# OVERCOMING IMMUNODEFICIENCY IN CHRONIC LYMPHOCYTIC LEUKAEMIA: CURRENT KNOWLEDGE AND PERSPECTIVES

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## ABSTRACT

While the standard of care for chronic lymphocytic leukaemia (CLL) leads to high overall response rates and a long progression-free survival, it can be highly toxic for many patients, particularly in the elderly who often present concurrent diseases with associated morbidities. Treatment-related immune system burden and complications are challenging as most CLL patients already show immunodeficiency and are at high risk of infection. The latter are the main cause for increased morbidity and mortality and are correlated with disease severity and type of therapy. In the last few years, many new approaches and innovative agents such as second-generation anti-CD20 monoclonal antibodies, lenalidomide, B cell receptor signalling inhibitors, and novel cellular therapies have advanced the outlook for CLL management. Indeed, novel therapies could soon be addressing the need to promote immune reactivation and re-sensitise the immune system. By doing so, they could reach two main objectives, namely lowering the high proportion of patients at risk of infection, and acting as effective tools for the immune system to overcome its defects and fight malignant cells.

Keywords: Chronic lymphocytic leukaemia (CLL), immunodeficiency, immunotherapy, cellular therapies.

## INTRODUCTION

Chronic lymphocytic leukaemia (CLL) is the most common leukaemia in adults in the Western world, and is characterised by the progressive accumulation of mature CD5-positive B lymphocytes within the blood, bone marrow, lymph nodes, and spleen.[1-3] Over the past decade, significant advances in the understanding of the pathogenesis of the disease have led to the development of a range of novel treatment options. In young patients without significant comorbidities, immunochemotherapy with fludarabine, cyclophosphamide, and rituximab (FCR) has been established as the first-line standard of care treatment.[4,5] While this regimen leads to high overall response rates (ORR) and a long progression-free survival (PFS), it is unsuitable for certain subgroups of patients; these include 'poor risk patients' with p53 abnormalities[6] and elderly patients with comorbidities unable to tolerate FCR-associated toxicities.[7] In the latter, a recently published pivotal Phase III trial by the German CLL Study Group (GCLLSG) showed that the Type 2 glycoengineered antibody obinutuzumab (also known as GA101) was superior to rituximab when each was combined with chlorambucil.[8] This led to the FDA approval of obinutuzumab in combination with chlorambucil in previously untreated CLL patients. In addition, several recent Phase I and II studies demonstrated that agents interfering with B cell receptor (BCR) signalling are very active for the treatment of relapsed or fludarabine-refractory CLL and

might overcome the issues associated with current treatment approaches.[9] BCR activation is a central stimulus in CLL and increases CLL cell survival by activating different tyrosine kinases such as Bruton's tyrosine kinase (BTK), spleen tyrosine kinase, ZAP70, Src family kinases, and phosphatidylinositol 3-kinase (PI3K), in part via activation of transcription factors such as NF-κB.

Currently, the only curative – yet only suitable for a selected small group of patients - treatment option is allogeneic hematopoietic stem cell transplantation (HSCT).[10] HSCT takes advantage of the graft-versus-leukaemia (GVL) effect mediated by differentiated transplanted effector cells which are capable of mounting an anti-tumour immune response. Targeting the immune system to induce durable disease eradication therefore seems to be an attractive treatment approach, especially if achievable by improving autologous anti-tumour immune responses. This is particularly interesting as CLL is now increasingly understood as a disease that is highly dependent on interactions with its microenvironment and the immune system.[11] Various cellular components such as macrophages, T cells, dendritic cells, and stromal cells provide pro-survival and anti-apoptotic signals and conditions, and influence CLL cell trafficking, survival, and proliferation. These interactions are often bidirectional, and CLL cells have developed several mechanisms to compromise the microenvironment to continuously provide a pro-tumour environment.

