CD20 as target for immunotherapy

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2

The evolution of immunotherapy: lessons learned from targeting CD20

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INTRODUCTION

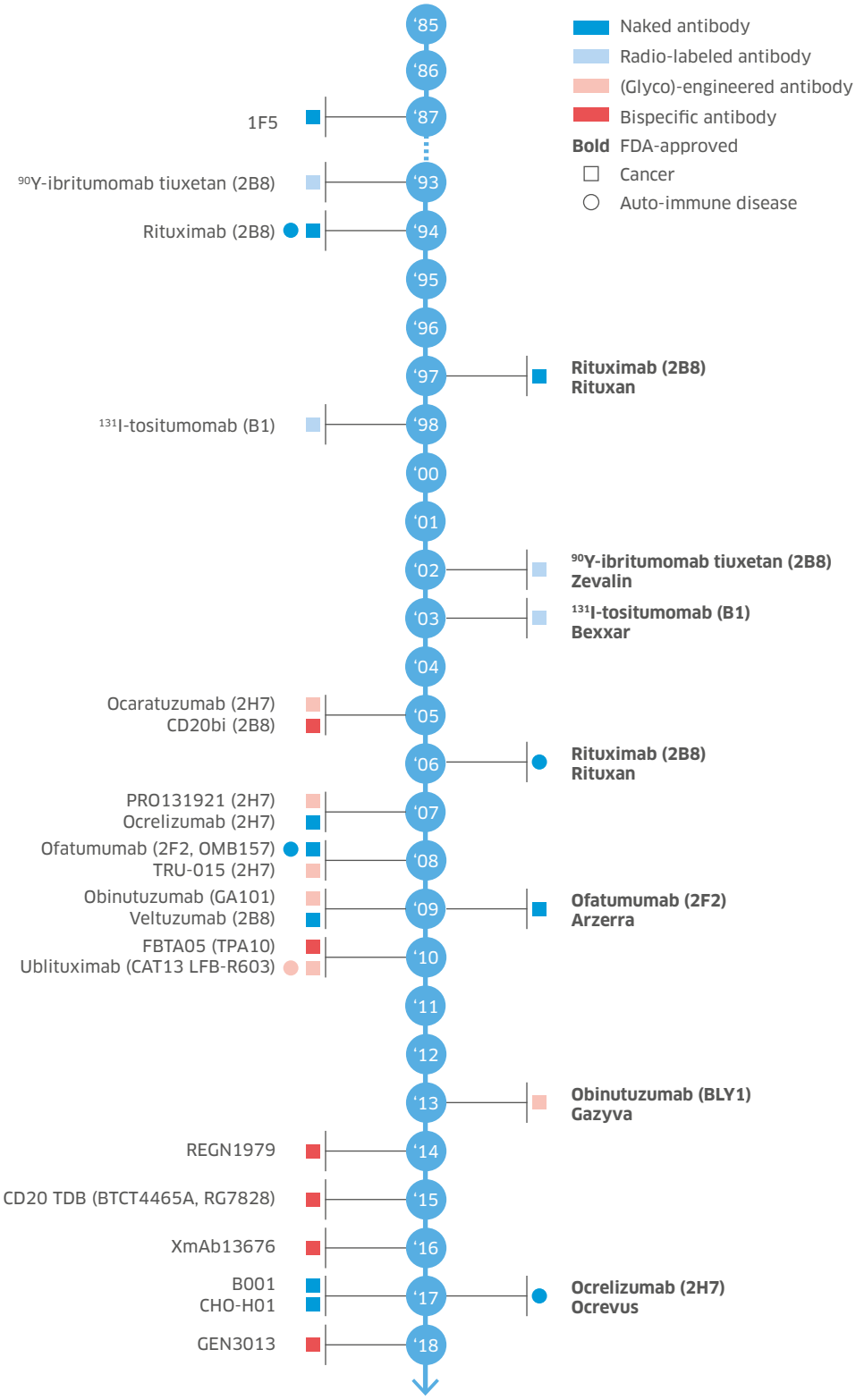
CD20 represents one of the most successful immunotherapy targets. The inclusion of rituximab (RTX; Rituxan®, MabThera®) into the treatment regimen of lymphoma patients significantly improved the overall survival of these patients. This revolutionized the immunotherapeutic landscape by showing the potential of monoclonal antibodies (mAbs). Since the initial approval of RTX in relapsed and/or refractory low-grade non-Hodgkin's lymphoma (NHL), label expansions followed. RTX continues to be the backbone for first-line treatment of B-cell lymphoma and leukemia despite newly approved CD20 mAbs, and is one of only three antibodies on the WHO essential medicines list (www.who.int/medicine/publications/essentialmedicines; 6th list, Amended March 2017). Despite the success of CD20-targeted therapies, significant numbers of patients still fail to respond or become refractory upon treatment, even though the target antigen generally continues to be expressed on their tumor cells. This is stimulating research into new and improved CD20-directed immunotherapies (see Figure 1, Table 1). CD20 expression is restricted to B cells, but is not expressed on lymphoid progenitor cells, and expression is lost when B cells differentiate into plasma cells. CD20-targeting antibodies do not distinguish between malignant and non-malignant cells, but depletion of healthy $CD20^+$ cells is well tolerated. Clinical research with CD20 antibodies was also expanded to other diseases in which a role for B cells was hypothesized (e.g. rheumatoid arthritis (RA) and multiple sclerosis (MS)).

