New and Old Monoclonal Antibodies for the Treatment of Chronic Lymphocytic Leukemia

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Abstract: Over the last few years, several new agents have been under evaluation in preclinical studies and clinical trials, showing promise in treating chronic lymphocytic leukemia (CLL). Among these agents, monoclonal antibodies (mAbs) such as rituximab and alemtuzumab have changed the natural course of the disease. Nowadays there are several new promising monoclonal antibodies under investigation against the CD20, CD23, CD37 and CD40 molecules. Application of newer monoclonal antibodies represents an area of ongoing clinical research in CLL.

Keywords: CD20, CD23, CD37, CD40, CD52, chronic lymphocytic leukemia, monoclonal antibodies.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Europe and North America. The natural clinical course of CLL is highly variable, and chemotherapy is usually not indicated in patients with early stage or stable disease, while patients with progressive disease or more advanced CLL require treatment. The standard of care has evolved from single-agent therapy with an alkylating agent through the combination of purine analogs and cyclophosphamide, which is more active in terms of overall response rate (OR), complete response (CR) and progression free survival (PFS) [1-4]. Recently the introduction of monoclonal antibodies (mAbs) such as rituximab and alemtuzumab has enhanced the therapeutical efficacy of chemotherapy, improving the clinical course of patients affected by chronic lymphocytic leukemia. Application of newer mAbs represents an area of ongoing clinical research in CLL; over the last few years, several new monoclonal antibodies have been investigated in preclinical studies and clinical trials for patients affected by CLL: the most promising are mAbs directed against CD20, CD23, CD37 and CD40 [5-13] (Table **1**) (Fig. **1**).

RITUXIMAB

Rituximab (Rituxan, Biogen IDEC, Cambridge, MA, and Mabthera, Hoffman-La Roche, Basel, Switzerland) is the first available chimeric murine/human monoclonal IgG1 antibody; it targets the CD20 antigen initiating immunologic reactions that mediate B-cell lysis [14].

CD20 is considered an ideal target for monoclonal antibody immunotherapy of B-cell malignancies, as it is expressed on almost all B cells, but not on haematological stem cells and it persists on the cell surface in the presence of the monoclonal antibody [15]. Functional activities of anti-CD20 antibodies consist of complement-dependent cytotoxicity (CDC), antibody dependent cellular cytotoxicity (ADCC) mediated by cells displaying Fc_ receptors, and 'direct cell death' by growth inhibition and non-classic apoptosis [16-18].

Immunotherapy with anti-CD20 mAbs may be limited by depletion of complement and exhaustion of cellular cytotoxicity caused by high burdens of IgG opsonized cells. Moreover ineffective killing of targeted cells may lead to reductions in CD20 surface expression. These mechanisms decrease the efficacy of anti CD20 mAbs stimulating the development of more potently recruit effector function [19].

Depending on the CD20 epitope that is bound and/or the binding mode, CD20 antibodies are classified in 2 major types termed type I and type II [18, 20, 21] (Table **2**).

Type I antibodies show stronger C1q binding and potent induction of CDC, but only low levels of direct cell death. In contrast, type II antibodies exhibit reduced binding to C1q and lower levels of CDC, but they potently induce direct cell death [18].

The majority of anti CD20 antibodies as rituximab and ofatumumab are of type I, whereas the prototype type II antibody is the murine antibody tositumomab [22, 23] (Fig. **2**).

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Table 1. Monoclonal Antibodies for Chronic Lymphocytic Leukemia

Antibody	Antigen	Description	Clinical status
Rituximab	CD20	Chimaeric	Approved
Alemtuzumab	CD52	Chimaeric	Approved
Ofatumumab	CD20	Humanized	Approved (FDA)
Lumiliximab	CD23	Chimaeric	Phase III
GA-101	CD20	Humanized	Phase III
TRU-016	CD37	Humanized	Phase I/II
Lucatumumab	CD40	Humanized	Phase I
Dacetumumab	CD40	Humanized	Phase I
Epratuzumab	CD22	Humanized	Phase I/II (NHL)
Galiximab	CD80	Chimaeric	Phase I/II (NHL)

