Review

Chronic Lymphocytic Leukemia: Current Concepts

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Abstract. *Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in adults, and while in early, asymptomatic stages treatment is not indicated, the threat to the quality of life and increased mortality of patients posed by more advanced-stage disease necessitate therapeutic intervention. Guidelines of when and how to treat are not well-established because CLL is a disease of the elderly and it is important to balance preservation of functional status and control of the disease. Advances in molecular and genetic profiling has led to the ability to identify sub-groups of patients with CLL whose disease may respond to selected therapy. This review discusses current standard therapies in the major sub-groups of CLL based on age and functional status, in both the front-line and relapsed/refractory settings. It also provides a concise review of novel agents that have shown considerable efficacy in CLL.*

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in the Western world (1). It affects around 4 in 100,000 of the population in the United States, with more than 15,000 people being diagnosed with CLL each year, and it is estimated that 4,500 die of the disease per year. It is more common in males, with a median age of diagnosis of 70 years (2). Caucasians are affected more than African–Americans, Asians and Pacific Islanders. Patients may have an ethnic or familial predisposition for developing CLL, as the incidence of CLL is higher among patients with an affected first-degree relative, and approximately 5%-10% of patients with CLL have a family history of lymphoid malignancies (3).

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Diagnosis

CLL and small lymphocytic lymphoma (SLL) are genetically similar, but SLL lacks peripheral blood lymphocytosis. Typically, CLL presents as lymphocytosis, with symptoms related to lymphadenopathy, splenomegaly, cytopenia, or fatigue. The iconic finding on peripheral blood smear is the 'smudge' cell, a fragile B-cell which appears smudged when smeared on a slide. Typically, CLL cells seen on peripheral blood smear are small, mature-appearing lymphocytes (3, 4). The diagnosis of CLL is usually established on peripheral blood using flow cytometry. CLL is characterized by the presence of a monoclonal population of B-cells expressing low levels of surface immunoglobulin, either kappa or lambda light chains, as well as expression of CD19, CD20, and CD23 with aberrant expression of CD5 (a T-cell marker), and lack of CD10 expression (3, 5, 6). The immunohistochemical staining pattern of CLL and other non-Hodgkin lymphomas are summarized in Table I. On lymph node biopsy, small lymphocytes with condensed chromatin and round nuclei are found along with larger lymphoid cells (prolymphocytes), clustered in pseudofollicles or proliferation centers (3). The diagnostic criteria for CLL that were developed by the International Workshop on chronic lymphocytic leukemia (IWCLL) are summarized in Table II (6).

Prognostic Factors

Traditionally, the Rai and Binet staging systems, in addition to laboratory parameters and patient characteristics have been used for prognostication in CLL (3, 7, 8). However, more recently, a new group of prognostic parameters has been established.

Immunoglobulin variable heavy chain gene (*IGVH*) hypermutation is seen in 50-55% of patients with CLL. Patients with unmutated *IGVH* have a shorter median survival of 5-10 years compared to 10-20 years in patients with mutated *IGVH* (9). *IGVH* status does not appear to change with disease progression. Seventy percent of patients with

Table I. *Flow cytometry patterns of non-Hodgkin lymphoma.*

Differential	sIg	CD20	CD5	CD23	CD10	CD103
Chronic lymphocytic leukemia	Dim	Dim	+	+	−	−
Lymphoplasmacytic Lymphoma	Mod	+	−/+	+/−	−	−
Mantle zone lymphoma	Mod	+	+	− (Partial)	−	−
Marginal zone: nodal/MALT lymphoma	+	+	−	−/+	−	−
Splenic marginal zone lymphoma	+	+	−/+	−/+	−	−/+
Follicular lymphoma	+	+	−	−/+	+/−	−
Hairy cell leukemia	+	+	−	−	−	+

Table II. *Diagnostic criteria for chronic lymphocytic leukemia, monoclonal B-cell lymphocytosis and small lymphocytic lymphoma according to the International Workshop on chronic lymphocytic leukemia guidelines (6).*

a: B-Cell count ≥5,000 μ/l (5×10^9/l) in peripheral blood for >3 months, with a dominance of morphologically mature-appearing small lymphocytes
b: Flow cytometry demonstrating B-cell number, and clonality of B-cells with a particular phenotype:
- Low level of surface immunoglobulin
- Either κ- or λ-light chain expression
- $CD5^+$, $CD19^+$, $CD20^+$ (dim), $CD79b^+$ (dim)

germline IGVH express CD38, and 70-80% express zeta-chain associated protein kinase 70 (ZAP70), an intracellular tyrosine kinase. CD38 and ZAP70 expression are considered poor prognostic indicators, and their expression can change throughout the disease course.

The median survival for patients with ZAP70 expression is 8-9 years *versus* 24 years for those patients who lack ZAP70 expression (3, 10-12). ZAP70 is typically expressed in T- and natural killer (NK)-cells, and is thought to mediate T-cell receptor signaling. The mechanism by which ZAP70 is expressed in CLL B-cells is unknown, but it appears to augment B-cell receptor signaling, resulting in a more aggressive CLL phenotype (13). ZAP-70 expression appears to be a surrogate marker for unmutated IGVH status, but it does occur in mutated IGVH CLL (14).

While ZAP70 expression is currently used for risk stratification, *ZAP70* methylation status appears to be a stronger prognostic indicator based on the results of a recently published study. Claus and colleagues discovered differential methylation of a single CpG dinucleotide 223 bp downstream of the transcription start site (CpG+223) in exon 1 of *ZAP70* in CLL cells. Methylation status was determined with MassARRAY pyrosequencing in 295 untreated patients with CLL and its impact on clinical outcome was evaluated in comparison to other known prognostic markers, ZAP70 expression, CD38 positivity, and *IGVH* mutational status. It was found that low methylation status was associated with positive ZAP70 expression and worse outcome. Interestingly, 57 out of 135 (42%) patients without ZAP70 expression had low levels of methylation. This group of patients, traditionally considered at low risk based on ZAP70 expression negativity, had worse outcome compared to their ZAP70-positive counterparts (15). Thus, *ZAP70* methylation assessment is being further studied in CLL trials, including an ongoing phase III study of ibrutinib in untreated, older patients with CLL (NCT01886872).

Fluorescent *in situ* hybridization (FISH) studies are also helpful in providing prognostic information in CLL. Over 80% of patients have chromosomal abnormalities, most commonly involving 13q15 (45-55% of cases), 11q22-23 (18%), chromosome 12 (16%), and 17p13 (7%) (16). Table III summarizes current, well-established molecular prognostic indicators (16, 17).

