Anti-CD20 monoclonal antibodies in chronic lymphocytic leukaemia: Mechanisms, resistance, and the evolution of targeted immunotherapy

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Anti-CD20 monoclonal antibodies (mAbs) have been the gold standard in the treatment of B-cell malignancies since their inception, most notably in the case of rituximab and related chimeric antibodies. However, a particular subtype of non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukaemia (CLL), has shown comparatively poor outcomes. Its slow-paced, indolent nature makes diagnosis difficult, while its evasive nature complicates therapy. The rise in the incidence of CLL, in South Africa in particular, has emphasised the need for ongoing research. Despite the availability of revolutionary therapies that have transformed outcomes in other NHL subtypes, treatment of CLL remains largely experimental. A comprehensive and systematic literature review was therefore conducted to illustrate the impact of anti-CD20 and to explore potential avenues for improvement in the field. Studies illustrate that the treatment landscape has grown considerably, from the introduction of newer mAbs such as obinutuzumab to altogether different modalities such as bispecifics, chimeric antigen receptor (CAR) T cells, and even a combination of these immunotherapy regimens. However, the largely experimental nature of these strategies and the ability of CLL to manufacture its tumour microenvironment continue to limit progress. It is evident that although great strides have been made, future breakthroughs via exploitation of the molecular pathways and mutations driving CLL will be essential to refine therapeutic approaches and advance the treatment of CLL and B-cell malignancies more broadly.

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Chronic lymphocytic leukaemia (CLL) is a blood cancer that primarily affects B lymphocytes. The activity of the disease is usually insidious and indolent, with 25 - 50% of patients being asymptomatic at the time of presentation.[1] On a molecular basis, CLL has been characterised by the proliferation of mature yet incompetent B cells which co-express the surface cluster of differentiation (CD) antigens CD19, CD20 and CD23, as well as CD5, which is the most prominent diagnostic marker. CD20, a commonly overexpressed marker in other non-Hodgkin's lymphomas (NHLs), is uncharacteristically suppressed in CLL.[2] Despite this, CD20 remains a relevant therapeutic marker even as its diagnostic salience remains limited. Its pertinence is largely possible owing to the great strides made in the treatment of NHL using anti-CD20 therapies, in particular rituximab and obinutuzumab.[3] While the incidence of CLL remains relatively low in sub-Saharan Africa, specific prevalence data for the region are limited. In South Africa, however, the prevalence is among the highest in Africa, with CLL accounting for approximately 43% of reported B-cell malignancies, emphasising the need for expanded research on treatment strategies.[4] Immunologically, CLL remains unique among NHLs owing to its dependence on its microenvironment for survival, and therefore poses significant challenges in terms of its management and curability.[5] Therapies currently available include anti-CD20 monoclonal antibodies (mAbs) such as rituximab, but treatment is evolving towards more dynamic modalities, including bispecific antibodies (BiTEs) that target two epitopes simultaneously, as well as chimeric antigen receptor (CAR) T cells that are designed to directly eliminate malignant cells. Despite these advancements, however, CLL remains persistently resistant to these therapies, confirming the need for in-depth analysis of the immune mechanisms underlying the use of anti-CD20 mAbs, its resistance mechanisms and therapeutic evolution.

Search strategy

A comprehensive literature review using PubMed was conducted to collect the data used in this study. Eight key concepts were defined and mapped to appropriate keywords to ensure a comprehensive and targeted search, these being:

1. **CLL.** "Leukemia, Lymphocytic, Chronic, B-cell"[MeSH] OR "chronic lymphocytic leukemia"[tw] OR "CLL"[tw] OR "B-cell leukemia"[tw] OR "mature B-cell leukemia"[tw] OR "indolent leukemia"[tw]
2. **CD20 antigen.** "Antigens, CD20"[MeSH] OR "CD20 antigen*"[tw] OR "CD20 expression*"[tw] OR "CD20 target*"[tw] OR "B-cell marker*"[tw] OR "B cell marker*"[tw]
3. **Monoclonal antibodies.** "Antibodies, Monoclonal"[MeSH] OR "anti-CD20 monoclonal antibod*"[tw] OR "Anti-CD20 mAb"[tw]
4. **Mechanisms of action.** "Cell Death"[MeSH] OR "ADCC"[tw] OR "CDC"[tw] OR "apoptosis" OR "immunomodulation"[tw]
5. **Resistance to anti-CD20 therapy.** "Immune Evasion"[MeSH] OR "CD20 downregulation"[tw] OR "resistance mechanism*"[tw] OR "Fc receptor polymorphism*"[tw] OR "immune evasion*"[tw]
6. **Targeted immunotherapy.** "Molecular Targeted Therapy"[Mesh] OR "targeted immunotherap*"[tw] OR "immunotherapy in lymphoma*"[tw] OR "targeted therap*"[tw] OR "biologic*"[tw] OR "BITE"[tw] OR "bispecific antibod*"[tw] OR "CAR-T"[tw] OR "chimeric antigen receptor T cell"[tw] OR "CD3-CD20"[tw] OR "CD3 CD20"[tw] OR "CD20-CD3"[tw] OR "T cell engager"[tw]
7. **Evolution of therapy.** "Clinical Trials as Topic"[MeSH] OR "second-generation antibod*"[tw] OR "next-generation immunotherap*"[tw] OR "clinical trial*"[tw] OR "novel agent*"[tw] OR "therapy development*"[tw] OR "pipeline drug*"[tw]
8. **Combination strategies.** "Drug Therapy"[Mesh] OR "immunochemotherap*"[tw] OR "combination therap*"[tw] OR "combination regimens"[tw]

To further ensure a broad search scope, the American spelling of leukaemia (leukemia) was used, as well as Boolean operators and truncation (e.g. "*").