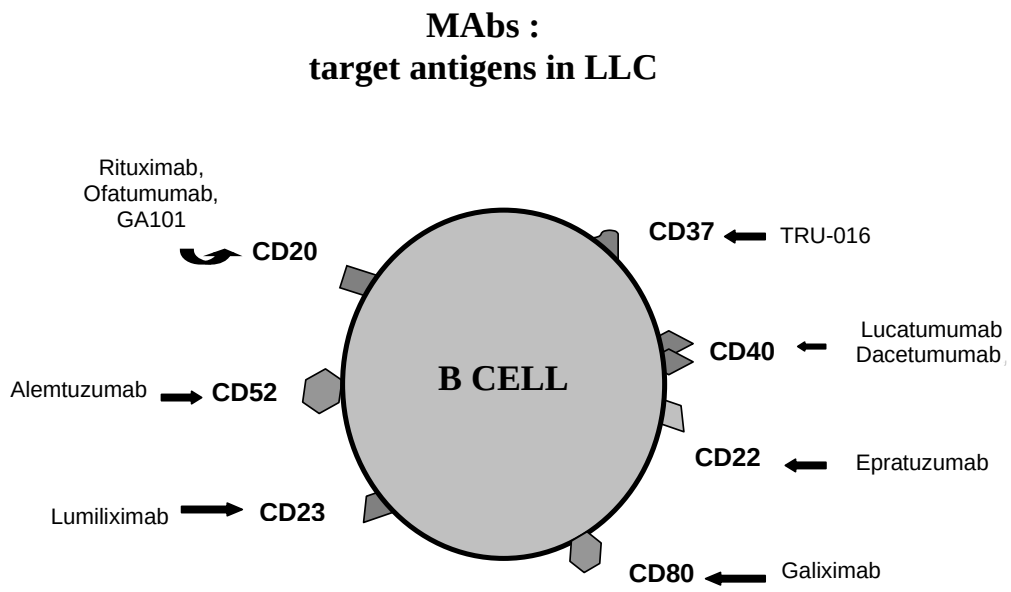

Fig. (1). Cell-Surface Antigens on the B Cell.

Table 2. Biological Property of Anti-CD20 Type I and Type II Antibodies

Type I	Type II
Rituximab Ofatumumab	GA101 Tositumomab
CD20 localization into lipid rafts	Lack of CD20 localization into lipid rafts
High CDC	Low CDC
Low direct cell death	High direct cell death
High ADCC and phagocytosis	High ADCC and phagocytosis

In contrast to B-cell lymphomas which express CD20 strongly, CD20 expression on CLL cells is weak. Rituximab induces both ADCC and CDC, but induction of apoptosis seems to play a more important role in CLL than in B-Non Hodgkin Lymphomas (NHL) [24-27].

Rituximab was the first monoclonal antibody (mAb) to be approved by the Food and Drug Administration (FDA) for treatment of a human disease in 1997 and since then has become the most important new treatment for B cell malignancies in the last decade.

