and 500 mg/m^2 on day 1 during subsequent courses for up to six courses. Overall, these results suggest that when compared to FCR, BR is somewhat less active, yielding lower CR rates, but it is also less myelotoxic. Moreover, no advantage has been demonstrated in those patients characterized by the presence of del(17p). Preliminary results by GCLLSG currently comparing BR to FCR in a randomized phase III trial, the CLL10 protocol, were presented at the last American Society of Haematology (ASH) meeting.[44] The results of the interim analysis confirm that FCR seems more efficient than BR in the first-line treatment of fit CLL patients with regard to higher CRR, as well as longer PFS and EFS. These advantages might be balanced by a higher rate of severe adverse events, in particular neutropenia and infections, associated with FCR. In particular, the elderly population seems to show no disadvantage from the use of BR.

Heterogeneity and Treatment

Heterogeneity in the clinical course of the disease is one of the hallmarks of CLL. The use of novel biological and genetic parameters allows to separate some patients in the high-risk subgroups, with a median survival <3 years, from those with a very mild course with a median survival >25 years. Studies determining how to integrate the results on the multiple new prognostic factors simultaneously are expected to further refine our ability to predict the natural history of CLL and permit tailoring of treatment to individual patients in the future. The analysis of genomic aberrations by Fluorescent In Situ Hybridation (FISH) reveals that approximately 7% of previously untreated patients have a deletion of the short arm of chromosome 17 (17p deletion) corresponding to the TP53 gene locus. Most patients with 17p deletion will have a mutation of the TP53 gene on the other allele and therefore no normal p53 protein. Some patients will have no obvious 17p deletion but will have two TP53 mutations, one on each allele, and therefore have inactivate p53. Moreover, several acquired somatic mutations have been identified in CLL using next-generation sequencing techniques. The most common somatic mutations, found in 5–15% of patients, affect SF3B1, TP53, and NOTCH1. The presence of these mutations is of prognostic value with many treatment modalities. For example, p53 is required for the normal cellular response to the DNA damage resulting from chemotherapy, ie DNA repair, cell cycle arrest, or apoptosis, meaning that these patients do not respond well to chemotherapy, resulting in much poorer outcomes compared with patients with intact p53.[45]

The addition of rituximab to FC or bendamustine does not improve the outcome in patients with p53 mutation or del17p. Because results with the above combination treatment are unsatisfactory, alternative treatments such as alemtuzumab or investigational therapies should be considered.[24,43,46] These patients seem to benefit particularly from the p53 independent activity of the anti-CD52 monoclonal antibody alemtuzumab treatment.

A phase III, randomized, multicenter, international clinical trial comparing alemtuzumab versus chlorambucil in untreated CLL (CAM307) demonstrated superior ORR, CR rates, and PFS.[47] Patients with del17p treated with alemtuzumab had three-fold better ORR and nearly five-fold improvement in median PFS albeit not statistically significant. The major problem with the use of alemtuzumab is its inferior activity in bulky lymph nodes. The larger the lymph node, the inferior the response.[48] The recent trials for 17p deleted CLL combine alemtuzumab with high-dose steroids, prednisone in the UK NCRI CLL206 trial,[49] and dexamethasone in the GCLLSG CLL2O trial,[50] and result in higher response rates compared with historical experience with conventional chemotherapy, even including rituximab-FC. Alemtuzumab has been now approved for use in previously untreated CLL, having been approved initially for fludarabine-refractory patients. Unfortunately, because of the fact that the pharmaceutical company is now developing the drug for use in multiple sclerosis, they have removed the license for CLL, exclusively for marketing reasons. However the drug can be obtained completely free by an international access program. Hopefully, this will be feasible in more studies with careful biological stratification, and adequate clinical and biological long-term follow-ups are required to answer whether single or combined treatment with alemtuzumab might be the approach of choice for first-line therapy in these patients.

On the contrary, in patients carrying mutated NOTCH1, there was no benefit from the addition of rituximab to FC. As the observation that mutations in NOTCH1 may predict a lack of benefit from rituximab awaits confirmation, it would also be important to investigate whether mutated NOTCH1 affects the treatment outcome with other anti-CD20 antibodies or monoclonal antibodies in general.[51]

In summary, 17p deletion and TP53 mutations predicted a particularly poor outcome with CIT; mutated NOTCH1 was associated with no benefit from the addition of rituximab to chemotherapy; and SF3B1 mutations, although neutral in regard to treatment response, were associated with more rapid disease progression in this prospective cohort of patients treated according to standard criteria.