This review gives an extensive overview of pre-clinical and clinical investigations with CD20-targeting immunotherapeutic regimens, with the focus on lymphomas and leukemias, and provides future perspectives.

CD20 EXPRESSION AND FUNCTION

Expression on normal B cells and protein structure

CD20 was initially described by Stashenko *et al.* in 1980 as B1 [1], but has also been denoted as leukocyte surface antigen 16 (Leu-16), B1 polypeptide of 35-kDA (Bp35) [2] and membrane-spanning 4-domain family, subfamily A (MS4A) [3]. CD20 was the first cell-surface antigen on human B cells to be identified by a mAb and as such became part of the cluster of differentiation (CD). CD antigens are cell surface molecules expressed on leukocytes and other immune cells [4]. The CD nomenclature provides a unified designation system for mAbs and for the cell surface molecules they recognize. The 1st international workshop on human leukocyte differentiation antigens was in 1982 and since then, more than 350 CDs have been assigned [5]. A subset of pre-defined CDs is used to phenotype the different human leukocyte subsets. CD20 is part of the CD-markers used to distinguish B-cells from other leukocytes [6].
Although CD20 was discovered more than three decades ago, its exact structure has not been solved by crystallography due to the complex membrane topology of the protein. CD20 spans the membrane four times [7,8], with a small extracellular loop of


Naked antibody
Radio-labeled antibody
(Glyco)-engineered antibody
Bispecific antibody
Bold FDA-approved
Cancer
Auto-immune disease
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1F5
90Y-ibritumomab tiuxetan (2B8)
Rituximab (2B8)
131I-tositumomab (B1)
Ocaratuzumab (2H7)
CD20bi (2B8)
PRO131921 (2H7)
Ocrelizumab (2H7)
Ofatumumab (2F2, OMB157)
TRU-015 (2H7)
Obinutuzumab (GA101)
Veltuzumab (2B8)
FBTA05 (TPA10)
Ublituximab (CAT13 LFB-R603)
REGN1979
CD20 TDB (BTCT4465A, RG7828)
XmAb13676
B001
CHO-H01
GEN3013
Rituximab (2B8)
Rituxan
90Y-ibritumomab tiuxetan (2B8)
Zevalin
131I-tositumomab (B1)
Bexxar
Rituximab (2B8)
Rituxan
Ofatumumab (2F2)
Arzerra
Obinutuzumab (BLY1)
Gazyva
Ocrelizumab (2H7)
Ocrevus

FIGURE 1 **Timeline of clinical development and approval of CD20 targeting antibodies.**

The figure provides an overview of clinical stage therapeutic antibodies. Timing of first entry into clinical development is shown below, and timing of FDA approval is shown in bold above timeline. Indications where anti-CD20 therapy is investigated /approved is indicated in symbols (☐ = cancer, ○ = auto immune disease). The original clone from which the antibody was derived is shown in parenthesis, or (in case of bispecific molecule) the current development name is provided. Color coding refers to the antibody format used.