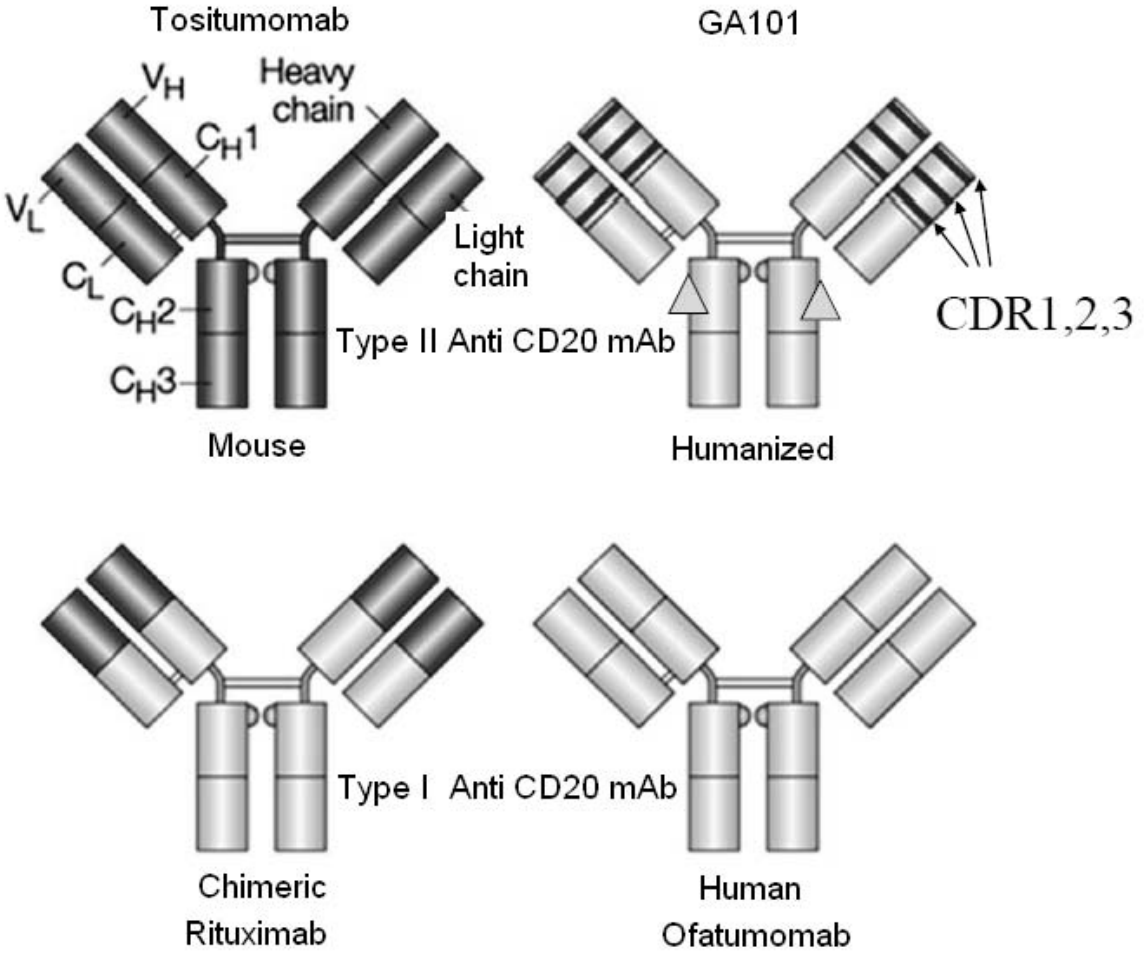

Fig. (2). Anti-CD20 monoclonal antibodies.

It is considered to be well tolerated, even if more than 90% of patients experience some infusion-related reaction, generally mild to moderate in severity [28, 29]. Patients with CLL and others with a markedly increased number of circulating lymphocytes may be at an increased risk of more serious adverse events, including respiratory insufficiency, tumour lysis syndrome and a rapid tumour clearance syndrome [30].

While the phase III pivotal approval study of rituximab in NHL demonstrated promising clinical activity, rituximab at standard dosages (375 mg/m^2) has limited single agent activity in previously treated or untreated CLL [29, 31, 32]. The weak activity of rituximab in CLL may be in part a result of reduced tumor expression of CD20 and the presence of soluble CD20 serving as a decoy receptor [33, 34]. Two trials administered either thrice weekly doses or higher doses of rituximab weekly (up to 2250 mg/m^2 per dose) to relapsed CLL patients with improved response, establishing a role for rituximab in CLL and encouraging subsequent trials of rituximab as a single-agent and in combination strategies with chemotherapy [35, 36].

A phase II study of fludarabine and rituximab in 33 previously treated or untreated patients with CLL resulted in an OR of 87% and a median duration of response of 75 weeks [37]. CALGB investigators performed a randomised phase II trial comparing concurrent with sequential fludarabine and rituximab in 104 untreated CLL patients confirming that the concomitant use of chemo-immunotherapy was superior to sequential administrations [38].