A new comprehensive prognostic index has been validated based on data combined from three prospective, randomized studies of 1,948 patients with CLL. On multivariate analysis, eight factors were found to be independent predictors of overall survival (OS) including sex, age, eastern cooperative oncology group (ECOG) status, deletion 17p, deletion 11q, *IGVH* mutational status, serum β2-microglobuin level, and serum thymidine kinase level (18). These factors formed the basis for a new risk scoring system and classification of patients into four prognostic groups, as shown in Table IV.

Pathophysiology and Molecular Mechanisms

CLL is thought to originate from antigen-exposed B-cells in lymphoid tissue. *IGVH*-mutated CLL is thought to arise from germinal center B-cells that have undergone T-cell-mediated somatic hypermutation, a process by which B-cell affinity to

Table III. *Prognostic markers.*

Good prognosis	Intermediate prognosis	Poor prognosis
Mutated *IGVH* Sole 13q deletion	Normal karyotype Trisomy 12 11q Deletion	Germline IGVH +CD38 +ZAP70 Multiple chromosomal abnormalities 17p Deletion

IGVH, Immunoglobulin variable heavy chain gene.

Table IV. *New chronic lymphocytic leukemia risk scoring system: del 17p=6 points; serum thymidine kinase level>10 U/l=2 points; β2-microglobulin level>3.5 mg/l=2 points; male gender, age>60 years, ECOG performance status>0, del(11q), and unmutated immunoglobulin variable heave chain gene each=1 point (18).*

Risk group	Risk score	6-Year overall survival
Low	0-2	94.8%
Intermediate	3-5	80.4%
High	6-10	55.6%
Very high	11-14	15.0%

antigen is amplified, and clonal expansion in the dark zone of the germinal center, followed by further selection of antigen-binding B-cells in the light zone. In *IGVH*-unmutated CLL, naïve B-cells undergo a T-cell-independent process that does not involve somatic hypermutation that increases B-cell affinity to antigen. In CLL, resultant antigen-experienced memory/marginal zone B-cells from both pathways become continuously activated by chronic antigen stimulation and acquisition of genetic lesions, leading to monoclonal B-cell lymphocytosis (MBL). Further accumulation of genetic abnormalities and oncogenic transformation of MBL cells lead to frank CLL (19). Interestingly, MBL has been reported in 6% of the elderly population, and 1-2% of patients with CLL are thought to have had a preceding MBL (20).

There is emerging data in gene-expression profiling that suggest a key role of auto-antigen activation of the B-cell receptor (BCR) pathway in the pathogenesis of CLL (21). The BCR is a ligand-binding structure composed of surface membrane immunoglobulins that are paired with a signal transduction structure composed of CD79a/CD79b. When a ligand binds to the BCR, phosphorylation of CD79a/CD79b occurs *via* spleen tyrosine kinase (SYK) and the tyrosine-protein kinase "LYN". This leads to the activation of Bruton's tyrosine kinase (BTK) pathway. Concurrently, LYN tyrosine kinase phosphorylates and activates the cytoplasmic portion of CD19, leading to the recruitment and activation of phosphoinositol 3-kinase (PI3K). Activation of the BCR and its downstream pathways leads to changes that promote B-cell survival and proliferation (22). In normal cells, the BCR can be activated by or be independent of ('tonic' BCR signaling) antigen-binding, both of which lead to a signaling cascade event allowing for selection, proliferation, and differentiation of the B-cell for antibody production against a specific antigen (23). In contrast to other B-cell lymphomas, CLL exhibits a phenomenon of BCR stereotypy. Analysis of large pools of CLL tumors from different patients has led to the discovery that all CLL tumors express a specific set of BCRs, regardless of *IGVH* mutational status. These data suggest that there may be a group of specific antigens that induce clonal proliferation of CLL cells (19). Our increasing knowledge of the BCR pathway has led to the discovery and development of new therapeutic targets in CLL, as will be discussed shortly.

Treatment Strategies

Newly-diagnosed CLL. In newly-diagnosed CLL, the clinician's challenge is to identify the patient who needs treatment. A meta-analysis performed in 1999 showed that patients with asymptomatic early-stage disease (Rai 0, Binet A) do not appear to benefit from alkylating agent-based therapy immediately upon diagnosis (24). Interestingly, it has been suggested that even patients with Rai intermediate risk or Binet stage B may be monitored without the initiation of therapy in the absence of active disease. Therefore, the IWCLL developed guidelines to better-define active CLL and to outline indications to start therapy, as summarized in Table V (6).

When treatment is indicated, first-line therapy is often determined by the patient's performance status and risk group. According to the National Comprehensive Cancer Network (NCCN) guidelines, high-risk disease is defined by the presence of deletion in 17p, and performance status is determined by the joint assessment of the patient's age and comorbid conditions (25). The current literature supports the use of chemo-immunotherapy (CIT) over single-agent therapy given the clear OS benefit with CIT. Purine analogs, such as fludarabine and pentostatin, and alkylating agents, including bendamustine, and cyclophosphamide combined with the targeted anti-CD20 immunologic agent, rituximab, represent the most commonly used regimens (26).

For otherwise healthy patients less than 70 years old without 11q or 17p deletion, first-line treatment options include the following regimens: fludarabine, cyclophosphamide, and rituximab (FCR); fludarabine and rituximab (FR); pentostatin, cyclophosphamide, and rituximab (PCR); bendamustine and rituximab (BR); and obinutuzumab with chlorambucil (O-Clb). A large randomized phase III trial compared fludarabine and cyclophosphamide (FC) to single-

Table V. *Indications to treat chronic lymphocytic leukemia according to International Workshop on chronic lymphocytic leukemia guidelines (6).*

- Bone marrow failure: worsening of anemia, thrombocytopenia, or both
- Enlarging, symptomatic, or massive splenomegaly defined as ≥6 cm below left costal margin
- Enlarging, symptomatic, or massive lymphadenopathy defined as ≥10 cm in longest diameter
- Progressive lymphocytosis
    - Increase of >50% over a 2-month period#
    - Lymphocyte doubling time of <6 months*
- Autoimmune anemia, thrombocytopenia, or both
    - Unresponsive to corticosteroids or other standard therapy
- Constitutional symptoms
    - Unintentional weight loss of ≥10% within 6 months
    - Significant fatigue
    - ECOG PS 2 or worse
    - Fevers ≥38.0˚C for 2 or more weeks#
    - Night sweats for ≥1 month#

*This criterion cannot be used if initial lymphocyte count is $<30\times10^9$/l; in any event should not be used as a sole indication to treat. #Infections should be excluded.