A New Paradigm: Targeted Therapy

B-cell receptor signaling pathway. The past decade brought significant achievements related to the understanding of the molecular aspects of CLL and at the same time revealed new potential targets that resulted in development of novel and more effective therapies to treat this disease. Advances in whole-genomic sequencing identified the relationship between recurrent mutations and clinical evolution of the disease. It is most likely that future therapeutic approaches will incorporate genomic sequencing as part of risk-stratification in CLL for therapeutic purposes. Therefore, treatment algorithms will start to shift from the traditional "one-size-fits-all" approach to an individualized strategy to treat CLL. With this background, it is most likely that in the next few years it will become increasingly challenging to select the correct treatment strategy. We can expect that targeted therapies will be tailored for specific subgroups of patients, using drugs that ideally target either directly or indirectly the genetic and biochemical abnormalities underlying the different forms of malignancy. The BCR signaling pathway represents an exciting potential target in CLL. Signaling through the BCR is thought to be deregulated in CLL and a key mediator of CLL survival proliferation and trafficking. BCR signaling is a complex process, and two types of signals emanate from the BCR: a "tonic" survival signal and an antigen-induced activation signal[52] (Fig. 1). Antigenic stimulation through the BCR is also implicated in the pathogenesis of CLL. The concept here is to interrupt the proliferative and survival signals initiated by the ligation of the BCR. An interesting phenomenon that occurs with the use of kinase inhibitors acting in the BCR signaling cascade is that because of a decrease in chemokines, there is initially a compartment shift so that lymphocytes migrate from lymph nodes into the blood. This results in a dramatic shrinkage of lymphadenopathy but also at the same time an increase in the absolute lymphocyte count. It is important to recognize this pattern of response so that this lymphocytosis is assumed to represent a progressive disease (Table 2).

Bruton tyrosine kinase (BTK) inhibitor. Although BCR signaling antagonists are exciting, the complexity of this pathway suggests that there may be many options for targeted inhibition, and the ideal target or specific kinase inhibitor may have yet to be identified. Moreover, it is possible that CLL and different types of NHL might have divergent ideal target based on their specific disease biology. BTK is essential for activation of several constitutively active pathways of CLL-cell survival, including the Akt extracellular signal-regulated kinase (ERK), and nuclear factor kappa light-chain enhancer of activated B-cells (NF-κB) pathways.[53] Ibrutinib is an orally bioavailable, potent covalent inhibitor of the BTK that binds at cysteine potently inhibiting enzyme activity.[54] The responses to ibrutinib observed in the studies published so far were more durable than expected on the basis of previous experience with other single-agent therapies for relapsed CLL.[55–57]

In a phase 1b-2 multicenter trial, ibrutinib was investigated in order to assess safety, efficacy, pharmacokinetics (PK), and pharmacodynamics (PD) in 85 patients with relapsed or refractory CLL, the majority considered to have high-risk disease. Patients received ibrutinib orally once daily; 51 received 420 mg and 34 received 840 mg. Toxic effects were predominantly grade 1 or 2 and included transient diarrhea, fatigue, and upper respiratory tract infection; hematologic toxicity was minimal.[54] The ORR was the same in the group that received 420 mg and the group that received 840 mg. Ibrutinib was associated with a high frequency of durable remission independent of clinical and genomic risk factors present before treatment, including advanced-stage disease, the number of previous therapies, and the 17p13.1 deletion.

In an open-label phase 1b/2 trial, 31 previously untreated patients aged at least 65 years (median age: 71 years) with CLL or SLL requiring therapy received once daily ibrutinib 420 mg or ibrutinib 840 mg.[55] Objective response was achieved in 22 patients (71%) while 4 of them (13%) had a complete response, 1 (3%) had a nodular partial response, and 17 (55%) had a partial response. Toxicity was mainly of mild-to-moderate severity (grades 1–2). This result could be considered encouraging considering that it was obtained by monotherapy.

In the first comparative open-label, multicenter, phase 3 trial, 391 patients with relapsed or refractory CLL or SLL were randomly assigned to receive daily ibrutinib or the anti-CD20 antibody ofatumumab.[57] The primary end point was the duration of PFS, with the duration of OS and the ORR as the secondary end points.

Ibrutinib at a median follow-up of 9.4 months significantly improved PFS; the median duration was not reached in the ibrutinib group compared with a median of 8.1 months in the ofatumumab group. Ibrutinib also significantly improved OS. The ORR was significantly higher in the ibrutinib group than in the ofatumumab group.

