Monoclonal Antibodies in Chronic Lymphocytic Leukemia

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ABSTRACT: Monoclonal antibodies (MoAbs) play a pivotal role in the treatment of chronic lymphocytic leukemia (CLL). Rituximab, a MoAb against CD20, was initially used as a single agent in the treatment of CLL before being incorporated into newer combination regimens. Integration of rituximab into chemotherapy regimens has led to an improvement in the response rate and overall survival when used in frontline therapy. Despite this, CLL remains an incurable disease, and treatment of relapsed CLL, particularly after failure of purine analog-based regimens, remains challenging. Technological advances relating to development of chimeric and humanized MoAbs are supporting the role of antibody-based regimens for many diseases, including CLL. Currently, MoAbs represent an integral component of CLL therapy. The antitumor efficacy of many therapeutic MoAbs can be potentially improved upon by their use in combination with new targeted therapy agents.

KEYWORDS: monoclonal antibodies, chronic lymphocytic leukemia

CITATION: Picardi et al. Monoclonal Antibodies in Chronic Lymphocytic Leukemia. *Lymphoma and Chronic Lymphocytic Leukemias* 2016:6 1–11 doi:10.4137/LCLL.S18491.

TYPE: Review

RECEIVED: December 1, 2015. **RESUBMITTED:** February 18, 2015. **ACCEPTED FOR PUBLICATION:** February 26, 2016.

ACADEMIC EDITOR: Mitchell Smith, Editor in Chief

PEER REVIEW: Three peer reviewers contributed to the peer review report. Reviewers' reports totaled 1,106 words, excluding any confidential comments to the academic editor.

FUNDING: Authors disclose no external funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Paper subject to independent expert single-blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Provenance: the authors were invited to submit this paper.

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Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common hematological malignancies. The disease manifests as lymphocytosis, usually with characteristic phenotype on B-cells (CD5- and CD23-positive markers). The median age at diagnosis is 72 years with 56.5% of cases aged between 65 and 84 years, with a median age at death of 79 years.[1] The National Cancer Institute estimates that 15,680 Americans were diagnosed with CLL and 4,580 died of the disease in 2013. Since incidence increases with age, one could expect in the coming years a rise in the overall number of CLL cases, which is also due to increasing life expectancies. Diagnosis requires a documented absolute number of B-lymphocytes ≥5,000/μL with ≤55% prolymphocyte count in peripheral blood. CLL is an extremely heterogeneous disease. A significant proportion of CLL patients never require treatment or can be managed effectively with palliative chemotherapy. In contrast, other patients have a very aggressive clinical course and suffer early disease progression and death. With the advent of molecular profiling, several new prognostic features have been identified for a better prognostic stratification. Mutational status of immunoglobulin (Ig) heavy chain variable region (IGHV), chromosome abnormalities identified by fluorescence in situ hybridization analysis, and TP53 mutation have been recognized as important prognostic factors proved to be useful in predicting which patients in the good prognosis group will more likely develop a progressive disease.[2] Moreover, they may predict responses to treatment and provide prognostic assessment such as response duration and survival.

Traditionally, chemotherapy with alkylating agents has been the treatment of choice for patients with advanced and progressive CLL. Since the introduction of purine nucleoside analogs, alkylating agents such as chlorambucil, cyclophosphamide, and bendamustine may no longer be considered as elective first-line treatment for young healthy patients. The combination of purine analogs and alkylating agents, principally fludarabine plus cyclophosphamide (FC), has been demonstrated to be superior to both as monotherapy. Nevertheless, improved understanding in the past two decades of the molecular differences between malignant and normal cells has led to the elaboration of therapies that more specifically target human tumors. These include monoclonal antibodies (MoAbs) owing to their high affinity and specificity, with a differential target antigen expression in tumor cells versus normal cells, making an ideal agent for cancer immunotherapy.[3] MoAbs directed against specific proteins expressed on B-cells have changed the management of patients with non-Hodgkin's lymphoma (NHL) in general and, more recently, in CLL patients. Since the human-murine Ig G1 anti-CD20 MoAb rituximab (R) was approved, several studies have evaluated the activity of other MoAbs in the

management of B-cell malignancies either alone or in combination with chemotherapy and currently in combination with novel agents. The aim of this review is to summarize the knowledge on pharmacokinetics, mechanism of action, and clinical use of MoAbs in CLL.

MoAbs Anti-CD20

CD20 is a 297-amino acid transmembrane phosphoprotein expressed as a cell surface antigen on more than 90% of normal and malignant B-cells. CD20 is firmly restricted to the B-cell lineage and it is not expressed in stem cells and plasma cells. Nevertheless, CD20 is differently expressed in B-cell malignancies. In fact, in B-cell lymphomas, CD20 is uniformly and strongly expressed, while in CLL, relatively low levels of CD20 are typically expressed.[4] The mechanism of action of anti-CD20 antibodies includes activation of complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and the induction of direct cell death.[5]

Rituximab

Rituximab is a chimeric human/mouse anti-CD20 MoAb with antileukemia action, including CDC, ADCC, and direct induction of apoptosis. The chimeric structure of rituximab incorporates murine variable regions and human constant kappa and Fc regions to diminish the development of anti-mouse Ig, antibody side effects, and possible resistance.[4] The predominant mechanism for the clearance of neoplastic cells in lymphomas seems to be ADCC, and FC-γ receptors are critical for the in vivo actions of rituximab. Based on FcγRIIIa polymorphisms, the importance of ADCC in the in vivo action of rituximab is supported by the difference in response rates observed among lymphoma patients.[6] In contrast, FcγRIIIa polymorphisms are not predictive of response in CLL. With regard to rituximab, CDC mechanism in in vitro studies has shown a more rapid and potent induced cell death with CDC than ADCC or apoptosis. Complement activation is relevant, as increased expression of complement inhibitors CD55 and C59 determined rituximab resistance in B-NHL cell lines and CLL cells.[7] In addition to the induced proapoptotic signal via the cell surface target structure, several studies have also pointed out the activity of rituximab in promoting cellular response against tumors.