Global immune defects, however, are a hallmark of CLL; hypogammaglobulinaemia, increased susceptibility to infections, increased incidence of autoimmune cytopaenias, and impaired responses to vaccinations are observed in the majority of CLL patients and are often aggravated by anti-tumour treatment. In addition, CLL-induced humoral and cellular immune defects often minimise anti-tumour immune responses and enable the malignant cells to escape from immune recognition.[12] Several preclinical studies and early clinical data, however, indicate that some novel agents and immunotherapy approaches have the potential to restore autologous immune responses. This review aims to briefly summarise the current knowledge on intrinsic and therapy-related immune deficiency in CLL and how this can be potentially overcome by novel treatment approaches.

## IMMUNE DEFECTS AND DYSFUNCTION IN CLL

The interactions between intrinsic and extrinsic immune defects in CLL and their clinical manifestations are very complex and still not fully understood. In general, immune dysfunction can be both disease and treatment-related, and clinical manifestations include severe and recurrent infections, hypogammaglobulinaemia, autoimmune anaemia, and thrombocytopaenia.[13] Infectious complications are the main cause for increased morbidity and mortality in CLL patients; in retrospective analyses, they account for up to 50% of CLL-related deaths.[14-17] This can be further aggravated by treatment with steroids, cytotoxic drugs, and monoclonal antibodies (mAbs), and several studies indicate that the main risk factor for infections seems to be the number of previously received chemotherapy lines.[18-20] Patients receiving purine analogues (e.g. fludarabine), with and without mAbs, appear to be espcially at risk of prolonged infections and cytopaenia.[21,22]

From a clinical point of view, treatment-related immunosuppression and especially opportunistic infections are a challenge in the day-to-day management of CLL patients. Purine analogues are linked to *Listeria*, *Candida*, *Aspergillus*, and herpes virus infections or reactivations. Alkylating agents frequently cause respiratory tract infections caused by typical and atypical bacteria (e.g. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*).[18-21,23-25] Alemtuzumab has been linked with an increased risk for herpes viruses, *Candida*, and *Aspergillus* infections, while rituximab is commonly associated with reactivation of hepatitis B/C viruses in sero-positive patients.[23,24] Furthermore, cytomegalovirus or cryptococcal infections following combination chemotherapy with mAbs have also been reported.[26-28]

As the underlying mechanisms, several quantitative and qualitative immune defects have been described, and they include both humoral and cellular immune responses. Early data implicated that an impaired complement system might be involved in the pathophysiology of CLL and its infectious complications.[29] Complement plays a crucial role in the control of some bacterial infections, and opsonisation with complement is necessary for subsequent interactions with neutrophils. Although CLL patients appear to

have normal serum concentrations of many complement factors, defects in C3b binding to *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Escherichia coli* were observed.[29] In addition, low activity of the classical complement pathway predicted survival in another study.[30]

Cellular immune defects are observed in nearly all immune cell types. Among B cells, the clinically most apparent defect is hypogammaglobulinaemia.[23] In general, the severity tends to increase with the duration and stage of disease.[23,31,32] More recent data however, indicate that neither hypogammaglobulinaemia nor pure immunoglobulin G (IgG) subclass deficiency are significant risk factors for infectious complications,[33,34] but this needs to be confirmed in larger and more homogenous series.

After early studies showed that clonal CLL cells have a limited ability to present antigen to T cells,[35,36] largely due to an inadequate costimulatory capacity,[37-39] a wide range of defects in the T cell compartment itself has been described. These include altered subset composition, changes in cytokine secretion and surface molecule expression, as well as profound functional defects.[40] T cells from patients with CLL also show severe gene expression profile changes, particularly in genes involved in actin cytoskeleton formation and stabilisation of the immune synapse.[41] To date, it remains unclear as to what extent these global changes represent an immune response to the malignant cells, as opposed to cells that have been compromised to 'help' the tumour cells evade immune recognition. Recently published data highlight the potential role of immune checkpoint pathways such as PD-1:PD-L1, CD160:HVEM, and CD200:CD200R in mediating these defects,[42-44] but the exact underlying mechanisms are still poorly understood.