agent fludarabine. The use of FC improved the overall response rate (ORR) and complete response rates (CR), without a significant increase in OS (27). The cancer and leukemia group B (CALGB) 9712 trial studied the efficacy of FR, and outcomes were compared to those of the CALGB9011 trial, which studied fludarabine alone (28-30). FR resulted in improved 2-year progression-free survival (PFS) and OS probabilities with similar risk of infection when compared to fludarabine alone (31). A phase III trial compared FCR to FC, and showed a benefit in overall response rates (ORR) and CR rate, although toxicity was increased in the FCR group, such as grade 3 or 4 neutropenia. These results led to the United States Food and Drug administration approval of the use of rituximab in combination with FC and fludarabine in the first-line treatment of CLL in this population. The Oncology Research group studied FCR *versus* PCR where both regimens yielded similar ORR and side-effect profiles, with the most severe reported adverse event (AE) of neutropenia (32). Bendamustine is an alkylating agent with different cytotoxic mechnisms of action, and hence it has low cross-resistance with drugs in the same class (33). When single-agent bendamustine resulted in improved CR rate and PFS compared to chlorambucil, combination BR underwent further study by the German CLL Study Group (34). BR resulted in a high ORR (88%) and CR rate (23%), but had decreased efficacy in patients with deletion of 17p (35).

Obinutuzumab, an anti-CD20 monoclonal antibody, was approved as first-line therapy in combination with chlorambucil after preliminary outcome data were reported from the German CLL Study Group CLL11 trial. Of note, the median age of enrolled patients was 73 years and included those with comorbid conditions. The median Cumulative Illness Rating Scale (CIRS) was 8 (range=0-22). The three-arm trial compared obinutuzumab and chlorambucil O-Clb, chlorambucil alone, and rituximab plus chlorambucil (R-Clb). The use of O-Clb improved ORR, CR, and PFS when compared to chlorambucil alone. O-Clb was also superior to rituximab plus chlorambucil in terms of ORR (36). Updated results of the CLL11 trial were recently published (37). There was a statistically significant OS benefit with the use of O-Clb and R-Clb over chlorambucil monotherapy, but no significant OS difference between the two monoclonal antibody arms, attributed to immature data. However, the PFS and time to next antileukemic treatment was significantly improved by 14 months and 10 months, respectively, with O-Clb compared to R-Clb.

For healthy patients with CLL less than 70 years of age who have 11q deletion, CIT such as FCR, BR, PCR, or O-Clb, is recommended. While the presence of the 11q deletion is generally deemed a poor prognostic factor, it appears that cyclophosphamide-containing CIT regimens can overcome the adverse influence of this genetic abnormality (38, 39).

FCR, FR, high-dose methylprednisolone with rituximab (HDMPR), O-Clb, and alemtuzumab-based regimens are among recommended first-line options in young, healthy patients with high-risk disease defined by the presence of 17p deletion. FCR, FR, and O-Clb, as discussed above, are active in high-risk CLL with 17p deletion. However, responses are often not durable and most patients will need second-line therapy and consideration of allogeneic stem cell transplantation (HSCT) upon disease relapse. Alemtuzumab with rituximab (AR) and HDMPR have been well studied in patients with relapsed or refractory CLL, including those with 17p deletion. Given their activity in patients with relapsed or refractory CLL with 17p deletion, which will be discussed in further detail in the next section, AR and HDMPR have been approved for use as front-line therapy in these high-risk patients (40, 41). Ibrutinib is a novel oral BTK inhibitor that recently approved for use in the first-line setting in patients with 17p deletion and will be discussed in further detail.

Although generally not used in the first-line setting, HSCT can be considered in a select group of younger patients with CLL, given the sub-optimal outcomes with standard CIT (42).

Relapsed/refractory CLL. In spite of the recent progress that has been made in established first-line treatment, CLL remains incurable, with disease progression occurring in most cases. There is no standard approach in the management of patients with relapsed or refractory CLL. Indications to treat in the relapsed or refractory setting are generally the same as in the front-line setting, as described by the IWCLL (6). Asymptomatic patients or those with Rai stage 0 or Binet stage A should be managed with active surveillance. Treatment choice depends, among other factors, on the patient's age, performance status, risk group, previous therapy, and duration of response to previous therapy. Re-treatment with previously used agents, commonly purine-based therapy, can be considered in those patients who achieved a durable response for over 1-2 years. Unfortunately, options are more limited for patients who have 17p deletion or *TP53* mutation. Patients with fludarabine-refractory disease have a much poorer prognosis compared to their fludarabine-sensitive counterparts, with a median survival of less than one year for the former group (43). Interphase FISH is helpful in confirming the presence of high-risk mutations, such as del11(11q12) and del17(17p13) that imply resistance to conventional chemotherapeutic agents and shorter median survival of less than 2-3 years (38). Thus, the identification of these mutations is imperative prior to re-initiation of chemotherapy because these patients are often best served by enrolling them into a clinical trial or considering them for an HSCT.

Among commonly-used regimens in relapsed/refractory Chronic lymphocytic leukemia (R/R-CLL), is the FCR regimen which has been shown to induce high response rates in this setting. An earlier study evaluated 177 patients with previously-treated CLL who were treated with FCR. The ORR was 73%. The estimated median survival for all patients was 42 months. Median survival for CRs and nodular partial remissions (PRs) were not reached, but were estimated to be greater than, or equal to, 45 months, and 30 months, respectively (44). The results of the Rituximab in the Study of Relapsed Chronic Lymphocytic Leukemia (REACH) study, a randomized, phase III trial comparing FCR with FC in 552 patients with previously treated CLL were published in 2010. All patients were treated with fludarabine and cyclophosphamide and 276 of these patients were randomly assigned to receive rituximab. Both arms included patients who were alkylator-refractory and fludarabine-exposed. At a median follow-up time of 25 months, FCR was shown to significantly improve the median PFS by 10 months, compared to FC. The addition of rituximab also improved ORR, CR rate, and median duration of response. Time to new CLL treatment and median OS were not reached for the FCR group, compared to 34.3 months and 52 months, respectively for the FC arm, though the difference in median OS was not statistically significant. The proportions of patients who discontinued treatment as a result of an AE were similar in both arms. Grade 3 and 4, serious, and fatal AE rates were higher in the FCR group, however the overall incidence of infections were similar in the two arms (45). Another study evaluating the use of FCR in patients with relapsed or refractory CLL, the ORR was 74% with a CR rate of 30%. Overall, the median PFS was 21 months and the estimated median OS was 47 months, however patients with fludarabine-refractory disease had a lower ORR and CR rate, as well as a significantly decreased median PFS and OS rates compared to fludarabine-sensitive patients. Also appreciated in this study were worse outcomes in patients with chromosome 17 abnormalities treated with FCR. In this sub-group of patients, the ORR was 35%, with no CRs; the median PFS was 5 months and the median OS was 10.5 months (46). Based on these findings, the investigators in that study concluded that FCR should be used in carefully-selected patients with R/R-CLL who are considered fludarabine-sensitive, have received fewer than four prior regimens, and do not have chromosome 17 abnormalities.