The REACH Trial compared fludarabine and cyclophosphamide (FC) to FC plus rituximab (FCR) in 552 patients with relapsed/refractory CLL. Treatment with FCR resulted in an OR of 70%, versus 58% for FC alone, and the CR was 24% versus 13% respectively. PFS in the FCR arm was 30.6 months compared with 20.6 months in the FC arm. Hematologic toxicities were the most significant adverse events [39]. Recently in a randomized, open-label, phase 3 trial comparing front line therapy in CLL patients with 6 cycles of FC versus FCR, the addition of rituximab at a dose of 375 mg/m^2 in the first course, and 500 mg/m^2 from the second to the sixth course led to increase both progression-free survival and overall survival (OS). Chemo-immunotherapy was more frequently associated with grade 3 and 4 neutropenia and leucopenia while other side-effects, including severe infections, were not increased [40]. Collectively, these two phase III studies provide justification for the use of rituximab as part of combination chemo-immunotherapy in both newly diagnosed and relapsed CLL fit patients. Rituximab has also been used successfully to

treat complications of CLL, including autoimmune hemolytic anemia, immune thrombocytopenia and pure red cell aplasia [41-43]. It was approved for treatment of rheumatoid arthritis and is being examined in other autoimmune disease [44]. Rare cases of Hepatitis B or JC poliomavirus reactivation have been reported with rituximab administration [45, 46].

ALEMTUZUMAB

The second approved mAb is alemtuzumab (Campath-1H, Genzyme, Cambridge, MA), a recombinant humanized IgG1k mAb targeting the CD52 antigen expressed on normal and malignant human peripheral blood B and T lymphocytes as well as natural killer cells, monocytes and macrophages [47-49].

Mechanism of action of alemtuzumab includes CDC, ADCC [50, 51]; some studies also reported direct cytotoxicity, but this was not confirmed in other studies [52-56].

It is active in advanced or refractory CLL and has proven efficacy in patients with high-risk genetic markers such as deletions of chromosome 17p (del 17p), and p53 mutations [57-61].

Alemtuzumab was approved by the FDA following the pivotal CAM 211 trial in which 93 patients with relapsed or refractory CLL who had failed prior therapy with fludarabine and an alkylating agent were treated with stepped-up dosing followed by 30 mg three times weekly for a total of 12 weeks. The response rate with alemtuzumab was 33%, even if no benefit was observed in patients with bulky lymph node [59]. In studies involving patients with relapsed/refractory CLL treated with alemtuzumab the most common adverse events were cytopenias and infections as a consequence of profound cellular immune suppression. Reactivation of Herpes viruses including cytomegalovirus (CMV) were the most common opportunistic infections observed. Some authors reported that periodic analysis of CMV reactivation by antigenemia and PCR allowed prompt starting of pre-emptive antiviral therapy avoiding progression to overt CMV disease [62]. Anti-tumor effects of alemtuzumab were more significant in blood and bone marrow than in lymph nodes (especially bulky disease), because of poor bioavailability of the drug in bulky lymph node or due to the variability in the immune effectors' mechanisms in lymph nodes compared with other sites [59]. More recently, alemtuzumab has been approved by the FDA for the initial treatment of CLL based on a randomized trial conducted by Hillmen and colleagues, which included 297 patients that received either chlorambucil or alemtuzumab 30 mg thrice weekly for up to 12 weeks. The antibody demonstrated a statistically significant improvement in the overall response rate, allowing to reach negativization of minimal residual disease. Toxicity was not substantially different between the two arms with the exception of neutropenia, lymphopenia and CMV reactivation, that were more frequent after alemtuzumab therapy. Also, alemtuzumab was shown to be effective in patients with del 17p [63].

Despite pre-medication with acetaminophen and diphenhydramine, intravenous infusion is associated with rigours in 90% of patients, often severe; fever has been noted in 85% of patients frequently coupled with nausea, vomiting and rash [59].

Because of the high risk of tumour lysis syndrome for the intravenous (IV) route and the observation that subcutaneous alemtuzumab had comparable biological activity with diminished infusion-related events, the subcutaneous administration has progressively increased. In a phase II trial in untreated CLL patients alemtuzumab was administered subcutaneously (SC) at a dose of 30 mg 3 times weekly for a maximum of 18 weeks obtaining a response rates and duration of response similar to those observed with the IV route of administration [63, 64]. Although 90% of patients display local injection site reactions, most are only grades I–II in severity and generally disappear within 2 weeks; infusional reactions are relatively mild [64].