Several studies have reported functional and numerical alterations in natural killer (NK) cells in CLL patients, which are particularly pronounced in advanced disease[45-47] and an independent predictive factor of disease progression in patients with newly diagnosed CLL.[48] In contrast, a more recent publication indicated that peripheral NK cells from CLL patients maintain partial functionality and are able to degranulate and exert antibody-dependent cellular cytotoxicity (ADCC), although some variability was observed.[49] Cellular immune defects are also observed in neutrophils, monocytes and macrophages, and dendritic cells, compromising their effector functions and phagocytic and bactericidal function as well as migration and chemotaxis.[50-52]

The clinical relevance of peripheral absolute monocyte count (AMC) has recently been demonstrated by the finding that patients with low AMC had a shorter time to treatment (TTT) and immune dysregulation leading to increased infection-related mortality. High AMC patients also had a shorter TTT compared to intermediate AMC patients.[53] NK cells in CLL are downregulated and their action against malignant cells is impaired. The capacity of monocytes/macrophages[53] and neutrophils in terms of phagocytosis, granulocyte function, and chemotaxis is also damaged: this results in an increased risk for bacterial and fungal infections.[50,54-56]

The stromal environment seems to be involved in CLL cell trafficking and homing to lymphoid tissues. The CXCR4/CXCL12[57-59] and CXCL13/CXCR5[60] axes are important therapeutic targets as both are involved in pleotropic effects, survival signals, and enhanced chemotaxis in CLL cells. Several recommendations on the prophylaxis and management of clinical immune defects exist, but these are reviewed elsewhere and will not be further discussed in this article.[13,61]

## THERAPEUTIC PERSPECTIVES

As outlined above, a plethora of *in vitro* and preclinical *in vivo* studies have highlighted the importance of interactions between CLL cells and humoral and cellular components of the immune system and microenvironment. While this has led to a paradigm shift in the management of CLL, the mechanisms leading to infections and poor anti-tumour immune responses are still poorly understood. Preclinical data and correlative studies however indicate that novel agents and treatment approaches have the potential to correct immune defects and to promote immune activation, potentially re-sensitising the immune system and restoring its ability to mount anti-tumour and anti-infection immune responses. This review focuses on most clinically relevant drugs, but Figure 1 provides an overview of current and experimental agents.

## ANTI-CD20 MONOCLONAL ANTIBODIES

After the first-generation mAb rituximab, several new mAbs targeting the B cell antigen CD20 have