Among the purine analogs, which include fludarabine, cladribine and pentostatin, the latter appears to cause less myelosuppression than the more widely-studied fludarabine, making the PCR regimen an attractive option. Rituximab was added to the combination of pentostatin and cyclophosphamide (PC) based on the activity of PC in heavily pre-treated patients with CLL demonstrated in a small prospective study of 23 patients who had received a median of three prior therapies. The response rate was 75%, including four CRs, 12 PRs, and one nodular PR (47). The addition of rituximab to PC was then studied in 32 previously treated patients with CLL. Rituximab was omitted for the first cycle to prevent severe infusion reactions that appear to more commonly occur in patients with severe lymphocytosis (48). Although this was a single-arm study of PCR, this regimen appeared to be superior based on previously reported outcomes of PC therapy alone, given the improved response duration and survival outcomes with the addition of rituximab. Of the 32 patients with CLL that were treated with PCR, 24 had a response, including eight patients who had a CR. Responders had a median duration of response of 25 months and the overall median OS for all treated patients was 44 months. PCR was generally well-tolerated, with myelosuppression being the most common grade 3 to 4 toxicicty. Grade 3 to 4 infections occurred in nine patients, eight of whom had pneumonia and one died from pneumonia (49).

The remaining regimens shown to be active in R/R-CLL that are briefly discussed are combination of oxaliplatin, fludarabine, cytarabine, and rituximab (OFAR), BR, HDMPR, and lastly, alemtuzumab-based regimens. The OFAR regimen was studied in a phase I/II trial of 30 fludarabine-refractory

patients with CLL and 20 patients with Richter's Syndrome. The median number of OFAR cycles completed was two and the ORR was 33% in the heavily pre-treated, fludarabine-refractory CLL group. There were two CRs and eight PRs in the 26 patients with CLL that received OFAR with the maximum tolerated oxaliplatin dose. Of note, 15 of the patients with CLL had documented 17p deletions, and among these high-risk patients, one achieved a CR and four achieved a PR. The 6-month survival rate was 89% for all patients and 86% in those with 17p deletion. Twelve patients with CLL and two with Richter's Syndrome underwent HSCT after OFAR and 10 of these patients were alive at 1 year. The most common toxicities were hematological in nature, with fatigue and nausea as the most common non-hematological adverse events. OFAR appeared to be well-tolerated in patients of 70 or more years of age (n=17) and the ORR in this subgroup of patients was 50% (50).

The combination of bendamustine and rituximab is also active in relapsed or refractory CLL, as demonstrated in a German phase II trial of 78 patients who were treated with planned six cycles of BR. The ORR was 59%, including 9% who achieved a CR. The ORR in the fludarabine-refractory group of 22 patients was 45.5%. At a median follow-up of 24 months, the OS was 34 months, but patients with the 17p deletion had worse outcomes and an OS of only 16 months (51). The final analysis of a phase III study comparing BR with chlorambucil, plus rituximab in patients with CLL is pending, but results from an interim analysis were reported at the American Society of Hematology Annual Meeting in 2012. Out of the 126 patients that were included in this interim analysis, 41 patients had received one prior therapy. There was a trend toward improvement in ORR and CR rate in the BR arm compared to the chlorambucil arm (52).

In a small study of 14 advanced, heavily pre-treated patients with CLL who received HDMPR, the ORR was 93%, which included a CR rate of 14%. The median survival was 20 months. Despite the use of infection prophylaxis, the most common AE was infection, including fungal pneumonias, varicella zoster virus infection, and septicemia (53).

Alemtuzumab is a humanized IgG1γ monoclonal antibody to CD52 that was FDA-approved for use in relapsed or refractory CLL in 2001 based on the results of CAM211, a phase II trial of single-agent therapy in 93 patients with relapsed or refractory CLL. The ORR was 33% and stable disease was achieved in 54% of patients. At a median follow-up of 29 months, 27 patients were alive and the median OS was 32 months for responders. The disease responded across all prognostic sub-groups, including fludarabine-refractory and heavily pre-treated patients. Predictors of poor response to alemtuzumab therapy were Rai stage IV disease, WHO performance status of 2, and bulky lymphadenopathy (presence of at least one lymph node more than 5 cm in diameter). The most common adverse events were infusion-related, grade 1 or 2 in severity and included rigors, nausea, vomiting, and rash. These infusion-related events generally decreased over time (54). Alemtuzumab has also been studied in combination with fludarabine, FC (FC-CAM), (56) and FCR (FCAR), all which have shown promising results with overall response rates ranging from 53-82% (55, 56, 57). However, the combination of systemic chemotherapy to alemtuzumab is fraught with significant toxicity. The consequences of alemtuzumab combination chemotherapy were demonstrated when recruitment for a phase III trial comparing FCR and FC-CAM in medically-fit, untreated patients with CLL (n=165) was halted prematurely due to excess toxicity of the FA-CAM regimen as evidenced by eight deaths in this treatment arm. This trial also failed to show superiority of FC-CAM over FCR in the front-line setting of fit patients with CLL (58). It is well-known that alemtuzumab-based regimens are plagued by a high rate of infectious complications, including opportunistic infections with Pneumocystis jirovecii and cytomegalovirus, such that anti-infective prophylaxis is imperative in patients receiving alemtuzumab. In an effort to improve upon the FCR regimen, FCAR was evaluated in a phase II study of 80 heavily pre-treated, patients with relapsed or refractory CLL with high-risk disease. Of the 79 patients who were assessable for response, there were 21 CRs (27%), three nodular PRs (4%), and 29 PRs (37%), adding up to an ORR of 67%. The median OS and PFS were 16.6 and 10.6 months, respectively, for all patients, and for those who achieved a CR, median OS and PFS were 67 and 28 months, respectively. As one would expect, the rate of grade 3-4 hematological toxicities and infections, most commonly pneumonia, was significantly high. Seven patients died of infectious complications during FCAR therapy and in the follow-up period. In spite of these results, it remains unclear whether FCAR is superior to FCR for salvage therapy as the two regimens were not directly compared and a matched-pair analysis by the investigators failed to demonstrate an improvement in survival, even in high-risk patients (59).