Single agent rituximab. Food and Drug Administration (FDA) approval of rituximab for the treatment of relapsed/refractory CD20+ NHL was based on the pivotal trial reported by McLaughlin et al, in which single-agent rituximab brought about significant response rate in heavily pretreated patients with indolent lymphoma.[8] In the cohort of patients with small lymphocytic lymphoma (SLL), a different form of chronic lymphocytic leukemia localized to the tissues, a response rate substantially lower than that observed in patients with follicular lymphoma (12% vs 60%, respectively) was observed.

The relatively weak activity of rituximab in patients with pretreated CLL in comparison to patients with other indolent B-NHL has been confirmed in subsequent studies. It was apparent that not only was there a low antigen density in SLL cells but also the antibody half-life was short and this short half-life correlated with a low response rate.[8] The difference in response rate can be explained by the fact that standard dose of rituximab is insufficient to saturate the large tumor mass and associated soluble CD20 molecules. Indeed, pharmacokinetic studies carried out in pivotal trials showed lower postinfusion rituximab levels in patients with SLL and/or circulating neoplastic cells.[9] In order to overcome the short half-life of rituximab, Byrd et al tested a dose-intense schedule of thrice weekly rituximab in patients with pretreated CLL/SLL. The increase in the conventional dose of 375 mg/m^2 three times a week led to a response rate of 45%–50% range.[9] Similarly, O'Brien et al demonstrated a dose–response effect using a dose-escalation strategy in relapse/refractory CLL patients.[10] The first dose was 375 mg/m^2 for all patients; escalated doses were from 500 to 2.250 mg/m^2. Response was correlated with dose: 22% for patients treated at 500–825 mg/m^2, 43% for those treated at 1,000–1,500 mg/m^2, and 75% for those treated at the highest dose of 2,250 mg/m^2.

Better results with standard-dose rituximab have been demonstrated in frontline therapy achieving a 51% response rate, substantially superior to that observed in pretreated patients.[11]

Although rituximab used in first-line treatment was more effective than in the setting of relapsed/refractory patients, the drug is still less active in CLL/SLL than in follicular lymphomas. Furthermore, in spite of more toxicity, fludarabine in monotherapy achieves better quality of response and more progression-free survival (PFS) (Table 1).

Combination therapy. The major interest of rituximab in CLL lies in the effect of MoAb used in combination therapy with purine analogs. As demonstrated by in vitro studies, when rituximab is combined with chemotherapeutic agents, the apoptosis is enhanced in an additive or synergic manner.[12,13] Experiments on cells of B-lymphoid malignancies showed that tumor cells, otherwise resistant to killing by fludarabine or other cytotoxic agents, were sensitized by rituximab.[14] This effect does not require the complete saturation of target CD20 sites or the presence of complement. Other investigators have demonstrated synergy between fludarabine and rituximab-induced complement lysis in vitro, at least in part through downregulation on the CD55 and CD46 complement inhibitors, in a malignant B-cell line.[15]

This evidence made the development of combination programs possible.

The Cancer and Leukaemia Group B (CALGB) conducted a randomized Phase II clinical trial to determine the ideal administration schedule of rituximab plus fludarabine in untreated patients (trial CALGB 9712).[16] Patients were randomized to receive six monthly courses of concurrent

Table 1. Monoclonal antibodies as single agents in CLL.

AUTHORS	NO. EVALUABLE pts	DISEASE STATUS	SCHEDULE	RESPONSE
			RITUXIMAB	
Byrd et al[9]	29	Pretreated	250 mg/sqm/TIW for 4 wks	48% PR
		Untreated	375 mg/sqm/TIW for 4 wks	4% CR
O'Brien et al[10]	24	Pretreated	375 mg/sqm wk 1, 500–825 mg/sqm wks 2, 3, 4	21% PR
	7		375 mg/sqm wk 1, 1,000–1,500 mg/sqm wks 2, 3, 4	43% PR
	8		375 mg/sqm wk 1, 2,250 mg/sqm wks 2, 3, 4	75% PR
Hainsworth et al[11]	43	Untreated	375 mg/sqm/w for 4 wks	51% OR
				4% CR
			OFATUMUMAB	
Wierda et al[35]	59	F and A refractory	Dose 1: 300 mg, dose 2–12: 2,000 mg	58% OR
	79	F refractoty and bulky disease		47% OR
			ALEMTUZUMAB	
Keating[55]	93	Pretreated	30 mg iv TIW, 12 wks	2% CR
				31% PR
Lundin[56]	41	Untreated	30 mg sc TIW, 18 wks	19% CR
				68% PR
Hillmen[58]	148	Untreated	Chlorambucil 40 mg d 1 every 28, 12 courses	2% CR, 53% PR
			A 30 mg iv TIW, 12 wks	24% CR, 59% PR

Abbreviations: w, weekly; wks, weeks; PR, partial response; CR, complete response; OR, overall response; pts, patients; TIW, thrice weekly; F, fludarabine; A, alemtuzumab.