5 amino acids (aa)[1] and a large extracellular loop of 44 aa The N- and C-terminus are located in the cytoplasm.
Attempts to recombinantly produce soluble CD20 were only partially successful, since binding of CD20-targeting mAbs required specific conformations that were not retained in the protein produced [9, 10]. RTX requires an intact disulfide bridge within the large extracellular loop to recognize CD20. Reducing the disulfide bridge, by treating the CD20 antigen with dithiothreitol (DTT), resulted in loss of RTX binding, which was restored after re-oxidation [9]. As no other disulfide bridge is present in CD20, the small loop does not have such a stability-providing structure and thus can only partially be mimicked by constrained peptides [11, 12] indicating the complexity and dynamics of the molecule.

The exact function of CD20 remains elusive. Despite the close association of CD20 expression with B cell development, absence of CD20 protein only resulted in a mild phenotype. In CD20 knock out mice a small reduction in number of B cells was observed, but normal B cell development and antibody responses were retained [13]. In a patient that expressed non-functional CD20 due to homozygous mutations, normal B-cell development occurred, although a reduced capacity to elicit antibody responses to specific antigens was observed [14]. It has been well established that CD20 is involved in mediating transmembrane calcium fluxes [15, 16] and is important for B-cell activation [17], differentiation [8, 17] and proliferation [17, 18].
In normal physiology, CD20 is expressed on more than 90% of the B cells in peripheral blood and in lymphoid organs. Although it has been suggested that CD20 was expressed at intermediate to low levels in a small subpopulation of normal $CD3^+$ T cells [19-24], this was later found to be incorrect. CD20 expression in T cells was the result of trogocytosis, extraction of surface molecules from other cells during contact [4, 25], a phenomenon that impacts diagnosis and mAb-based therapies of malignancies [26, 27]. CD20 is first expressed at the pre-B cell stage, before IgM is expressed on the cell surface. The expression of CD20 con-

1 Inconsistencies exist in literature in the reported number of surface-exposed amino acids in the small loop. Based on the properties of the amino acids (hydrophobic Isoleucine and rigid proline), we define, in this review, IP-PI as the boundary of the transmembrane stretches, resulting in five surface-exposed amino acids in the small loop.