The first three core concepts (i.e. CLL, CD20 antigen, and monoclonal antibodies) were fixed and searched in combination. These were then individually combined with each of the five remaining concepts to refine the search and ensure comprehensive coverage of the relevant literature. This strategy ultimately yielded 87 articles by the end of June 2025, of which 63 were deemed relevant based on the following criteria:

Inclusion criteria:

- Published within the past 10 years (with exceptions for foundational older studies)
- Peer reviewed
- Human studies (including animal models if mechanistically relevant)
- English language
- Included clinical trials, reviews, or mechanistic studies.

Exclusion criteria:

- Articles that excluded CD20 as a relevant target
- Articles that did not explicitly focus on CLL
- Articles that did not discuss monoclonal antibodies (mAbs).

Additional references were included to support context or background where relevant.

Mechanisms of action of anti-CD20 therapies in CLL

Monoclonal antibodies have transformed the treatment landscape for NHLs, including CLL, and are often combined with chemotherapy to enhance therapeutic efficacy. They target specialised cell surface antigens known as CD markers, commonly used in normal cell expression to differentiate between various cell types, as well as to determine the maturation stage of a cell.[6] Despite CLL's rather dim expression of CD20, its response to medications that select against this particular antigen (commonly known as anti-CD20 therapy) has been shown to be sufficiently effective and specific to justify continued use of this target.[7]

There are four main anti-CD20 mAbs currently used to treat CLL. These are rituximab, ublituximab and ofatumumab (type I mAbs) and obinutuzumab (type II mAb), which are often classified by their distinct mechanisms of action.[8,9]

The binding of an mAb to CD20 usually elicits one of three general pathways across NHLs:

1. Antibody-dependent cell-mediated cytotoxicity (ADCC)
2. Complement-dependent cytotoxicity (CDC)
3. Direct cell death.[10]

In the context of CLL, however, these pathways may have reduced or even antagonistic effects. For example, ADCC – which can be briefly characterised by the recognition of the fragment crystallisable (Fc) region of the antigen by Fc receptors (natural killer (NK) cells, neutrophils or eosinophils), followed by the release of pore-forming, cytotoxic granules – seems to require only a moderate number of CD20 mAb complexes for effective performance.[10] NK cells in particular play a larger role in inducing this pathway, while the other aforementioned cells have minimal cytotoxic activity. The dependence on NK cells poses a rather glaring problem in CLL, as NK-mediated ADCC is often reported as suboptimal. Some studies therefore highlight an alternative mechanism known as antibody-dependent cellular phagocytosis (ADCP) as a primary, which may be more effective in CLL owing to its alternative use of macrophages and the relatively lower concentrations at which it is activated compared with standard ADCC dosages.[11,12]

CDC, on the other hand, requires a significant amount of CD20 expression – more so than ADCP or even ADCC – which would therefore require combined chemotherapy in the case of CLL or any other indolent NHL.[10] The complement cascade on which it depends has three main pathways, these being the classical, alternative and lectin pathways. These converge at the formation of complement component 5 (C5) and C3 convertase complexes, which are instrumental in assembly of the membrane attack complex (MAC), release of chemoattractants (C5a and C3a) and overall opsonisation of the target pathogen. Despite its reduced efficiency, however, CDC remains a key mechanism for activity in anti-CD20 mAbs in CLL.[10] Some studies indicate that in the case of rituximab, CDC and ADCC may even work antagonistically, owing to inhibition by the C3b component.[13]

The third method, direct cell death, can be induced in a multitude of ways, including apoptosis, reactive oxygen species (ROS)-dependent death and caspase-independent methods. However, in the CLL microenvironment, ROS produced by monocytes may actually protect CLL cells indirectly by impairing NK cells and limiting ADCC, reducing overall therapeutic efficacy. As a result, targeting ROS production may enhance both direct and immune-mediated killing.[10,14]

Resistance mechanisms in CLL

As mentioned, CLL is notoriously characterised by its decreased CD20, which further complicates clinical treatment. This marked decrease in expression is largely attributed to mechanisms such as C-X-C receptor type 4 (CXCR4) signalling and, more recently, mutations of the *NOTCH1* and *S3FB1* genes.[15] While these pathways appear to drive constitutive downregulation, treatment-induced suppression of CD20 also significantly affects therapeutic efficacy and may further exacerbate mutations. For example, studies have indicated that exposure to rituximab significantly reduces CD20 expression, with levels only recovering 24 - 48 hours after a brief 1 - 2-hour *ex vivo* exposure.[16]