fludarabine and rituximab followed by four rituximab consolidation courses or sequential treatment of fludarabine alone followed by four infusions of rituximab as consolidation. With the concurrent regimen, 47% of complete response (CR) was observed compared with 28% with sequential regimen. The overall response rate (ORR) and PFS were not significantly different between the two groups with a median follow-up of 23 months. Long-term follow-up of patients enrolled in the CALGB 9712 trial demonstrated extended overall survival (OS) and PFS with an estimated 13% of responders free of progression at almost 10 years of follow-up.[17]

Higher response rate and time-to-treatment failure of fludarabine in combination with cyclophosphamide compared with fludarabine alone has been demonstrated in several studies.[18,19] In a trial conducted at the MD Anderson Cancer Center, 224 patients with progressive or advanced CLL were enrolled to receive fludarabine, cyclophosphamide, and rituximab (FCR) combination regimen as first-line therapy. The patients received a median of six courses of FCR achieving OR and CR rates of 95% and 70%, respectively. Flow cytometric analysis performed on 207 patients to evaluate residual disease showed that 67% had less than 1% CD5- and CD19-coexpressing cells.[20] At five years of follow-up, no evidence of progression was shown in 68% of patients.[21] This regimen was tested by the same group on 177 patients with relapsed or refractory CLL. Most of the patients had been previously exposed to fludarabine.[22] The ORR observed was 73% (25% CR) with a median time to progression in responding patients of 28 months. The most common reason for discontinuing treatment was myelosuppression. In comparison to patients treated with fludarabine and cyclophosphamide, a similar incidence of neutropenia, thrombocytopenia, and infection was observed, suggesting that the addition of rituximab did not significantly increase toxicity.

Other prospective randomized trials either in previously treated or in untreated patients were designed to test the benefit of the combination rituximab, fludarabine, and cyclophosphamide.[23,24]

The randomized REACH trial was designed to compare FCR to FC alone in relapsed CLL patients.[23] Among the 552 patients enrolled, the group receiving rituximab showed a significant improvement in terms of PFS, ORR, and CR compared with the group randomly assigned to the arm without MoAb. The post trial cross-over to rituximab and a relatively short follow-up prevented the demonstration of a survival advantage.

The evidence that FCR not only improved response rate but also conferred a survival advantage compared with the previous gold standard FC was first demonstrated in the randomized CLL8 trial,[24] where 817 untreated patients were enrolled. FCR regimen induced a higher ORR than FC alone (90% vs 80%), more CRs (44% vs 22%), and longer median PFS (51.8 vs 32.8 months). As already mentioned, this is the first randomized study demonstrating superiority in OS (at 37.7 months, 84.1% vs 79.0%) between the two treatment arms. Hematological toxicity was higher in patients treated with FCR, even though the mean number of courses

delivered in the two groups was similar: 5.2 courses in the FCR arm versus 4.8 courses in FC arm. Updates from the German CLL Study Group (GCLLSG), CLL8 trial indicates that patients treated with FCR as their initial therapy have a median PFS of 56.8 months. FCR improved PFS and OS compared to FC. The del(17p) subgroup showed the shortest median PFS but still had treatment benefit from FCR (11.2 vs 9.1 months).

Therefore, FCR is now the gold standard therapy for patients who have no significant comorbidities.

Given the fact that FCR is a safe regimen only for fit patients, other chemotherapeutic agents, such as bendamustine, were evaluated in combination with rituximab in relapsed/refractory and naïve CLL patients.

The GCLLSG initiated the CLL2M Phase II study to investigate the combination of bendamustine (B) plus rituximab in 78 relapsed and/or refractory CLL patients.[25] The ORR of 59% with a CR rate of 9% were observed. Of note is that a high ORR (92%) was observed among patients with del11q. Major but tolerable treatment toxicities were myelosuppression and infections. Grade 3–4 infections were documented in 12% of all given cycles.

The same group also considered bendamustine plus rituximab as a first-line treatment in a multicenter Phase II study.[26] Results on 117 CLL patients showed an ORR of 88% with a CR in 23% of cases. After 27 months of median time observation, event-free survival was 33.9 months, and 90.5% of patients were alive. As in the previously treated patients, BR confirmed its efficacy even among patients with adverse prognostic features such as del11q and unmutated IgVH status, leading to ORR of 90% and 89%, respectively. Patients with del17p achieved only partial remissions (37%).

Considering the low toxicity profile of bendamustine-containing regimens, CLL10 was designed to evaluate the efficacy and safety of BR versus FCR in frontline therapy of fit patients without del17p. The final analysis shows that FCR remains the standard treatment in very fit CLL patients due to a higher CR rate, more minimal residual disease (MRD) negativity, and longer PFS in comparison with BR. However, in elderly fit patients, high toxicity rates and infection rates result into dose reduction, leading to similar efficacy between both arms (Table 2).[27]

Rituximab in combination with novel agents. The two major classes of novel agents with substantial activity across all genomic subgroups of CLL are the BCR signaling inhibitors (idelalisib and ibrutinib) and the BCL2 antagonists (venetoclax). These drugs are orally bioavailable and show dramatic efficacy and favorable tolerability compared with chemoimmunotherapy.

Idelalisib and ibrutinib have been approved by the FDA and EMA for relapsed/refractory CLL patients and as frontline therapy for patients harboring del17p.

CLL patients with high-risk cytogenetic abnormalities (deletion 17p, TP53 mutation, or deletion 11q) can particularly benefit from new kinase inhibitors, given the high response rates to therapy with the BTK inhibitor ibrutinib and the PI3 kinase delta idelalisib in combination with rituximab.[28,29]

Rituximab in combination with novel agents was investigated in a multicenter, randomized, double-blind, placebo-controlled, Phase III study, which assessed the efficacy and safety of idelalisib, in combination with rituximab versus rituximab plus placebo in relapsed CLL patients less able to undergo chemotherapy.[30] The combination of idelalisib and rituximab, when compared with placebo and rituximab, significantly improved PFS (median not reached vs 5.5 months), response rate (81% vs 13%), and OS (92% vs 80% at 12 months).