Lenalidomide in combination with rituximab is another active regimen in relapsed or refractory CLL. Based on the activity of single-agent lenalidomide noted in several phase II studies showing ORRs ranging from 32% to 47, a phase II trial evaluated the addition of rituximab to lenalidomide in 59 patients with relapsed or refractory CLL (60). The ORR was 66% which included 12% of patients who achieved a CR. The median time to treatment failure was 17 months and the median OS was not reached. The 3-year OS was estimated to be 71%. Remarkably, the ORR for the sub-group of patients with the 17p deletion did not significantly differ from patients without this high-risk chromosomal aberration. However, patients considered fludarabine-refractory appeared to flare worse than patients who were fludarabine-sensitive with ORR of 33% and 70%, respectively, in each sub-group (61).

Unfortunately, lenalidomide monotherapy and lenalidomide-based regimens are fraught with significant AEs, including tumor lysis syndrome and tumor flare reactions, and therefore are not widely used.

Ofatumumab is a monoclonal antibody to human CD-20 with well-documented activity in patients with fludarabine-and alemtuzumab-refractory CLL. Ofatumumab was FDA-approved in the United States in 2009 for use in patients with relapsed or refractory CLL based on results from an interim analysis of an international, single-arm study of 138 patients with CLL. Patients were considered fludarabine and alemtuzumab-refractory (n=56) or fludarabine-refractory with bulky lymphadenopathy (n=79). ORR were 58% and 47% in each subgroup, respectively. Median OS was 13.7 months in the fludarabine and alemtuzumab-refractory group and 15.4 months in the fludarabine-refractory with bulky lymphadenopathy group (62). Results from the final analysis of the study were reported in 2010, which included 206 enrolled patients. The ORR by Independent Endpoint Review Committee evaluation was 51% for the fludarabine and alemtuzumab-refractory group and 44% for the group with bulky lymphadenopathy. All responses were PRs except for two patients in the group with bulky lymphadenopathy who achieved a CR. OS was 14.2 months and 17.4 months in the two groups, respectively. Ofatumumab was generally well-tolerated. Grade 1-2 infusion reactions were common after the first and second doses of ofatumumab. Fatal infections occurred in 8% of patients, 13% in fludarabine and alemtuzumab-refractory group and 5% in the group with bulky lymphadenopathy (63).

There is no standard approach to the treatment of relapsed or refractory CLL, but as described above, clinical responses and improved survival can be achieved with the use of conventional combination regimens. Unfortunately, responses are often not durable and the majority of patients with relapsed or refractory CLL who respond to salvage chemotherapy will experience subsequent relapse without a HSCT. However, there are a number of novel biological agents that have shown unprecedented efficacy and are changing the landscape of relapsed and refractory CLL and providing a way for patients to enjoy both longevity and quality of life while avoiding the rigors of undergoing HSCT. These novel agents will be discussed in further detail later in our review.

Frail and elderly patients with CLL. The treatment of frail and elderly patients with CLL remains a challenge, often because of poor performance status, comorbid conditions, as well as their advanced age, which is a predictor of poor outcome in patients with CLL (64,65). About 70% of patients are diagnosed at an age of 65 years or more (66, 67). Patients with comorbid conditions or of advanced age are generally under-represented in clinical trials, but data are available on the use of some of the aforementioned regimens in this specific cohort of patients. It is also worth mentioning that published studies suggest that standard front-line regimens, including FCR and PCR, should be considered in elderly patients with minimal comorbidity, good functional status, and with reasonable life expectancy (68, 69). In fit, elderly patients, the management approach should take disease stage and prognostic factors, including high-risk molecular and cytogenetic features, into consideration as with younger patients with CLL. The CIRS and creatinine clearance are used as eligibility criteria for clinical trials by the German CLL Study Group, who investigated the use of the CIRS in predicting mortality and treatment toxicity among 817 patients who were enrolled in the CLL8 trial (70, 71). Eight percent of patients had more than three organ systems affected by comorbidity and 18% with at least one organ system affected by comorbidity of moderate or higher grade. Higher CIRS scores were associated with increased mortality and, more specifically, the number of involved organ systems and organ-specific severity scores were the most powerful independent predictors of mortality (72). Thus, CIRS can be a useful tool for identifying elderly patients with CLL who are potential candidates for more intensive treatment regimens.

The number of treatment options for patients with significant comorbidities continues to expand. The findings of one analysis published in February 2013 specifically looked at the outcome of elderly patients from four frontline CALGB CLL studies: 9011, 9712, 19901, and 10101 (30, 29, 73, 74). Evaluated treatment regimens included single-agent chlorambucil or fludarabine on CALGB 90114, FR on CALGB 97127, fludarabine with alemtuzumab consolidation on CALGB 1990114, and FR with alemtuzumab consolidation CALGB 1010115. This study showed that although fludarabine improves response rates, it does not appear to offer a survival advantage in comparison to chlorambucil in patients aged 70 years or older. This finding was consistent with the results of a previous study comparing fludarabine and chlorambucil (75). In a multivariate analysis, age was not a significant predictor of response across all treatment groups. While OS was significantly different across the treatment regimens for patients younger than age 70 years, there were no differences in OS for patients 70 years of age or older. Overall, the addition of rituximab to fludarabine improved OS, which was not dependent on age. The addition of alemtuzumab consolidation to fludarabine or FR did not provide a substantial OS benefit across all patients, as well. The authors of that study concluded that single-agent chlorambucil is a safe and effective treatment in frail patients with CLL, but that the combination of R-Clb warrants further investigation (76). The results of a phase II trial evaluating R-Clb with and without rituximab maintenance in the first-line treatment of elderly patients with CLL (n=97) were recently published (77). At the end of induction with R-Clb, responding patients were randomized to observation (n=32) or rituximab

maintenance (n=34). The ORR to induction in the intent-to-treat (ITT) population was 82.5%, which included 16.5% CRs and 60.0% PRs. These results compared favorably with previous trials of single-agent chlorambucil, with ORRs ranging from 31-55% (75, 78, 79). The ORR was similar across all Binet stages and age groups. The median PFS was 34.7 months and median OS was not reached at a median follow-up time of 34.2 months. Rituximab maintenance did not appear to provide significant additional clinical benefit, but there was a trend toward improved PFS in those who received maintenance compared to those who were observed after induction. Overall, induction with R-Clb was well-tolerated with minimal grade 3-4 toxicity and there did not appear to be an increased rate of AEs with those that underwent rituximab maintenance.

Other CIT approaches, most notably BR and O-Clb, which were discussed in previous sections of this review can also be considered in older patients with or without comorbidities (25). As is the case for relapsed or refractory CLL, the management approach of frail, elderly patients with CLL is rapidly changing with better-tolerated, novel targeted-agents.