Signaling through the B-cell receptor is mediated in part by the activation of the delta isoform of phosphatidylinositol 3-kinase (PI3Kδ). The delta isoform is one of four catalytic isoforms (α, β, γ, and δ) that differ in their tissue expression, with PI3Kδ being highly expressed in lymphoid cells and the most critical isoform involved in the malignant phenotype in CLL.

PI3K inhibitor. Another BCR inhibitor currently tested in pivotal trial is idelalisib an agent that targets PI3Kδ.[58] In a multicenter, randomized, double-blind, placebo-controlled, phase 3 study, 220 patients with decreased renal function, previous therapy-induced myelosuppression, or major coexisting illnesses

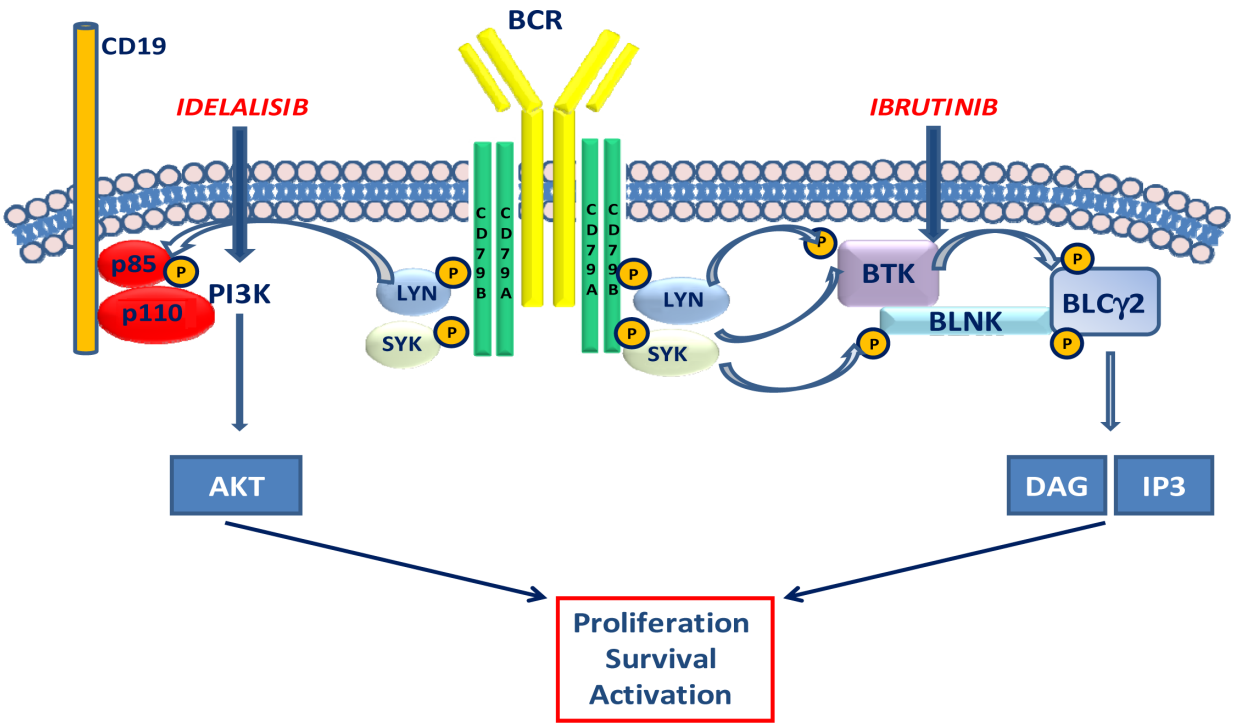

Figure 2. Chronic activation of the BCR engages multiple intracellular pathways.
Notes: PI3K is inhibited by idelalisib; BTK is inhibited by Ibrutinib.

were randomly assigned to receive orally. Idelalisib 150 mg twice daily in combination with rituximab versus rituximab plus placebo.[59] The primary end point of the study was PFS. At the first interim analysis, the study was stopped early because of the overwhelming efficacy reported. The median PFS was 5.5 months in the placebo group and not reached in the idelalisib group. Serious adverse events occurred in 40% of the patients receiving idelalisib and rituximab and in 35% of those receiving placebo and rituximab.

Major criticism of this study is that comparator arm rituximab as monotherapy is not accepted, at least in Europe, as standard treatment for CLL because of its demonstrated low efficacy.