In order to evaluate idelalisib in combination with rituximab as initial therapy, a Phase II trial was recently conducted in 64 naïve CLL older patients treated with rituximab 375 mg/m^2 weekly × 8 and idelalisib 150 mg bid continuously for 48 weeks.[29] Results showed an ORR of 97% with 19% of CR. ORR was 100% in patients with del(17p)/TP53 mutation and 97% in those with unmutated IGHV.

Prospective studies of these agents in the frontline setting are currently under way.

Ibrutinib in combination with rituximab was investigated in a single-arm Phase II trial that enrolled 40 patients with high-risk CLL (defined as previously treated patients with short remission duration or presence of 17p deletion/TP53 mutation, or naïve patients with 17p deletion/TP53 mutation).[31] With regard to efficacy, ORR was 95% (87% PR, 8% CR) and two of the three patients who achieved a CR were patients with del17p or TP53 mutation who were previously untreated. At 18 months, an estimated 78% of patients was free of progression. In patients with del17/TP53 mutation, PFS was 72% (Table 3).

The results of venetoclax in CLL have been presented in abstract form.[32,33] Both as single agent and in combination with rituximab, venetoclax achieved high response rates of approximately 80%, and importantly—and differently from the results of BCR antagonists—complete remission rates of approximately 25% were reported even in the relapsed/refractory 17p setting.

Ofatumumab

Since CD20 has proved to be a highly successful target antigen for immunotherapy in lymphoproliferative disorders, the development of novel anti-CD20 antibodies with a different principal activity from that of rituximab is investigated. Furthermore, despite its success, rituximab limitations have become apparent, particularly when used alone in advanced-phase CLL, thus creating a need for new agents, including new generation anti-CD20 MoAbs with enhanced activity. Among those agents under evaluation, the furthest ahead in terms of clinical development in CLL is ofatumumab.

Ofatumumab is a second-generation fully human anti-CD20 MoAb that functions as a Type I (rituximab-like) antibody, which operates via both CDC and ADCC, but does

Table 2. Anti CD20 monoclonal antibodies in combination with chemotherapy.

AUTHORS	COMP. STUDY	NO. EVALUABLE pts	DISEASE STATUS	TREATMENT REGIMEN	CLINICAL RESPONSE	
				RITUXIMAB	CR (%)	OR (%)
Byrd et al[16]	Yes	104	Untreated	Sequential: F 25 mg/sqm × 5 d 6 cycles, after 2 mo R 375 mg/sqm 4 weekly dose	47	90
				vs		
				Concurrent: F 25 mg/sqm × 5 d 6 cycles, R 375 mg/sqm d 1 and 4 cycle 1, d 1 of cycles 2–6 after 2 mo R 375 mg/sqm 4 weekly doses	28	77
Keating et al[20]	No	224	Untreated	R-FC: R 375 mg/sqm first cycle, 500 mg/sqm d 1 cycles 2–6, F 25 mg/sqm and CTX 250 mg/sqm d 2–4 cycle 1, d 1–3 cycles 2–6	70	95
Badoux et al[22]	No	284	Pretreated	R-FC: R 375 mg/sqm first cycle, 500 mg/sqm d 1 cycles 2–6, F 25 mg/sqm and CTX 250 mg/sqm d 2–4 cycle 1, d 1–3 cycles 2–6	30	74
Robak et al[23]	Yes	552	Pretreated	FC: F 25 mg/sqm and CTX 250 mg/sqm d 1–3	13	58
				vs		
				R-FC: R 375 mg/sq first cycle, 500 mg/sqm d 1 of the cycles 2–6, F 25 mg/sqm and CTX 250 mg/sqm d 1–3	24	70
Hallek et al[24]	Yes	817	Untreated	FC: F 25 mg/sqm and CTX 250 mg/sqm d 1–3	22	88
				vs		
				R-FC: R 375 mg/sqm first cycle, 500 mg/sqm d 1 of the cycles 2–6, F 25 mg/sqm and CTX 250 mg/sqm d 1–3	44	95
				OFATUMUMAB		
Wierda et al[39]	Yes	61	Untreated	O-FC: O 500 mg/sqm d 1, F 25 mg/sqm and CTX 250 mg/sqm d 2–4, 6 cycles	30	77
				vs		
				O-FC: O 1,000 mg/sqm d 1, F 25 mg/sqm and CTX 250 mg/sqm d 2–4, 6 cycles	50	73
Hillmen et al[40]	Yes	447	Untreated	Chlorambucil 10 mg/sqm d 1–7 of every 28 d cycles	1	69
				vs		
				Chlorambucil 10 mg/sqm d 1–7 of every 28 d cycles	12	82
				O cycle 1: 300 mg d 1, 1,000 mg d 8; O subsequent cycles maximum 12: 1,000 mg d 1		
				OBINUTUZUMAB (GA-101)		
Goede et al[53]	Yes	663	Untreated	Chlorambucil 0.5 mg/kg d 1, d 15 mg/sqm every 28, 6 cycles	21	78
				GA-101 100 mg d 1, 900 mg d 2, 1,000 mg d 8 and d 15 of cycle 1, 1,000 mg d 1 cycle 2–6		
				vs		
				Chlorambucil 0.5 mg/kg d 1, d 15 mg/sqm every 28, 6 cycles	7	65
				Rituximab 375 mg/sqm first cycle, 500 mg/sqm d 1 of the cycles 2–6		