In the fludarabine-refractory CLL setting, the GCLLSG protocol enrolled 103 patients who received SC alemtuzumab at dose of 30 mg three times per week for up to 12 weeks; an overall response rate of 34% with efficacy in the del 17p subgroup confirmed the applicability of the anti CD52 mAb in this setting of patients [60, 61, 65].

In order to reduce toxicity some authors tried to use a lower dose schedula of alemtuzumab. Two Italian groups administered IV or SC alemtuzumab at dosage of 10 mg three times per week (30 mg week), for 10 to 18 weeks, to patients with heavily pre-treated/fludarabine refractory CLL, demonstrating a favourable toxicity profile, and a good response rate [66-68].

Immunological recovery after alemtuzumab therapy showed a constant long lasting immune depletion with reduced counts of all lymphoid subsets, especially $CD4^+$ lymphocytes, both in previously untreated and heavily pre-treated B-CLL patients; this side effect seems to be independent of the alemtuzumab dose and route of administration [69, 70].

Alemtuzumab has been combined with other active agents including fludarabine. A randomised trial was performed in 335 patients treated at second line with fludarabine alone compared with fludarabine and alemtuzumab (FluCam) given at a dose of 30 mg on days 1–3 of each 28-day cycle. OR and CR rates were 84.8% versus 67.9% and 30.4% versus 16.4%, respectively. The PFS was longer in FluCam arm (29.6 vs. 20.7 months). Adverse events including cytopenias and infections were similar in both treatment arms, except for the occurrence of CMV infection uniformly associated with the FluCam arm [71].

Attempts to further intensify this regimen with the addition of cyclophosphamide to FluCAM resulted in increased infectious morbidity [72].

The currently approved schedule of alemtuzumab administration involves a dose of 3 mg delivered IV on day 1, escalating to 10 mg on day 2 and then to 30 mg three times weekly as tolerated, for a total of 8-12 weeks [57].

OFATUMUMAB

Ofatumumab (HuMax-CD20; Arzerra, GlaxoSmithKline/ Genmab), is a newly approved fully human type I, anti-

CD20, IgG1k mAb with a molecular weight of approximately 149 kDa [73-78].

Ofatumumab induces killing of a panel of tumor B-cell lines and primary tumor cells *via* activation of CDC and ADCC *in vitro* [79, 80]. Compared with rituximab, ofatumumab has similar ADCC but increased binding of C1q and stronger CDC as well as a slower off-rate and more stable CD20 binding [15, 74, 80].

It appears to bind a different epitope of CD20 than rituximab composed of both small and large extracellular loops on the CD20 molecule [15] (Fig. **3**). The potent CDC with ofatumumab may be a result of the greater proximity of the small-loop binding site to the cell membrane, potentially leading to more effective deposition of complement on the cell surface [15, 79-81]. In CLL, it is likely that high initial plasma ofatumumab concentrations will be required to eliminate B cells located in tissues, and subsequently, lower plasma concentrations may be sufficient to saturate CD20 epitopes and to maintain depletion of B cells in peripheral blood [82]. As expected the major ofatumumab toxicity observed in nonclinical studies was the severe and prolonged depletion of B lymphocytes both in the circulation and in the major lymphoid organs [77, 78]. In patients with CLL refractory to fludarabine and alemtuzumab, after the eighth and twelfth ofatumumab infusions, there were 91% and 85% median decreases in circulating $CD19^+$ B cells [83]. The time to recovery of normal levels of lymphocytes is as yet undetermined [83]. A phase I/II study of ofatumumab at doses of 2000 mg in 33 relapsed/refractory CLL patients, demonstrated an overall response rate of 50%. Infusion-related adverse events were similar to rituximab and 51% of patients developed infections, including one which was fatal [73].