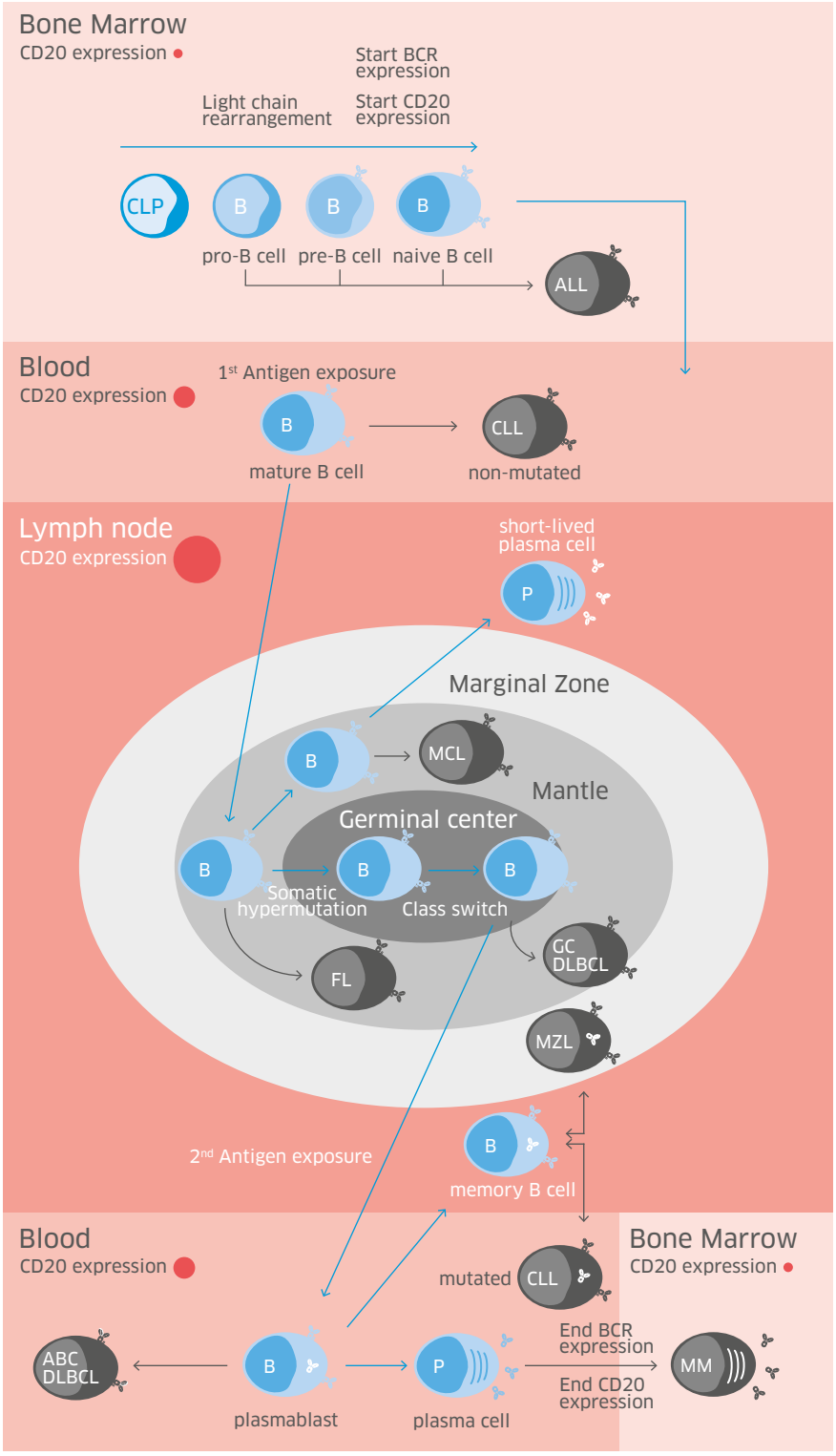
Bone Marrow
CD20 expression
Start BCR expression
Light chain rearrangement
Start CD20 expression
CLP
B
B
B
pro-B cell
pre-B cell
naive B cell
ALL
Blood
CD20 expression
1st Antigen exposure
B
mature B cell
CLL
non-mutated
Lymph node
CD20 expression
short-lived plasma cell
P
Marginal Zone
B
MCL
Mantle
Germinal center
B
B
B
Somatic hypermutation
Class switch
FL
GC DLBCL
MZL
2nd Antigen exposure
B
memory B cell
Blood
CD20 expression
Bone Marrow
CD20 expression
mutated
CLL
ABC DLBCL
B
P
plasmablast
plasma cell
End BCR expression
End CD20 expression
MM

FIGURE 2 Stages of B cell Development and associated malignancies.

B cells arise from common lymphoid progenitor (CLP) cells and undergo various maturation stages. Each step could give rise to a specific malignancy. ALL = Acute lymphatic Leukemia; CLL = Chronic lymphocytic leukemia; FL = Follicular lymphoma; GC DLBCL = Germinal center Diffuse large B cell lymphoma; ABC DLBCL = Activated B cell Diffuse large B cell lymphoma; MM = Multiple myeloma.

tinues throughout B cell maturation until the plasmacytoid immunoblast stage. CD20 is not expressed on hematopoietic stem cells, pro-B cells, plasma cells or on other normal non-B cell lineage tissues [28,29]. The normal B cell development stages are reflected in B cell malignancies, which often echo dominant clonal expansion of a specific maturation stage (Figure 2) [6], although antigen exposure and stimulation may also play a key role in the onset of B-cell malignancies [30].