Abbreviations: Comp., comparative; CR, complete response; OR, overall response; OS, overall survival; F, fludarabine; R, Riuximab; CTX, cyclophosphamyde; d, days; mo, months; wks, weeks; pts, patients.

not directly induce apoptosis. Ofatumumab demonstrates increased binding of C1q and more potent CDC than rituximab, even in cells with low CD20 expression levels, including freshly isolated CLL cells and complement-resistant B-cell lines.[34]

Ofatumumab single agent. Ofatumumab in monotherapy was investigated in an international, multicenter trial (study 406) for patients with fludarabine (F) and alemtuzumab (A) refractory CLL (FA-ref) and CLL patients with fludarabine-refractory but considered not suitable for alemtuzumab due to

bulky lymphadenopathy (BF-ref). An interim analysis of 138 subjects (FA-ref = 59, BF-ref = 79) demonstrated an ORR of 58% and 47% in the FA-ref and BF-ref cohorts, respectively.[35] The median PFS time was 5.7 and 5.9 months in the FA-ref and BF-ref groups, respectively. These results have shown significant activity providing meaningful clinical improvements in poor-risk patients. A subsequent ad hoc analysis showed that ofatumumab was effective even when previously treated with rituximab (Table 1).

Recent data from GEN416 study[36] were aimed to inquire into efficacy of ofatumumab retreatment and maintenance. This trial enrolled patients from study 406, who had at least a stable disease following ofatumumab monotherapy and who had subsequently progressed. Twenty-nine patients were retreated with eight weekly infusions followed by monthly infusions for upto two years. The ORR after eight weeks of induction retreatment was 45%. Response duration was 24.1 months, time to next therapy was 14.8 months, and PFS was 7.4 months, suggesting that this therapy is feasible in patients with heavily pretreated CLL.

At the 2014 American Society of Hematology Meeting, Österborg et al presented the results from the Phase III study OMB114242.[37] One hundred and twenty patients with bulky fludarabine-refractory disease were randomly assigned to ofatumumab (79 cases) vs physician's choice therapy (43 cases). Ofatumumab as salvage treatment, in this selected high-risk population, obtained a 37% ORR, a slightly inferior result than the pivotal trials. Furthermore, ofatumumab did not meet the primary end point of the study, not being able to demonstrate a clear superiority with respect to the best available therapies in terms of PFS (5.4 months with ofatumumab vs 3.6 months with physician's choice). The lack of a satisfactory prolongation of PFS and OS represents the most evident single agent ofatumumab limitation.

The advent of new targeted therapies led to the first open-label Phase III study RESONATE directly comparing ofatumumab and ibrutinib.[38] A clear superiority of the latter in terms of responses and survival was observed: ibrutinib demonstrated a statistically significant 78% reduction in the risk of progression or death and a 56% reduction in the risk of death compared with ofatumumab. ORR was significantly better in the ibrutinib group compared with the ofatumumab group (42.6% versus 4.1%).

Furthermore, ibrutinib, as well as other small molecules, was able to abrogate the negative impact 17p deletion. The addition of ofatumumab to the new targeted therapies could represent a promising association.

Combination therapy. The first study combining ofatumumab with FC as first-line treatment was a Phase II trial[39] in which two cohorts of patients were treated with FC combination added to ofatumumab 500 or 1,000 mg for up to six cycles. The ORR for the combined dose cohorts was 75% (CR = 41%). A statistical trend in favor of the latter 1,000 mg cohort was observed in terms of complete remission rate (32% versus 50%). Response rates were lower in the presence of Del17p or with beta2-microglobulin levels of ≥4 mg/L. Events of grade 3 or 4 neutropenia were detected in at least 87% of patients in each dose cohort during treatment. Fludarabine, cyclophosphamide, and ofatumumab combination, even with ofatumumab 1,000 mg, failed to demonstrate a clear superiority compared with historical data from FCR, in terms of both CRR (50% vs 44%) and ORR (73% vs 90%).

COMPLEMENT1 is a Phase III randomized trial looking at the efficacy of ofatumumab added to chlorambucil versus chlorambucil monotherapy in previously untreated patients with CLL.[40] Patients with CLL who required therapy and were considered inappropriate for fludarabine-based therapy due to advanced age and/or comorbidities were randomized (1:1) to receive either ofatumumab plus chlorambucil or chlorambucil. PFS was significantly prolonged in the ofatumumab plus chlorambucil arm (22 months) compared with chlorambucil alone (13 months). ORR was higher for ofatumumab plus chlorambucil versus chlorambucil alone (82% vs 69%), with a superior CR rate (12% vs 1%). Negative MRD was observed in 37% of ofatumumab plus chlorambucil subjects with an assessed CR. Median OS was not reached for ofatumumab plus chlorambucil or chlorambucil alone after a median follow-up of 29 months. These data suggested that addition of ofatumumab to chlorambucil led to better clinical outcomes in treatment-naive patients with chronic lymphocytic leukemia who were elderly or had comorbidities. However, it is important to note that similar results were reported in Phase II trial evaluating rituximab plus chlorambucil versus chlorambucil alone in unfit CLL patients.[41]

The Italian group of GIMEMA investigated the role of ofatumumab in addition to bendamustine in an open-label, noncomparative Phase II multicenter trial.[42] After six cycles of treatment, the ORR was 72.3% with 17% of CR. The response rate was not dependent on the following factors: IGHV status, 11q deletion, NOTCH1 mutations, BIRC3 mutations, age over 70 years, prior exposure to fludarabine and/or rituximab, and number of previous therapies. However, those with the 17p deletion or TP53 mutations had a markedly lower ORR of 30%.