Novel and Investigational Agents

The last decade has been marked by a surge of novel therapeutic agents that are active in CLL. The majority of these new drugs achieve clinical effect *via* the targeting of pathways involved in B-cell receptor signaling. Ibrutinib and idelalisib are just two such agents shown to be effective and safe in the treatment of CLL.

BTK inhibition. Ibrutinib is a potent, orally-active small molecule that irreversibly binds and inhibits BTK, which is involved in BCR signal transduction and malignant B-cell survival. Ibrutinib also inhibits 19 other kinases, including interleukin-2 inducible kinase which is involved in T-cell signaling. Upon discovery of its efficacy in CLL in an initial phase I study, it underwent phase Ib/II in cohorts of relapsed or refractory CLL (n=85) and older, untreated CLL (n=31) patients (80). Patients with relapsed or refractory CLL were treated with 420 mg or 840 mg Ibrutinib daily and previously untreated older patients with CLL received 420 mg Ibrutinib daily. ORR was 71% in both cohorts, with PRs making up the majority of the responses (68%) (81, 82). PFS was 76% in the relapsed or refractory CLL group at 26 months of follow-up and 96% in the previously untreated group at 24 months (81,82). Of note, the PFS for patients with 17p deletion was 57% at 26 months in spite of an increased relapse rate in these high-risk patients (81). OS was 83% at 26 months in the relapsed or refractory CLL group and 97% at 24 months in the untreated group (81, 82). Side-effects were generally mild, consisting mostly of grade I and II diarrhea, fatigue, and upper respiratory tract infection. Results from an independent 3-year analysis, post-initiation of ibrutinib monotherapy, were recently reported. The updated ORR was 78% for all patients (n=132). Median duration of response was not reached for all patients. Patients with 17p deletion (n=36) had an ORR of 56%. No new safety concerns emerged and 64% of patients remained on ibrutinib therapy at the time these results were reported (83). The phase II RESONATE trial, comparing ibrutinib to ofatumumab in 391 patients with relapsed or refractory CLL, was stopped early after meeting its primary PFS and secondary OS end-points. Ibrutinib was found to significantly improve PFS (median not reached) compared to ofatumumab (8.1 months) at a median follow-up of 9.4 months. At 12 months, OS rate was 90% in the ibrutinib group and 81% in the ofatumumab group. ORR was 42.6% and 4.1 in the ibrutinib and ofatumumab groups, respectively. Even patients with purine analog resistance and 17p deletion benefited with ibrutinib (84). Ibrutinib is currently undergoing further investigation in combination with rituximab, ofatumumab, BR, and FCR (NCT01292135) (85, 86, 87). Ibrutinib was FDA-approved for the treatment of CLL as monotherapy in patients who have received at least one prior therapy on February 12, 2014. On July 28th, 2014, the FDA expanded approved use of ibrutinib to patients with CLL with deletion in 17p.

CC-292 and ONO-4059 are BTK inhibitors currently under study. They are both more specific inhibitors of BTK than ibrutinib. So far, CC-292 monotherapy has undergone phase I study in relapsed or refractory CLL with promising results. As of June 30, 2013, 83 patients were enrolled who had received a median of three prior therapies: 67% of these patients had high-risk features including 17p deletion, 11q deletion, and unmutated IGVH status. There were four arms, each with different drug doses ranging from 375 mg to 1,000 mg twice daily. The proportion of total responders ranged from 55-67% and PR was achieved in 31-67% of patients depending on the drug dose. The drug was well-tolerated with the most frequent grade 3-4 AEs being neutropenia, thrombocytopenia, pneumonia, and anemia. Only 2.4% of patients discontinued treatment due to AEs (88). CC-292 is currently under phase I investigation in combination with lenalidomide in relapsed or refractory CLL. ONO-4059 is currently in early phase I study and preliminary results were reported at the American Society of Hematology annual meeting in 2013. Nineteen patients were enrolled to date and there were no dose-limiting toxicities reported. All patients had a rapid decrease in their lymphadenopathy and improvement in their hematological parameters after over 3 months of treatment (89).

Phosphoinositol 3-kinase inhibition. Idelalisib is a first-in-class, selective oral inhibitor of the delta isoform of phosphoinositol 3-kinase (PI3Kδ), which has a key role in B-cell proliferation and survival. A total of 54 patients with

heavily-pretreated CLL were included in a phase I study of idelalisib and 70% of these patients were considered to have refractory disease. The nodal response rate was 81%, PR rate was 39%, and PR with lymphocytosis rate was 33%. Those who were treated at the recommended phase II dose of 150 mg twice daily or higher had a median PFS of 29 months *versus* 17.1 months in the entire study population (90). The combination of idelalisib and rituximab was studied in 64 previously untreated patients with CLL in a phase II trial. Idelalisib was given at a dose of 150 mg twice daily for 48 weeks and rituximab at 375 mg/m^2 was given weekly for a total of eight doses. Patients who completed 48 weeks of idelalisib without progression, continued on an extension study. The ORR was 97% with a CR rate of 19% and PR rate of 93% at 24 months. All nine patients with 17p deletion had a response with three patients achieving CRs. At the time these results were reported, none of these patients had disease progression. Common toxicities included elevation in liver transaminases and diarrhea (91). Idelalisib, plus rituximab was compared to rituximab monotherapy in a phase III, randomized, placebo-controlled trial in patients with CLL with comorbid conditions or therapy-induced myelosuppresion who relapsed within 2 years of their last therapy. A total of 110 patients were enrolled in each arm and 44% of patients had 17p deletion. The ORR for the idelalisib arm was 81% (*versus* 13% in the placebo group) and the hazard ratio for PFS was 0.15 ($p<0.0001$). The median PFS was not reached in the idelalisib arm compared to 5.5 months in the placebo arm; 12-month OS was 92% and 80%, respectively (92). Given these results from the first interim analysis, the trial was stopped early for efficacy. A second interim analysis which included patients in the extension study again confirmed the superiority of idelalisib in combination with rituximab over rituximab monotherapy in relapsed CLL (93). A separate analysis of CLL sub-populations of poor risk factors was reported which included patients with 17p deletion, 11q deletion, *TP53* mutation, unmutated *IGVH* status, ZAP70 positivity, CD38 expression, and beta-2-microglobulin of greater than 4 mg/l. This analysis showed that even high-risk patients benefit from idelalisib and rituximab therapy, as evidenced by the ORR of 76.5% and PFS hazard ratio of 0.13 in patients harboring both 17p deletion and TP53 mutations compared to the ORR of 80.4 and PFS of 0.17 in patients without these cytogenetic abnormalities (94). Based on these results, the FDA approved the use of idelalisib, in combination with rituximab, for the treatment of relapsed or refractory CLL on July 23, 2014.