Efficacy of ofatumumab was confirmed by an international pivotal trial in 138 patients with CLL refractory to at least one fludarabine-containing regimen and either refractory to at least one alemtuzumab-containing regimen or to have bulky lymphadenopathy (> 5 cm) making them less suitable for alemtuzumab treatment. Patients received eight weekly infusions of ofatumumab followed by four monthly infusions during a 24-week period (dose 1 = 300 mg; doses 2 to 12 = 2000 mg). Hematologic events during treatment included anemia and neutropenia. The OR was 58% for the patients with CLL refractory to fludarabine and alemtuzumab and 47% for the patients refractory to fludarabine with bulky lymphadenopathy [75]. The preliminary results show promising efficacy of ofatumumab monotherapy in heavily-pretreated and poor prognosis patients with fludarabine-refractory CLL. Combination studies with chemotherapy, such as fludarabine and cyclophosphamide, are being done to enhance the therapeutic effect of the mAb in untreated patients [84]. Ofatumumab is approved in the US for the treatment of patients with CLL who are refractory to fludarabine and alemtuzumab or have bulky fludarabine-refractory disease [83, 85]. The recommended regimen is 12 doses of ofatumumab; the initial dose is 300 mg, followed 1 week later by 2000 mg administered weekly for seven doses, then monthly for four administrations [77, 83].

GA-101

GA-101 (RO5072759, Hoffman La Roche and Genentech) is a fully humanized anti-CD20 IgG1 mAb [16,

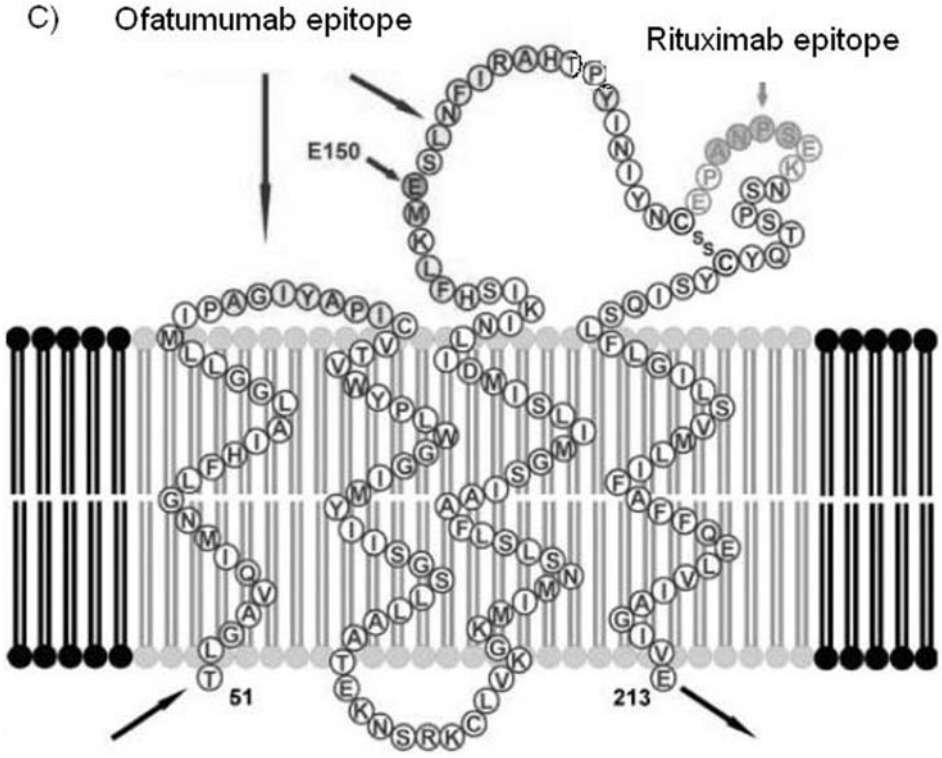

Fig. (3). CD20 epitopes recognised by anti-CD20 mAbs.

86-88]. In addition to an engineered variable region conferring type II CD20 binding, GA101 also harbours a glycol-engineered Fc segment that binds with increased affinity to Fc receptor displayed by immune effectors cells [87, 89].