Idelalisib was also evaluated in a phase 1 trial recruiting 54 patients with relapsed/refractory CLL with adverse characteristics including bulky lymphadenopathy, extensive prior therapy, treatment-refractory disease, unmutated Immunoglobuline Heavy Variable Group (IGHV), and del17p and/or TP53 mutations.[60] Patients were treated at six dose levels of oral idelalisib (range 50–350 mg once or twice daily). The most commonly observed grade ≥3 adverse events were pneumonia, neutropenic fever, and diarrhea. The ORR was 72%, all of them were partial remission with or without lymphocytosis. The median PFS for all patients was 15.8 months.

IPI-145 is another oral PI3K inhibitor that inhibits both δ and γ isoforms disrupting PI3K-δ, γ signaling within tumor cells and their interactions with the microenvironment inhibiting CLL-cell survival.[61] A phase 1 study was designed to evaluate the safety, maximum tolerated dose (MTD), PK, PD, and activity of orally administered IPI-145 twice a day in 28-day cycles for relapsed/refractory CLL.[62]

Two expansion cohorts are ongoing and examine 75 mg twice a day and 25 mg twice a day as MTD. To date, 44 CLL patients have been enrolled; there has been no dose-related increase in frequency or severity of adverse events. The most common grade ≥3 events have been transient neutropenia. Reduction in adenopathy occurred early with no apparent dose dependence. Best overall response in evaluable patients has been 52%. The PK/PD and clinical activity suggest that 25 mg twice a day is a biologically active dose in relapsed/refractory CLL, and this dose has been selected for an upcoming randomized phase 3 trial in the same setting.

BCL2 family. The BCL2 family members play an important role in regulating programed cell death and arbitrating the cellular fate through an accurate balance between proapoptotic and prosurvival factors. The opportunity to induce apoptosis by targeting BCL2 proteins is considered a potentially promising therapeutic approach in hematological malignancies, also in CLL where high BCL2 levels have been detected.[63]

ABT-199. ABT-199 is a selective, potent, orally bioavailable, small molecule BH3 mimetic that can trigger apoptosis in vitro, even in del(17p) CLL-cells. The prototype for ABT-199 was ABT-263: this agent binds to BCL-xl in platelets and resulted in thrombocytopenia. Thus, ABT-199 was designed to lack binding to BCL-xl. The main issue in patients with

Table 2. Results of published clinical trials evaluating targeted therapeutic agents used alone or in combination.

[REF.] TREATMENT	TARGETED THERAPY						
	TREATMENT STATUS	NO PTS	ORR* (%)	CR (%)	MEDIAN PFS (MONTHS)	DEL(17) (p13.1) ORR (%)	MEDIAN OS (MONTHS)
[55] Ibrutinib 420 mg/d Ibrutinib 840 mg/d	Relapsed/refractory	51 34	91 86	4 0	75% at 26 mo	68 (1 pts CR)	83% at 26 mo
[56] Ibrutinib 420 mg/d Ibrutinib 840 mg/d**	Untreated	27 4	71	13	96.3% at 24 mo	100 (2/2 pts)	96.6% at 24 mo
[57] Ibrutinib 420 mg/d vs Ofa 300 mg w1; 2000 mg/w for 8 w → 2000 mg/mo for 4 mo	Relapsed/refractory	195 196	62.6 4.1	0 0	Not reached at 9.4 mo 8.1 mo	n.s.	90% at 12 mo 81% at 12 mo
[59] Rituximab 375 mg/m^2 first dose →500 mg/m^2 every 2 w for 4 doses → every 4 w for 3 doses + placebo vs Rituximab + Idelalisib 150 mg bid	Relapsed	110 110	81 13	0 0	**5.5 mo Not reached at 24 mo	n.s.	80% at 12 mo 92% at 12 mo
[60] Idelalisib, 50 to 350 mg once or twice daily	Relapsed/refractory	54	72	0	15.8 mo	54	75% at 36 mo
[62] IPI-145, 8 to 75 mg bid	Relapsed/refractory	155	52[1] 53[2]	1/31 pts 1/19 pts	n.s.	n.s.	n.s.
[64] ABT–199, 150 to 1200 mg/d	Relapsed/refractory	56	84	20	n.s.	n.s	n.s.

Notes: *CR/PR/PR with lymphocytosis; **Arm closed before end of study; [1]for evaluable patients; [2]patients treated with ≤25 mg bid.
Abbreviations: Ofa, Oftumumab; ORR, overall response rate; CR, complete response; PFS, progression free survival; OS, overall survival; FA-ref, Fludarabine reractory; BF-ref, bulky-fludarabine refractory; D, day/s; w, week/s; mo, month/s; bid, twyce p day; pts, patients; n.s., not specified.