Another method by which CLL evades treatment is through its use of the protective tumour microenvironment (TME) located in the stromal layer of bone marrow. The TME has allowed the cancer to mitigate various types of treatments, including the rather revolutionary type II obinutuzumab, with increased dependence on direct contact as opposed to soluble factors. This method often co-opts CD20 downregulation and deflects against programmed cell death.[17]

Evolution of anti-CD20 therapies in CLL

In an attempt to combat the evasiveness of CLL, first-line treatments have evolved from type I mAbs such as rituximab, ofatumumab and ublituximab to the type II mAb obinutuzumab. The first-generation chimeric mAb, rituximab, revolutionised CLL therapy by enhancing responses when combined with chemotherapy, notably in the FCR (fludarabine, cyclophosphamide, rituximab) regimen.[9] However, its limited single-agent activity in CLL prompted the development of newer agents.[18,19]

Second-generation agents such as ofatumumab are fully human and bound to particular CD20 epitopes with slower dissociation kinetics, aiming to decrease use of CDC.[20] Third-generation mAbs such as obinutuzumab, on the other hand, are glycoengineered type II antibodies designed to enhance ADCC and induce direct cell death with less reliance on CDC.[21,22] Obinutuzumab has therefore shown greater depth of response and prolonged progression-free survival (PFS), particularly when combined with venetoclax or chlorambucil, compared with rituximab-based regimens.[23]

Despite advances, the variability in CD20 expression and resistance mechanisms, including antigen internalisation and trogocytosis, has motivated a shift towards non-CD20 targets, including CD19, CD22 and CD37.[22,24] These targets offer alternative avenues for immune engagement, particularly in patients with relapsed/refractory disease or even those previously exposed to anti-CD20 therapies.

Furthermore, the development of BiTEs such as mosunetuzumab, which simultaneously bind CD3 on T cells and CD20 on B cells, offers a chemotherapy-free and off-the-shelf immunotherapeutic option. These agents recruit and redirect polyclonal T cells to kill malignant B cells independent of antigen presentation or co-stimulation.[3] Early trials of mosunetuzumab in CLL show promise, although T-cell exhaustion and immune suppression in CLL still remain major barriers.[25]

Similarly, CAR T-cell therapy, which has revolutionised treatment in most other NHLs, has faced greater challenges in CLL. The CLL microenvironment promotes T-cell dysfunction and immune evasion, which compromises CAR T expansion and persistence.[22,25] Trials involving CD19-directed CAR T cells have reported variable efficacy, and combination strategies with immune checkpoint inhibitors or bruton tyrosine kinase (BTK) inhibitors (to reverse T-cell anergy) are still being explored.[26]

Clinical implications and future directions

The clinical superiority of obinutuzumab over rituximab still remains a topic of debate. While trials such as CLL14 demonstrate improved PFS when obinutuzumab is combined with venetoclax compared with rituximab combinations, the long-term overall survival benefits are still quite unclear. On one hand, obinutuzumab is associated with higher rates of infusion-related reactions.[22,23] On the other, some studies suggest that increasing the rituximab dose does not necessarily enhance efficacy, further complicating the comparison.[27]

Integration with BTK inhibitors, such as ibrutinib and acalabrutinib, has redefined CLL management. However, their interaction with anti-CD20 antibodies is complex. BTK inhibitors can impair ADCC and macrophage-mediated phagocytosis, reducing the efficacy of concurrent anti-CD20 therapies.[5,28] Despite this, combination regimens (e.g. ibrutinib and obinutuzumab) are still explored, particularly in high-risk or unfit patients, although some studies favour consecutive sequencing over simultaneous use.[26]

Personalised treatment strategies may also be gaining traction, with emphasis on molecular profiling (e.g. *TP53*, *IGHV* mutation status) and immune phenotyping to guide therapy selection.[17,25] For instance, patients with *TP53* aberrations may benefit more from targeted agents than from chemoimmunotherapy.

Finally, the future of BiTEs and CAR T therapies in CLL may hinge on overcoming immune exhaustion and microenvironmental suppression. While these immunotherapies are highly effective in some B-cell malignancies, in CLL they are currently best reserved for clinical trials or refractory cases owing to safety concerns, manufacturing logistics, and cost.[25,29]

Conclusion

To conclude, it is evident that the evolution of anti-CD20 therapies for CLL has revolutionised the treatment landscape, this being from the development of first-generation mAbs such as rituximab, to second-generation glycoengineered mAbs such as obinutuzumab, and now more recently to bispecifics and CAR T cells. These developments have allowed us to deepen our understanding of key mechanisms such as ADCC, CDC and direct cell death, enabling us to better tailor emerging therapies.

Despite these advancements, however, it is clear that challenges still remain: the resistance that persists via CD20 downregulation, as well as the inconsistency of response durability. These issues are not unique to CLL, and the research that goes into its molecular understanding will assist in paving the way for more targeted and durable treatment strategies across all B-cell malignancies.

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