Another oral PI3K inhibitor known as IPI-145 has shown promise in phase I analysis. IPI-145 is a potent inhibitor of the delta and gamma isoforms of PI3K. The dose established for further clinical study in CLL is 25 mg twice daily. As of July 2013, 44 patients with relapsed or refractory CLL were enrolled in the phase I study of IPI-145 safety and preliminary results have been reported in abstract form. Clinical activity was appreciated in patients with relapsed or refractory CLL at all doses studied from 8 mg to 75 mg twice daily, including patients with 17p deletion and TP53 mutations. Best OR in evaluable patients was reported as 52% (95). Recruitment for a phase III trial of IPI-45 compared to ofatumumab in relapsed or refractory CLL has begun (NCT02004522), and investigators are also planning a phase III study of IPI-145 in treatment-naïve patients with CLL.

B-Cell CLL/lymphoma 2 (BCL2) inhibition. BCL2 proteins regulate the apoptotic process and are universally overexpressed in CLL, making it a potential therapeutic target. ABT-199 is a potent, orally-active, selective BCL2 inhibitor that was engineered when a similar agent known as ABT-263 showed clinical activity in CLL, but at the expense of severe thrombocytopenia due to its less selective anti-BCL2 properties (96, 97). As of December 2013, 84 patients with relapsed or refractory CLL and SLL were enrolled in a phase I trial of ABT-199 with a median time on study of 14.7 months. The ORR in 63 evaluable patients was 79%, which included 22% who achieved CR/complete remission with incomplete blood count recovery (CRi). At 12 months, PFS rate was 91% in those who achieved a CR and 65% in patients with PR. The ORR was similar is patients who were fludarabine-refractory and harbored the 17p deletion. The most common AEs were diarrhea, nausea, and neutropenia. Grade 3-4 AEs included neutropenia, anemia, tumor lysis syndrome, and febrile neutropenia. Eight out of 28 patients who discontinued therapy did so due to AEs (98). Further investigation of ABT-199 is ongoing at this time and this agent is being studied specifically in combination with rituximab, as well as obinutuzumab in patients with CLL.

SYK inhibition. SYK is another regulator in the B-cell receptor signaling pathway that is a potential therapeutic target in CLL. GS-9973 is an oral selective inhibitor of SYK and has undergone phase II study with favorable results. Recent reports detail its use in 44 previously-treated patients with CLL at a dose of 800 mg twice daily in an ongoing phase II study. Patients were followed with imaging at 2-month intervals until 6 months of treatment, then every 3 months thereafter. At the time of initial analysis, 27 patients were still receiving treatment for a median of 22 weeks. Ninety-one percent of patients had a decrease in their tumor bulk, including 64% who had a decrease in tumor size of greater than 50%. Diarrhea, fatigue, and nausea were the most frequent AEs (99). GS-9973 is currently being studied in combination with idealisib in patients with relapsed or refractory CLL in another phase II trial.

CD19 targeted chimeric antigen receptor therapy. A novel approach involving the use of engineered autologous T-cells has shown success in the treatment of advanced and relapsed or

refractory CLL. Patients' own T-cells are harvested by leukapheresis, then manipulated to express a chimeric antigen receptor (CAR), thereby combining antibody-mediated targeting and cell-mediated killing in a single therapeutic strategy. Long-term tumor control and protection of recurrence can potentially be achieved *via* the integration of biological targeting and vaccine-like therapy. CD19 is expressed in malignant and normal B-cells, as well as a small proportion of immune cells, but is otherwise not expressed in normal cells, including hematopoietic stem cells (100). CD19 is felt to be a good target for CAR therapy in B-cell malignancies, including CLL. A CAR directed against CD19 is first introduced (usually *via* lentiviral or retroviral transduction) into autologous T-cells and these cells are expanded *ex vivo* over two weeks prior to intravenous infusion. CAR-modified T-cells continue to proliferate and persist *in vivo* for a long period of time. This approach was recently studied using autologous T-cells modified with a CAR directed against CD19 (termed CTL019 cells) in 14 patients with relapsed or refractory CLL at the University of Pennsylvania (101). Patients had received a median of four prior therapies. All patients received lymphodepleting chemotherapy consisting of either FC, PC, or single-agent bendamustine prior to CTL019 cell infusion. ORR was 57%, including four CRs and four PRs. All responding patients developed a delayed cytokine release syndrome, with five patients requiring intervention with an interleukin-6 (IL6) receptor antagonist, tocilizumab and/or corticosteroids. CAR-modified T-cells persisted based on detection by flow cytometry for over 3 years after infusion of the CTL019 cells in all six patients with ongoing responses (101). Preliminary results of a phase II dose optimization study comparing two different doses of CTL019 cells, so far, shows no significant relationship between dose and response/toxicity in 18 patients (102). Anti-CD19 CAR T-cells have been used to treat CLL, with slight differences among protocols, in several reported studies with promising results (103, 104). The main toxicities of CAR T-cell therapy are B-cell aplasia and hypogammaglobulinemia, delayed tumor lysis syndrome, and cytokine release syndrome (CRS). CRS usually occurs in patients with a disease response, as described earlier. Patients with CRS can present with high fever, nausea, myalgia, arthralgia, hypotension, and pulmonary infiltrates as a result of capillary leak due to increased cytokine release. Due to high levels of IL6 and interferon gamma noted during CRS, tocilizumab, an IL6 inhibitor, and corticosteroids are used to dampen the inflammatory response. Further study of CAR therapy is necessary in patients with CLL to define the best technique in administering this treatment, to find the best subset of patients with CLL who are likely to benefit, and to better assess for long-term toxicities. Additionally, it will be important to better define the role of CAR T-cell therapy in this era of novel, targeted agents that are proving to be more efficacious and less toxic than the cytotoxic chemotherapies that have been the mainstay of CLL treatment for many years.