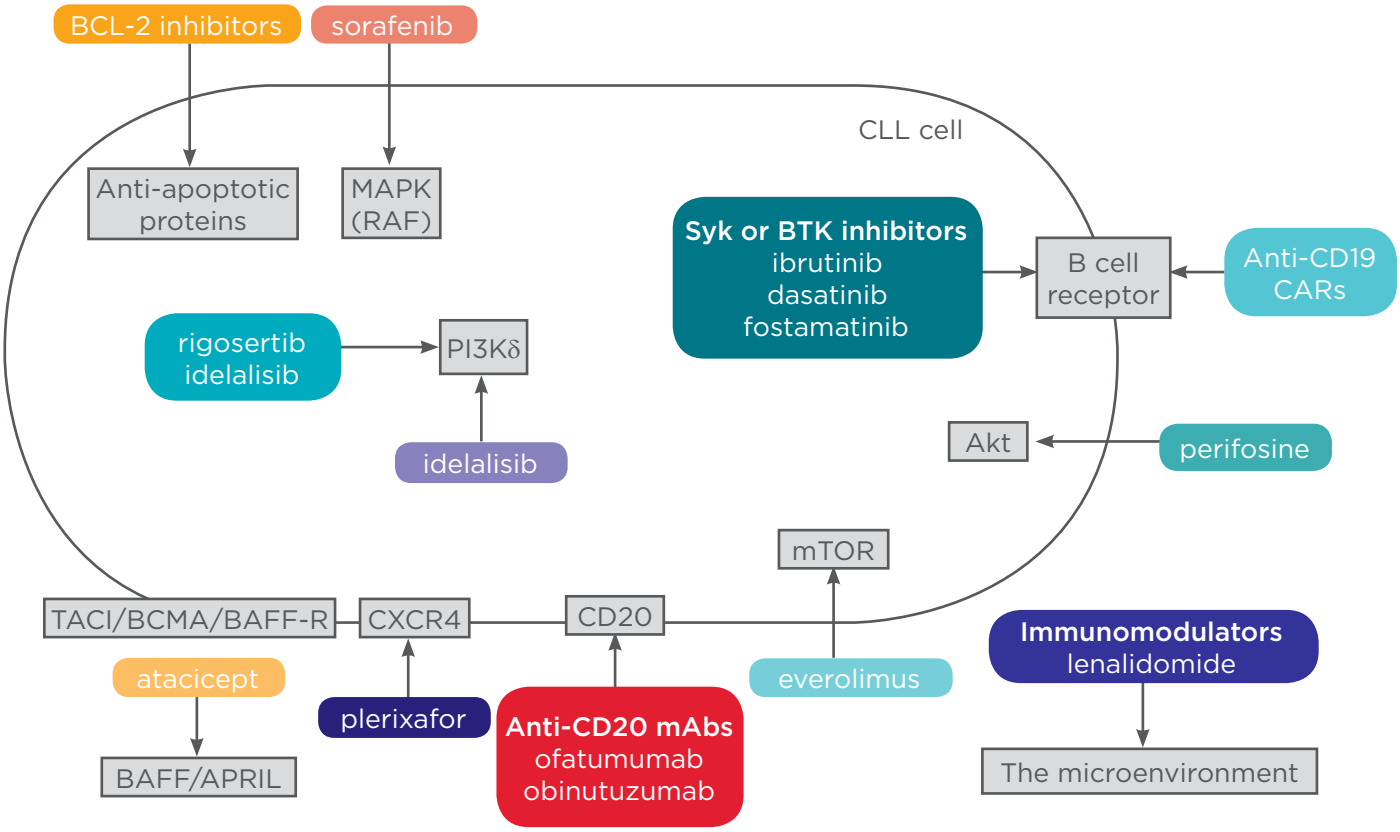


**Figure 1: Approved and experimental therapeutic options targeting CLL microenvironment.**
CLL: chronic lymphocytic leukaemia; Akt: protein kinase B; APRIL: a proliferation-inducing ligand; BAFF: B cell–activating factor of the tumour necrosis factor family; BAFF-R: B cell–activating factor of tumour necrosis factor family receptor; BCL-2: antiapoptotic protein B cell lymphoma 2; BCMA: B cell maturation antigen; BTK: Bruton tyrosine kinase; CD19: cluster of differentiation 19; CD20: cluster of differentiation 20; CXCR4: chemokine (C-X-C motif) receptor 4; MAPK: mitogen-activated protein kinase; mTOR: mammalian target of rapamycin; PI3Kδ: phosphatidylinositol 3-kinase delta; Syk: spleen tyrosine kinase; TACI: transmembrane activator and calcium modulator and cyclophilin ligand-interactor; CAR: chimeric antigen receptor; mAb: monoclonal antibody.
*Adapted from Herishanu Y et al.*[53]

been developed. Although representing a 'passive' immunotherapy, they display enhanced anti-tumoural activity by engaging the immune system through increased complement-dependent cytotoxicity and ADCC, and are now an integral component of CLL therapy.[62-64]

## Ofatumumab

Ofatumumab is a fully humanised anti-CD20 mAb that has recently been explored in patients with refractory CLL and as first-line treatment.[65-68] In fludarabine or alemtuzumab-refractory patients, ofatumumab yielded an ORR of almost 60%, and was even effective in patients pre-treated with rituximab.[65,68] This led to the accelerated approval by the US FDA in 2010 for the same clinical settings.[69] The combination therapy with chlorambucil is currently assessed in a Phase III study,[70] and other approaches include the combination with targeted agents such as idelalisib or lenalidomide.[71,72]