Ofatumumab and novel agents. Jaglowski et al[43] evaluated the efficacy and safety, tolerability, and efficacy of three different fixed-dose regimens of ibrutinib combined with ofatumumab in patients with relapsed/refractory CLL and related disease (prolymphocytic leukemia and Richter's syndrome): group 1 with ibrutinib lead-in, group 2 with concomitant administration (ofatumumab on day 1/ibrutinib on day 2), and group 3 with ofatumumab lead-in. These patients were heavily pretreated, and the majority had high-risk disease features. The ORR among patients with CLL/SLL was 100% in group 1, 78.9% in group 2, and 70.8% in group 3, suggesting the importance of a prompt start of ibrutinib. The estimated 12-month PFS rate was 83.1% for the entire study population,

88.7% in group 1, 85% in group 2, and 75% in group 3, demonstrating that ibrutinib and ofatumumab had high clinical activity in patients with relapsed/refractory CLL/SLL in all three dose administration sequences investigated. These results appear to be superior compared to ibrutinib as single agent and comparable to ibrutinib combined with rituximab.[31]

Early results of Phase III randomized trial comparing ofatumumab plus idelalisib versus ofatumumab alone for previously treated CLL were presented at the last EHA meeting.[44] Combination regimen showed a superior PFS and ORR compared with ofatumumab as single agent (75% versus 18%), including in high-risk subgroups. A median duration of response of 14.9 months was observed in the group with idelalisib as compared with 6.7 months in the ofatumumab monotherapy group (Table 3).

Obinutuzumab

Obinutuzumab (GA101) is a novel humanized Type II anti-CD20 MoAb that has been investigated and compared with rituximab.

Type I antibodies, inducing the translocation and stabilization of CD20 into lipid rafts, cause strong CDC but little direct cell death. In contrast, type II antibodies do not stabilize CD20 in lipid rafts and by consequence show a reduced binding to C1q, resulting in flat level of CDC.[45] Obinutuzumab is a unique, glycoengineered type II anti-CD20MoAb that recognizes a CD20 epitope overlapping with that of rituximab, but compared with type I anti-CD20 antibodies, it exhibits a different elbow hinge angle and binds CD20 in a different orientation, the latter of which could be the basis of the functional differences between type I and type II antibodies. Importantly, the type II antibody obinutuzumab 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. Obinutuzumab binds with high affinity to CD20 and induces up to one hundred times stronger ADCC compared with rituximab.[46–48]

Obinutuzumab single agent. As for clinical trials, a separate portion of the Phase I/II GAUGUIN study specifically investigated obinutuzumab monotherapy in relapsed/refractory CLL.[49] In the Phase I study, dose escalation of obinutuzumab (400–1,200 mg) was provided to 13 relapsed/refractory CLL patients, and 20 additional CLL patients received a fixed dose of 1,000 mg in the Phase II study. The observed ORR was 62% (Phase I) and 30% (Phase II). The median PFS was 10.7 months, with a median duration of response of 8.9 months. Most commonly, adverse events (AEs) were represented by infusion-related reactions (IRRs), neutropenia, lymphocytopenia, and thrombocytopenia with most of the AEs being grade 1–2.

Based on these initial studies, obinutuzumab proved to be well tolerated without major reported dose-limiting toxicities and no required dose reductions.

Phase II GAGE trial enrolled 80 previously untreated patients with CLL comparing alternative treatment schedule.[50,51] High rates of CR/CRi were observed in patients receiving 2,000 mg dose of obinutuzumab when compared with those receiving the standard dose of 1,000 mg (67% versus 49%). CR or CR with incomplete cytopenia response

Table 3. Anti CD20 monoclonal antibodies in combination with novel agents.

AUTHORS	NO. EVALUABLE pts	DISEASE STATUS	TREATMENT REGIMEN	CLINICAL RESPONSE	
			RITUXIMAB	OR (%)	PFS (MEDIAN)
Furman et al[30]	220	Pretreated	R 375 mg/sqm first cycle, 500 mg/sqm cycle 2–8 plus placebo	13%	5.5 mo
			vs		
			R 375 mg/sqm first cycle, 500 mg/sqm cycle 2–8 plus idelalisib 150 mg twice daily	81%	n.r.
O'Brien et al[29]	64	Untreated	R 375 mg/sqm weekly cycle 1–8 plus idelalisib 150 mg twice daily	97%	n.r.
Burger et al[31]	40	Pretreated Untreated (high risk)	R 375 mg/sqm weekly first cycle, once every 4 weeks cycle 2–6 plus ibrutinib 420 mg daily	95%	n.r.
			OFATUMUMAB		
Jaglowsky et al[43]	66	Pretreated	O 300 mg dose 1, 2,000 mg dose 2–12 plus ibrutinib 420 mg daily (ibrutinib lead-in)	100%	n.r.
			vs		
			O 300 mg dose 1, 2,000 mg dose 2–12 plus ibrutinib 420 mg daily (concurrent I and O)	78.9%	n.r.
			vs		
			O 300 mg dose 1, 2,000 mg dose 2–12 plus ibrutinib 420 mg daily (O lead-in)	70.8%	n.r.

Abbreviations: OR, overall response; PFS, progression free survival; R, Riuximab; O, Ofatumumab.

(20% versus 5%) favored 2,000 mg, suggesting that obinutuzumab as single agent has a marked efficacy even in the absence of combination with chemotherapy.