Allogeneic Hematopoietic Stem Cell Transplantation

The role of allogeneic HSCT in CLL remains unclear with the advent of highly effective targeted-therapies, but the use of HSCT in CLL continues to be an active area of clinical research. Allogeneic HSCT is generally thought to be the only reliable means by which a durable remission can be achieved in advanced or high-risk CLL. In a study of 28 patients with relapsed or refractory CLL younger than or equal to the age of 60 years were conditioned with high-dose cyclophosphamide and fractionated total body irradiation with the exception of one patient who received carmustine, etoposide, cytarabine, and melphalan due to prior radiotherapy. Twenty patients had Human Leukocyte Antigen-identical donors, seven patients had a matched unrelated donor, and one patient had a one-antigen mismatched sibling donor. The median follow-up time for surviving patients was 66 months. The 5-year PFS and OS from the time of transplant were 42% and 45%, respectively. The 5-year OS rate was significantly improved for patients considered to have chemo-sensitive disease (78%) compared to those with chemo-refractory disease (31%) (105). Due to high rates of total related mortality associated with myeloablative conditioning regimens, reduced-intensity conditioning has been studied in patients with CLL. The 5-year follow-up analysis of patients with fludarabine-refractory CLL treated with non-myeloablative conditioning followed by allogeneic HSCT were reported in 2008. In this study, 82 patients aged 42-72 years received total body irradiation alone or in combination with fludarabine followed by HSCT from either related or unrelated donors. Fifty-five percent of all patients achieved a CR, and those who had an unrelated donor had higher CR rates (67%). Among the 25 patients who initially achieved a CR, 21 patients were alive and remained in CR at 5 years. Five-year non-relapse mortality was 23%. At 5-years, 24% of surviving patients were receiving immunosuppressive therapy for chronic graft *versus* host disease. The only prognostic factors that appeared to have a significantly negative impact on transplant outcome were high hematopoietic cell transplantation-specific comorbidity index score and the presence of lymphadenopathy of 5 cm or more at the time of transplantation. Interestingly, poor-risk cytogenetic abnormalities including the 17p and the 11q deletions did not appear to negatively affect outcome (106). Other studies evaluating the use of allogeneic HSCT in high-risk patients with relapsed or refractory CLL, including those with ZAP70 expression, unmutated *IGVH* status, and unfavorable cytogenetics have shown promising results (107-109).

A recent study utilized minimal residual disease (MRD) quantification in 40 patients with CLL who underwent reduced-intensity allogeneic HSCT. It was found that MRD burden at several time points post-transplant was predictive of relapse and disease-free survival (110). The results of a six-year follow-up

analysis of the German CLL Study Group CLL3X trial were recently published, which specifically looked at outcomes of patients with *TP53,* splicing factor 3b subunit 1 (*SF3B1*), and *NOTCH1* mutations who underwent reduced-intensity allogeneic HSCT. These genetic abnormalities are thought to confer resistance to conventional CLL therapies based on prior studies, however in this post-transplant study population, the presence of these deleterious mutations did not appear to negatively impact event-free and overall survival (111). Lastly, a retrospective analysis comparing myeloablative *versus* reduced-intensity conditioning regimens in 297 patients with CLL undergoing matched sibling donor HSCT showed superiority of reduced intensity conditioning over myeloablative conditioning. Reduced intensity conditioning was associated with faster platelet count recovery and less treatment related mortality compared to myeloablative conditioning. In patients receiving HSCT after the year 2000, RIC was associated with improved PFS and OS, as well (112). There exists a large body of evidence to support the use of allogeneic HSCT in CLL. However, given the substantial risks of HSCT and the development of novel and generally well-tolerated agents, further studies need to be undertaken directly comparing outcomes of HSCT and pharmacotherapy in patients with CLL.

Future Perspectives

Targeted-therapy has gained a strong foothold on CLL management in recent years, so much so that CIT, the current standard in front-line therapy, may soon become an afterthought. Targeted biological agents discussed in this review, including ibrutinib and idelalisib, not only show exceptional efficacy, but also have relatively mild side effect profiles which allows for their use in heavily pre-treated and frail patients. A randomized phase III trial of untreated, older (65 years of age or more) comparing single agent ibrutinib, BR, and ibrutinib in combination with rituximab is currently underway (NCT01886872). These agents are also being extensively studied in the first-line setting in younger, fit individuals with the hope that it will provide a better means to treating these patients who are already immunocompromised as a result from their CLL by bypassing the risk of severe infection and myelosuppression that accompany traditional cytotoxic chemotherapy drugs. Ibrutinib and rituximab combination therapy, for example, is now being studied in a randomized phase III trial of untreated, younger patients in comparison with FCR (NCT02048813).

The optimal sequence and timing of therapies that can now be used in CLL is unknown. However, we anticipate future discovery of new treatment strategies that provide durable remissions *via* eradication of MRD in these patients. Up to 25% of patients who achieve a CR by standard criteria have residual disease detected molecularly or by flow cytometric assay (113). While there is no level 1 evidence to support the eradication of MRD in patients who have achieved a major response to therapy, recent studies have shown that the presence of MRD after chemotherapy is associated with inferior overall survival (113,114). One study reported the outcomes of previously-treated patients with CLL who received alemtuzumab with the goal of eradicating MRD. Patients in whom MRD-negativity was achieved appeared to have prolonged treatment-free and overall survival compared to patients who remained MRD-positive(114).

One proposed treatment approach, coined 'sequential triple-T' (tailored, targeted, total eradication of MRD) by Hallek *et al*. is a potentially less toxic, three-part strategy aimed at achieving MRD negativity (113). The first step would consist of using traditional systemic chemotherapy in short courses over 1-2 months to debulk the tumor. This would be followed by definitive treatment over 6-12 months in the second step of 'induction' with the use of a combination of targeted drugs, including monoclonal antibodies and kinase inhibitors. The last step of the triple-T strategy would be aimed at maintaining remission with single-agent therapy. MRD assessment should be done after CR is achieved in the induction phase and should continue during the maintenance phase to help the clinician decide when to stop and/or resume maintenance therapy (41). For now, the IWCLL recommends the use of MRD status as an exploratory end point in prospective clinical trials and advises against treatment of CLL on the basis of MRD positivity alone (6).

This management approach, as well as other new potential treatment strategies, have yet to be studied and validated in the CLL population. However, it is very encouraging to see the increasing number of relatively less toxic and highly efficacious therapies that will allow for novel treatment protocols leading to a cure, or at least a prolonged remission, with preservation of the quality of life of patients with CLL.

Executive Summary

CLL is the most common white blood cell malignancy in adults.

While diagnosis and staging of CLL is traditionally straightforward, risk stratification and management of CLL is becoming more complex with the discovery of new molecular and genetic prognostic markers.

Combination CIT has become the standard-of-care in the treatment of CLL with the development of rituximab, an anti-CD20 monoclonal antibody.

Novel agents that target the B-cell activation pathways are now changing the landscape of the management of non-Hodgkin lymphomas, including CLL. These targeted-agents are highly-effective even in the relapsed and refractory setting and have improved safety profiles compared to traditional cytotoxic drugs, which allow frail and elderly patients with CLL to benefit from these new drugs.

The optimal sequence and timing of therapies that can now be used in CLL is unknown, however, we anticipate future discovery of new treatment strategies that provide durable remissions *via* eradication of minimal residual disease.

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