## Obinutuzumab

Obinutuzumab is a Type 2 anti-CD20 mAb presently undergoing clinical investigation, and has demonstrated impressive early results. In the pivotal GCLLSG-CLL11 Phase III trial investigating chlorambucil plus obinutuzumab (Clb-O) or chlorambucil plus rituximab (Clb-R) against chlorambucil alone (Clb) in patients with previously untreated CLL and coexisting conditions, Clb-O or Clb-R as compared with Clb alone increased response rates and prolonged PFS. OS was significantly prolonged with Clb-O compared

with Clb alone. Treatment with Clb-O compared with Clb-R resulted in prolongation of PFS and higher rates of complete response (20.7% versus 7.0%) and molecular response.[8] The FDA approved obinutuzumab in combination with chlorambucil as a first-line therapeutic option in November 2013.[73]

### Lenalidomide

Lenalidomide is a thalidomide analogue that has been demonstrated to have pleiotropic effects on immune cells in both preclinical and early clinical studies. Its mechanism of action in CLL appears to function primarily by enhancing anti-tumoural immunity in T cells.[74] It induces T cell cytoskeletal genes and restores the ability to form immunological synapses with B cells both *in vitro* and *in vivo*.[75,76] Lenalidomide also enhances T cell motility and downregulates the expression of T cell-inhibitory molecules in CLL.[42,77] In CLL cells, it induces CD40L (CD154) expression, thus playing a key role in CD80-mediated T cell activation,[75,78] and sensitises cells to TRAIL-mediated apoptosis and costimulatory activation of normal B cells to produce anti-tumoural antibodies.[79] The latter has been consolidated by two clinical studies by the M.D. Anderson Cancer Center, which revealed that lenalidomide therapy can yield a sustained increase in Ig levels.[80,81]

Other clinical trials have demonstrated that this immunomodulatory drug has activity as monotherapy for CLL, but is associated with non-negligible toxicities such as tumour flare reaction and increased risk of opportunistic infections, making the potential combination with drugs such as fludarabine or alemtuzumab difficult.[82-87] The efficacy and safety of the combination with rituximab in relapsed or refractory CLL were explored in a Phase II trial.[84] The ORR was 66% with 12% of CR, for an estimated survival at 36 months of 71%. Severe toxicities included neutropaenia, thrombocytopaenia, and anaemia, while one patient suffered from a Grade 3 tumour lysis.[80,81,87] The combination of lenalidomide with ofatumumab is currently being investigated in relapsed or refractory CLL.[72,88]

## BCR SIGNALLING INHIBITORS

### Ibrutinib

Ibrutinib is a BTK inhibitor that primarily blocks BCR associated anti-apoptotic pathways. In addition, it affects BCR and chemokine-controlled retention and homing of CLL cells in their growth and survival-supporting lymph node and bone marrow microenvironment.[89-91] Thus, malignant B cells are driven out of their protective niches and are more accessible to cytotoxic therapy and potentially other immune cells.[91] This was supported by early clinical trials where treatment resulted in transient lymphocytosis associated with a decrease in lymphadenopathy, with very good safety profiles.[92]

To further explore these findings, a Phase I/II study was conducted in 85 heavily pre-treated patients with relapsed or refractory CLL.[93] The ORR was 71%, with 18% of PRs with lymphocytosis and 2% of CRs. The progression-free rate at 26 months was 75% with an OS rate of 83%. Overall toxicity was very mild with few serious adverse events. Marked changes were noted in terms of the reduction of lymph nodes and spleen sizes, and platelet and red blood cell counts were also improved. As a consequence, the FDA approved ibrutinib in February 2014 for the treatment of CLL as second-line therapy.[94] Recently published preclinical data indicate that ibrutinib also irreversibly binds the BTK isoform ITK, which is expressed in T cells, and can therefore be potentially used to correct T cell based immune responses.[95]

### Idelalisib

Idelalisib is an inhibitor of PI3Kδ, which is also a component of the CLL signalling pathways involved in cell survival, clonal expansion, and malignant cell retention in lymphoid tissues.[96,97] In a Phase I study conducted on 54 heavily-pretreated CLL patients with relapsed or refractory disease, 39% of patients achieved a PR, and 33% of patients achieved a PR with lymphocytosis; the safety profile of idelalisib was acceptable.[98]