Combination therapy. Phase Ib GALTON trial explored the safety and efficacy of obinutuzumab–bendamustine (O-B) or obinutuzumab fludarabine cyclophosphamide (O-FC) for the therapy of previously untreated fit patients with CLL.[52] Most common AEs were IRRs (88%, grade 3–4 20%) and neutropenia (55% on G-B arm versus 48% on G-FC arm). The ORR was 90% for G-B arm including 20% of CR and 62% for G-FC arm with 10% of CR. This trial confirmed that obinutuzumab can be safely administered with standard chemotherapy in previously untreated fit CLL patients.

CLL11 was a multicenter, randomized, Phase III, open-label trial that enrolled 781 previously untreated patients with comorbidities.[53] This study evaluated three treatments: data of chlorambucil alone, obinutuzumab + chlorambucil, and rituximab + chlorambucil, showing that chemoimmunotherapy with obinutuzumab + chlorambucil or rituximab + chlorambucil prolongs significantly PFS compared with chlorambucil alone (26.7 months vs 15.2 months vs 11.1 months). The ORR was 77.3% in the obinutuzumab arm vs 65.7% in the rituximab arm and 31.4% for chlorambucil monotherapy. OS benefits were achieved with O-chlorambucil compared to chlorambucil (9% death rate vs 20%), but no significant OS benefit was noted for R-chlorambucil compared to chlorambucil or with O-chlorambucil compared to R-chlorambucil. MRD rates were markedly increased in the obinutuzumab arm compared with the rituximab arm in both bone marrow and peripheral blood. IRR and neutropenia were more common with obinutuzumab + chlorambucil without an increase in infections (Table 2).

Due to this study, obinutuzumab in combination with chlorambucil has become an accepted comparator for unfit, previously untreated patients with CLL. Therefore, in order to assess the efficacy of novel agents (ibrutinib, ABT-199, and idelalisib) in combination with obinutuzumab, several comparative studies are accruing in older or frail CLL patients.

Alemtuzumab

Alemtuzumab (Campath-1H) is an unconjugated recombinant DNA-derived humanized IgG1 MoAb targeting the CD52 antigen, which consists of a glycosylated protein joined to the cell membrane by glycosylphospatidylinositol (GPI) anchor. CD52 is represented on normal and neoplastic lymphoid cells, either B- or T-lymphocytes, monocytes, and macrophages but not expressed on hematopoietic stem cells, erythrocytes, and platelets. The physiologic role of CD52 is unknown. However, signal transduction via the T-cell receptor induced by legation and cross-linking of T-cell CD52 have been shown after exposition to alemtuzumab.[53] Alemtuzumab induces CLL cell death through various mechanisms including ADCC, CDC, and direct apoptosis. The lowest expression of CD52 has been observed on normal B-cells, while the highest expression seems to be on T-prolymphocytic leukemia cells, B-cell CLL cells, and hairy cell leukemia cells.[54] Due to the small amount of pharmacological data, the dose of 30 mg three times a week is largely empirical and is not adjusted for surface area. In patients with a small tumor load, such as those being treated following allogeneic bone marrow transplantation, the half-life of alemtuzumab is longer. In patients in relapse, the administration of alemtuzumab intravenously three times a week for 12–16 weeks showed an ORR of approximately 33%.[54] Infusion-related AEs, such as fever and chills, are of common observation during the first 3–5 infusions of alemtuzumab, while in a longer period, the development of opportunistic infections, in particular reactivation of cytomegalovirus, could occur. The major problem with the use of alemtuzumab is its inferior activity in bulky lymph nodes. The larger the lymph node size, the inferior the response.[55]

Frontline therapy with subcutaneous alemtuzumab in CLL patients has been tested by Swedish investigators.[56] Response rate in 33 patients enrolled in the trial was 87% with 19% CRs. Despite the observation of local reactions after the first few subcutaneous injections, patients were then able to self-administer the MoAb. Subcutaneous administration was well tolerated, apart from local reactions. There was a very marked decrease in the number of infusion-related side effects.

Animal studies from MoAbs permitted the observation that they work at best with small tumor burden. This is the way the role of alemtuzumab in the management of residual disease after chemotherapy has been explored. Alemtuzumab is very effective in clearing residual blood and marrow disease and fairly effective in splenic disease. Patients who are able to reach eradication of MRD have much longer time-to-treatment failure and probably survival.[57]

CAM307, a Phase III, randomized, multicenter, international clinical trial comparing alemtuzumab versus chlorambucil in previously untreated patients with CLL, demonstrated higher ORR (83.2% vs 55.4%), CR rates (24.2% vs 2.0%), and PFS (14.6 vs 11.7 months).[58] Alemtuzumab also resulted in deeper responses; eradication of MRD was obtained in 11 (31%) of 36 complete responders to alemtuzumab, while among chlorambucil patients who attained a response, none of them showed MRD negativity. Patients with del17p treated with alemtuzumab had threefold better ORR (64% vs 20%) and nearly fivefold improvement in median PFS (10.7 vs 2.2 months), although not statistically significant.

NCRI CLL206 trial[59] and CLLSG CLL20 trial[60] evaluated alemtuzumab in combination with high-dose steroids, prednisone and dexamethasone, respectively, in 17p-deleted CLL patients. Results demonstrated higher response rates compared with historical experience with conventional chemotherapy, even including rituximab–fludarabine plus cyclophosphamide. Among patients treated with alemtuzumab plus prednisone in the frontline setting, 88% of them responded,

with 65% achieving a CR, and among those treated with alemtuzumab plus dexamethasone, 97% of previously untreated patients responded, with 20% experiencing a CR. The addition of alemtuzumab to chemotherapy may represent another treatment possibility of CLL. Six patients refractory to fludarabine or alemtuzumab, while treated with each agent alone obtained a high number of responses when the two drugs were combined, supporting the hypothesis of a synergistic effect. Fludarabine in combination with alemtuzumab was compared with fludarabine alone in a Phase III trial for relapsed CLL patients. Fludarabine plus alemtuzumab yielded clearly better response rates and improved OS than fludarabine monotherapy.[61]