CLL has been tumor lysis syndrome (TLS), and precaution must be taken to deal with this, particularly in patients with bulky disease. Results of a phase I, dose-escalation study have been recently reported with the aim to evaluate the safety and PK, determine a MTD, and a recommended phase 2 dose of ABT-199. Secondary objectives were to assess efficacy and to explore biomarkers for response.[64] Modifications were made to the dose-escalation scheme and TLS prophylaxis and monitoring schedule after TLS was observed in some patients. ABT-199 showed activity in patients with relapsed and refractory CLL with a response rate of 84% for the study population, including 20% CR/CRi. Notably, patients with high-risk CLL showed similar efficacy with a response rate of 82% in del(17p) and 78% in F-refractory disease. A phase 2 monotherapy study in patients with relapsed del(17p) CLL as well as combination studies with either rituximab or obinutuzumab in patients with relapsed CLL have commenced.

Adoptive Cell Therapy, Chimeric Antigen Receptor (CAR)

Among these numerous new investigational drugs and immunotherapies targeting CLL, a unique approach CAR is the genetic modification of T cells to B-cell antigens through the gene transfer of a CAR, which is composed of an antigen-binding component fused to T-cell signaling domains. A patient's own T cells are genetically modified and then adoptively transferred back to the patient to mediate killing of malignant, and normal, B-cells. Over the past 10 years, this technology has transitioned from preclinical models to clinical trials, with evidence of promising results.[65] In most trials, CAR-modified T cells are generated ex vivo and include an initial activation step followed by a gene-transfer step. Basically, the process consists of a leukapheretic isolation of leukocytes from the peripheral blood of the patient while T cells are enriched and activated with anti-CD3 and/or anti-CD28 ligation. Gene transfer can be accomplished by retroviral transduction, electroporation, RNA transfection, or via transposase activity. Afterward, CAR-modified T cells are expanded and ultimately adoptively transferred back into the patient.

The early reports from published trials in patients with relapsed and/or refractory CLL clearly demonstrate the potential of CAR-modified T-cell therapy.[66,67] Significant work is required to determine the optimal CAR design, prior conditioning regimen, and gene-transfer methodology to rationally design second-generation clinical trials to treat CLL patients. An optimized methodology could support the application of CAR-modified T cells at an earlier stage of disease progression by increasing the number of patients with complete molecular remissions, long-term disease control, and/or delaying the start of subsequent salvage therapies.

Conclusion

Two recently published reports demonstrate that survival in CLL patients has significantly improved over time, comparing cohorts of patients from the 1980s and 2000s.[68,69] Phase 3 trials conducted in the 1990s established that fludarabine-based therapy offered superior response rates and PFS compared with alkylating agent-based therapy. Randomized controlled trials subsequently demonstrated that the combination of FC further improved response rates and PFS. These advances were followed by the development of CIT combining anti-CD20 monoclonal antibodies (mAbs) with purine nucleoside analogs. A seminal phase 3 trial conducted by the GCLLSG demonstrated that the addition of the anti-CD20 mAb rituximab to the FC platform (FCR) improved not only response rates and PFS but also OS.

However, traditional chemotherapy is associated with acute and long-term toxicity and is considered undesirable by most patients. In recent years, interest in non-chemotherapeutic approaches has been stimulated by an increasing number of agents that target cell surface, intracellular pathways, and the tumor microenvironment. Despite significant progress, CLL remains an incurable disease, and the role of novel agents in the first line for both physically fit and frail patients or those who relapse after previous treatment is being explored in clinical trials. The challenge will be to develop scientifically rational combinations of these agents that will probably (that are likely to) vary on a disease and even patient basis. Nevertheless, effective, well-tolerated, non-chemotherapeutic approaches have great potential to lead to individualized strategies that will not only improve the outcome of patients with CLL but will render cytotoxic approaches as only of historical interest. Patients with high-risk, early stage disease have a high likelihood of early disease progression and death from CLL. Patients in this group should be referred for participation in clinical trials evaluating the efficacy of new treatment and perhaps early intervention. Apart from clinical trials, standard treatments, notably CIT in most patients with CLL, should remain the treatment of choice even if it is hoped that continued efforts to develop new agents and strategies will ultimately lead to the cure of this disease.

Author Contributions

Conceived the concepts: MM. Analyzed the data: MM and AT. Wrote the first draft of the manuscript: MM. Contributed to the writing of the manuscript: MM, AMF, PP and AT. Agree with manuscript conclusions: MM, AMF, PP and AT. Jointly developed the structure and arguments for the paper: MM and AT. Made critical revisions and approved final version: AT, AMF, PP. All authors reviewed and approved of the final manuscript.

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