It binds CD20 in a completely different orientation than rituximab and over a larger surface area, and initiates a caspase independent cell death *via* an actin-dependent lysosome-mediated mechanism that is reliant on cell-to-cell contact [90-92]. GA-101 binds with high affinity to the CD20 epitope and, as a result, induction of ADCC is 5-100 times greater than rituximab [88, 90, 91, 93, 94]. CDC activity is low due to the recognition of the CD20 type II epitope and the lack of CD20 localization into lipid rafts after binding of mAb to CD20 [21, 88]. In nonhuman primates it demonstrates superior B cell–depleting activity in lymphoid tissue, including in lymphonodes and spleen [16]. It causes depletion of CLL cells in whole blood samples that may be more potent than rituximab at similar concentrations [93, 94].

In a phase I/II study GA-101 at doses from 50 mg to 2000 mg was administered in heavily pretreated patients with a $CD20^+$ neoplasm, showing promising efficacy and a similar safety profile to rituximab [87]. GA-101 as single agent was relatively well tolerated in relapsed/ refractory CLL patients, with the most common grade 3-4 toxicity being transient neutropenia [95]. A phase III multicentre three arm randomized study is currently underway in untreated CLL patients with comorbidity comparing monotherapy with chlorambucil, chlorambucil plus rituximab, and chlorambucil plus GA-101.

LUMILIXIMAB

Lumiliximab (IDEC 152) is a genetically engineered (macaque variable regions, human constant regions) anti-CD23 IgG1κ mAb targeting CD23, a transmembrane glycoprotein expressed on the majority of CLL cells [96, 97]. It induces ADCC and CDC, producing similar levels of apoptosis to rituximab in CD23-bearing lymphoid cell lines [98]. As CD23 is expressed on a high proportion of CLL cells but is only minimally expressed on other cells, targeting this molecule provides a specific modality of treatment with the potential to minimize toxicity. In preclinical studies, lumiliximab was shown to enhance the effects of fludarabine and rituximab, providing a rationale for its use in chemo-immunotherapy regimens in clinical trials in CLL [98].

In a phase I, dose escalation trial performed in patients with relapsed and refractory CLL luminiximab demonstrated a transient disease reduction, with a favourable toxicity profile. The recommended dose for future studies of lumiliximab in combination with other agents was 500 mg/m^2 [99]. Pharmacodynamic studies showed dose-dependent increases in soluble CD23, but no down-regulation of CD23 antigen [99]. A phase I/II combination study with fludarabine, cyclophosphamide, and rituximab in patients with relapsed or refractory CLL showed a potential benefit, leading to a high CR rate and without evidence for enhanced toxicity in previously treated patients with CLL [100]. However, results from a phase III international study comparing FCR plus lumiliximab versus FCR in relapsed CLL, did not confirm benefit in terms of improved response or PFS and the study was recently prematurely closed [13].

TRU-016

TRU-016 (SMIP, CytoxB37G) is an anti-CD37 IgG fusion protein created by humanizing SMIP-016, a mouse/human chimeric protein with preclinical antitumor activity against lymphoid malignancies; it represents a structural modification of a CD37 antibody that lacks the CH1 domain [101, 102]. Among antibodies currently in clinical development for haematological malignancies, those targeting CD37 appear the most promising because CD37 is expressed on B cells and transformed mature B cell leukemias and lymphomas including CLL, where it is present in increased density compared with normal B cells.

In vitro studies with the chimeric version of TRU-016 have provided evidence that this molecule is a potent inducer of apoptosis and ADCC against CLL cells [103].

Results of a phase I/II clinical trial of TRU-016 in 28 pretreated CLL patients reported a favorable toxicity profile with adverse effects primarily of grade 1 or 2. Clinical activity was observed, including a reduction in circulating lymphocytes and lymph node size [104].

In a phase I study in relapsed and refractory CLL patients a reduced absolute lymphocyte count was observed in all cohorts above the 0.1-mg/kg dose [105].

DACETUZUMAB

Dacetuzumab (SGN-40) is an IgG1 humanized mAb directed at CD40 with partial agonist properties. In a phase I study in pre-treated patients with CLL it exhibited minimal clinical activity with mild or moderate toxicity, indicating that it may potentially be employed in combination therapies [106].