Although high response rates have been achieved with the combination of alemtuzumab–fludarabine plus cyclophosphamide in relapsed and refractory patients,[62] experiences with this combination in the first-line setting have led to some safety challenges. A randomized study for untreated CLL patients by the French group comparing the activity of alemtuzumab–fludarabine plus cyclophosphamide to rituximab–fludarabine plus cyclophosphamide was prematurely closed due to the excess of toxicity in the arm with the alemtuzumab combination. Moreover, alemtuzumab–fludarabine plus cyclophosphamide yielded a disappointing response rate compared with the other arm.[63] As several trials demonstrated the superiority of the combination FC over fludarabine alone, and rituximab and alemtuzumab appears to be effective together, the regimen of alemtuzumab–rituximab has been developed for refractory/relapsed CLL, obtaining an ORR of 52% (8% CR; 4% nodular PR, nPR; 40% PR). However, the time to relapse was still unsatisfactory Table 4.[64]

Other MoAbs

Transmembrane proteins such as CD23, CD37, CD40, and CD74 are being pursued as targets for MoAbs therapy and are at various phases of development.[65–68] This is also the case for CD19, a glycoprotein member of the Ig superfamily,[69] and HLA-DR, a class II antigen of the major histocompatibility complex.[70] In a Phase I/II study, 91 patients with relapsed/refractory NHL, CLL, and multiple myeloma were enrolled to receive MEDI-551, an antibody targeting CD19. In the CLL cohort, an ORR of 24% was observed. A randomized trial (bendamustine/MEDI-551 vs bendamustine/rituximab) in patients with relapsed/refractory is currently ongoing.

Lumiliximab, a chimeric CD23 targeting MoAb, was studied in combination with FCR in 31 patients with relapsed/refractory CLL and resulted in an ORR of 71% with a CR rate of 52% with an acceptable toxicity. However, in a randomized trial (FCR ± lumiliximab), lumilixumab failed to improve clinical outcomes.[71]

Additional targets being pursued include the immunosuppressive molecule CD200.[72]

Conclusion

The introduction of rituximab in the late 1990s has changed the treatment modality of lymphoproliferative disorders. The CLL8 German trial was the first study demonstrating the superiority in terms of prolonging OS by chemoimmunotherapy with FCR over the comparator, chemotherapy alone, FC. Despite its demonstrated efficacy, an important issue has been addressed about FCR: should FCR be considered the *standard* of care for all patients with CLL? In order to

Table 4. Alemtuzumab in combination with chemotherapy.

AUTHORS	COMP. STUDY	NO. EVALUABLE pts	DISEASE STATUS	TREATMENT REGIMEN	CLINICAL RESPONSE	
					CR (%)	OR (%)
Elter et al[61]	Yes	335	Untreated	Alemtuzumab 30 mg d 1–3, F 30 mg/sqm d 1–3 every 28 d 6 cycles	13	82
				vs		
				F 25 mg/sqm d 1–5 every 28 d 6 cycles	4	75
Montillo et al[62]	No	43	Pretreated	FCC: F 40 mg/sqm os and CTX 250 mg/sqm os and Alemtuzumab sc d 1-every 28 d, 6 cycles	30	67
Lepretre et al[63]	Yes	165	Pretreated	FCR: R 375 mg/sq first cycle, 500 mg/sqm d 1 of the cycles 2–6, F 40 mg/sqm os and CTX 250 mg/sqm os d 1–3, every 28 d, <6 cycles	34	91
				vs		
				FCCam: Alemtuzumab 30 mg sc and F 40 mg/sqm os and CTX 250 mg/sqm os d 1–3, every 28 d, <6 cycles	19	90
Faderl et al[64]	No	48	Pretreated	R 375 mg/sqm first cycle weekly for 4 w, Alemtuzumab 30 mg × 2/w for 4 w 2 cycles maximum	8	52

Abbreviations: Comp., comparative; CR, complete response; OR, overall response; PFS, progression free survival; F, fludarabine; R, Rituximab; CTX, cyclophosphamyde; Cam, Campath-1H; d, days; mo, months; w, weeks; pts, patients; sc, subcutaneous.

investigate less toxic regimens for elderly or unfit patients, other chemotherapeutic agents, such as bendamustine and chlorambucil, were investigated in combination with rituximab, confirming the superiority of chemoimmunotherapy when compared with chemotherapy alone. New anti-CD20 MoAbs considered to be more effective than rituximab on the basis of preclinical studies have been tested in the past few years. Both ofatumumab and obinutuzumab in Phase III randomized trials showed that the combination with chlorambucil improves the outcomes in previously untreated patients with CLL not suitable for fludarabine-based treatment and/or coexisting conditions.

Nevertheless, in the era of small molecular inhibitors, the therapeutic goal for lymphoproliferative disorders is an effective chemotherapy-free strategy to avoid the plague of non-targeted toxic drugs. The possibility of a chemotherapy-free world is a rapidly approaching reality, and MoAbs seem to represent a pillar of these forthcoming strategies.

Author Contributions

Wrote first draft of the manuscript: PP, MMontillo. Contributed to the writing of the manuscript: PP, MMontillo, AMF, AT, RC, MMazzucchelli. Agree with manuscript results and conclusions: PP, MMontillo, AMF, AT, MMazzucchelli, RC. Jointly developed the structure and arguments for the paper: PP, MMontillo. Made critical revisions and approved final version: PP, MMontillo. All authors reviewed and approved of the final manuscript.

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