A Phase III trial was then initiated in 220 patients with relapsed CLL receiving idelalisib in combination with rituximab versus rituximab plus placebo.[99] Due to overwhelming efficacy, the study was interrupted after the first interim analysis: the ORR was 81% for the combination therapy versus 13% for rituximab monotherapy, while OS at 12 months was of 92% and 80%, respectively. PFS was also greatly improved in the combination arm (93% versus 46%, respectively) and a higher proportion of patients presented a 50% or higher reduction of lymphadenopathy (93% versus 4%, respectively). The safety profiles were similar and acceptable within both groups, with

severe toxicities occurring in 40% and 35% of patients, respectively.

Additional investigation on idelalisib is currently ongoing, especially as a combination therapy with other agents: two Phase III trials on previously treated CLL patients are exploring the efficacy and safety of idelalisib with ofatumumab versus ofatumumab alone,[100] and of idelalisib with rituximab plus bendamustine versus rituximab/bendamustine and a placebo.[101] Preliminary results are expected in late 2015 and late 2016, respectively.

## CELLULAR THERAPIES

In contrast with 'passive' immunomodulation as discussed above, active immunotherapy is a reasonable option in some patients, and such strategies include HSCT and chimeric antigen receptor (CAR) T cell therapy.

### RIC-HSCT

As previously stated, HSCT is the only curative option for CLL, but is limited to a selected group of patients.[102] Aside from the rarity of available histocompatible donors, myeloablative conditioning is associated with high transplant-related morbidity and non-relapse mortality, as well as the occurrence of graft-versus-host disease (GVHD).[103,104] Thus, non-myeloablative or reduced-intensity conditioning (RIC) approaches have been and continue to be evaluated in CLL patients that would otherwise be ineligible to HSCT, namely elderly patients. Several clinical studies with long-term follow-up have demonstrated that HSCT can provide long-term disease control, even in patients with poor-risk CLL.[104-108] Nevertheless, GVHD remains a complication of RIC HSCT, and translational efforts are now focused on the modulation of GVHD towards the beneficial GVL effect.[109]

### Gene Therapy with Chimeric Antigen Receptor T Cells

CAR technology has recently emerged as a novel and promising perspective to specifically target malignant cells with precisely engineered T cells. It uses the single chain variable fragment from an antibody molecule fused with an internal T cell signalling domain to form a CAR, which is then transduced into T cells.[110] A major advantage of this approach is that it eliminates major histocompatibility complex restriction, enabling the same CAR to be used for several different patients.

In a pivotal report, a heavily pre-treated high-risk patient with refractory CLL received autologous T cells that had been modified with CARs directed at CD19, a B cell surface antigen, resulting in remission induction and lasting tumour control.[111] Since then, several clinical trials have reported impressive results with anti-CD19 CARs, both in CLL and acute lymphoblastic leukaemia.[111-115] However, it has also become clear that the success of CAR therapy depends on the inclusion of lympho-reducing conditioning chemotherapy and the choice of CAR design.[111,113,114,116]

In addition, CAR T cell therapy can be associated with severe complications such as cytokine release syndrome, a potentially lethal complication, and lasting normal B cell depletion,[113,117,118] which potentially requires continuous intravenous Ig administration. Taken together, CAR T cells show significant clinical activity but are unlikely to fully restore disease-related immune defects. Further studies are needed to fully investigate the clinical use of CAR T cell therapy and treatment-related toxicities, and its optimal combination with existing treatment approaches.

## CONCLUSION

While initial chemoimmunotherapies had offered promising high ORR and OS, real-life settings such as the complex management of comorbidities and complications in vulnerable or elderly patients or in immunosuppressed patients after multiple rounds of therapy is challenging. In the last few years, many new approaches and innovative agents have advanced the outlook for CLL management. Indeed, novel therapies could soon be addressing the need to promote immune reactivation and resensitise the immune system. By doing so, they could fulfil two main objectives, namely lowering the high proportion of patients at risk of infection and acting as effective tools for the immune system to overcome its defects and mount effective, strong, and lasting anti-tumour responses.

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