LUCATUMUMAB

Lucatumumab (HCD122, CHIR-12.12) is a fully human anti CD40 mAb that blocks CD40/CD40L interactions *in vitro* and inhibits CD40L induced proliferation of human peripheral blood lymphocytes, inducing higher ADCC than rituximab against CLL cells [107,108]. A phase I study of locatumumab in pretreated CLL patients has shown some activity with a favorable safety profile at a dose up to 3 mg/Kg [109].

APOLIZUMAB

Apolizumab (Hu1D10), is a humanized IgG1 mAb specific for a polymorphic determinant found on the Human leukocyte antigen-DR beta-chain (HLA-DRβ chain). The 1D10 antigen is present on normal and malignant B lymphocytes, dendritic cells, macrophages and some activated T lymphocytes. Apolizumab induces ADCC, CDC and apoptosis *in vitro* [110]. A phase I/II dose-escalation study of thrice-weekly apolizumab (1.5, 3.0, 5.0 mg/kg/dose) for 4 weeks in relapsed CLL showed relevant toxicity with lack of efficacy discouraging further development of the mAb [111].

EPRATUZUMAB

Epratuzumab (hLL2) is a humanized antihuman CD22 IgG1 antibody with clinical activity in patients with non-Hodgkin's lymphomas and autoimmune disorders. In investigations of epratuzumab's mode of action in comparison to rituximab, no CDC was detected, and ADCC was modest but significant with epratuzumab [112]. Epratuzumab exhibits highly efficacy to bind anti-CD22 and to induce CD22 internalization and can cause phosphorylation. Similar results were observed when epratuzumab was tested *in vitro* on primary normal B cells and neoplastic B cells separated from fresh peripheral blood samples from patients with chronic lymphocytic leukemia. Clinical studies are needed to evaluate the role of this agent in CLL [113].

GALIXIMAB

Galiximab. (IDEC-114) is a mAb with human IgG1 constant regions and Cynomolgous macaque variable regions that binds to CD80 on lymphoma cells and induces apoptosis, ADCC and a block in proliferation. There are ongoing clinical trials to evaluate its activity in relapsed/refractory NHL [114].

CONCLUSIONS

Chronic lymphocytic leukemia is a heterogeneous disease with a highly variable clinical course. Over recent years a better understanding of the genetic and biological groups in CLL has improved prognostic stratification of patients with this disease, allowing the search of new, more specific and active drugs in the optic of risk adapted treatment. While the immunoglobulin variable region heavy chain (IgVH) gene mutation status still has not changed treatment approaches, specific treatment for CLL patients has been developed based on cytogenetic risk.

The anti-CD20 monoclonal antibody rituximab is less active as a single agent in CLL than in B-cell lymphomas, unless very high doses or denser dosing regimen are used [35, 36, 115]. On the contrary, combinations of rituximab with chemotherapy as front-line therapy in CLL have proven for the first time to increase the overall survival in the chemo-immunotherapy group, suggesting that the choice of a specific treatment can change the natural course of the disease [40]. The presence of del 17p was the strongest negative prognostic indicator of overall survival irrespective of the treatment given [40].

Patients with del 17p benefit from alemtuzumab based therapy, which remains a reasonable first treatment option for these patients. Nevertheless, patients with chromosome 17 deletion still have a poor prognosis and according to recommendations of EBMT, physically fit patients with refractory CLL or with a del 17p should be offered an allogenic transplantation [116].

Other than rituximab two others anti CD20 mAbs demonstrated promising results in the treatment of CLL. Ofatumumab, was approved in the US for the treatment of fludarabine/alemtuzumab-refractory or bulky fludarabine-refractory disease, based on its proven efficacy especially in bulky disease [75]. GA-101 is currently in phase III of research; it is possible that it may have greater efficacy than rituximab, because it binds CD20 with higher affinity and induces significantly greater ADCC and direct cell death than rituximab.

Further studies should clarify whether the new mAbs, either alone or in combination chemo-immunotherapies, have the potential to improve the outlook for patients with CLL. However, it should not be forgotten that CLL is primarily a disease of the elderly that often requires tailoring the treatment according to the patients' fitness and co-morbidities. Therefore, future randomized studies should stratify patients both for fitness status and/or biological risk factors in order to ensure that the best treatment option for the individual CLL patient is established.

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