In resting B cells, CD20 and the B-cell receptor (BCR, i.e. surface-expressed immunoglobulin (Ig)) are uniformly distributed in the plasma membrane. CD20 is translocated to cholesterol- and sphingolipid-rich micro domains, referred to as lipid rafts or detergent-insoluble glycolipid-enriched structures (DIGS), upon cross-linking by a subset of CD20 antibodies, the so-called type I CD20 antibodies [31,32], and/or engagement of the BCR by antigen [33]. BCR and CD20 co-localize initially, but rapidly translocate to distinct lipid rafts, followed by endocytosis of the BCR [33]. A central feature of lipid rafts is their ability to selectively include or exclude membrane proteins. Translocation of CD20 and the BCR into lipid rafts is an extremely rapid process, and is directly followed by phosphorylation of typical raft proteins like Lyn, which initiate the BCR signaling cascades [34,35].

EXPRESSION IN B CELL MALIGNANCIES

A wide variety of studies have analyzed CD20 antigen expression in B cell malignancies and correlated this with expression on the normal B cell counterpart. More than 90% of human B cell lymphomas and most B cell leukemias express CD20. Expression of CD20 was found to be lower on B cell chronic lymphocytic leukemia (B-CLL) and B cell acute lymphoblastic leukemia (B-ALL) than on normal peripheral B cells or on other malignant NHL [36-41]. In B-ALL, CD20 expression is lower and more heterogeneous compared to other B-cell malignancies. CD20 expression heavily depends on the differentiation stage of the originator cell and CD20 on B-ALL thus reflects the low expression on pro-B-cells, early pre-B-cells and pre-B-cells from which the tumor derived [42]. B-CLL derives from antigen-experienced B lymphocytes and can be classified based on whether the cells have undergone somatic hyper mutation (mutated immunoglobulin heavy-chain variable region (*IGHV*)), or not (unmutated *IGHV*). Further stratification can be done based on other factors such as e.g. chromosomal abnormalities and BCR signaling. This heterogeneity of the disease is reflected in CD20 expression, which for B-CLL cells varies with the genetic subtype [43-45] and the anatomical location of the tumor cells.

Indeed, CD20 expression levels were higher on B-CLL cells obtained from peripheral blood than on cells isolated from bone marrow and lymph nodes aspirates [46].
In the WHO revision of the classification of lymphoid malignancies in 2016, a distinction is made in 35 different types of lymphoma and myeloma [47]. As it will go too far to describe the role of CD20 expression in all of these subtypes, we will focus on the more common types, such as follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL) and multiple myeloma (MM). The level of CD20 expression in these malignancies, from high to low, is DLBCL > FL > MCL > MZL > MM [38-40, 48]. Although generally plasma cells do not express CD20, it is well documented that a small portion of MM are CD20-positive, yet expression is lower than on MZL [49-53]. This is often associated with the t(11;14) translocation and/or IgG isotype [49, 50, 54, 55]. Waldenström Macroglobulinemia (WM; where CD20 expression is present, but lower than on other lymphoma subtypes [46]), can be considered a prestage of MM.

RELEVANCE OF CD20 EXPRESSION IN MALIGNANCIES FOR TREATMENT

The prognostic role of CD20 expression in ALL is still cause for debate, as in childhood-precursor B-ALL, CD20 expression is associated with increased survival, in contrast to adult B-ALL where this is not the case [56-58]. The discovery that the addition of prednisone to the treatment regimen resulted in the upregulation of CD20 expression in pediatric B-ALL might offer at least a partial explanation for this difference [59]. The addition of RTX to the hyperCVAD chemotherapeutic regimen (alternating cyclophosphamide, vincristine, doxorubicin plus dexamethasone with methotrexate and cytarabine) for previously untreated B-ALL patients, stratified for CD20 expression (cut off > 20% $CD20^+$ cells), impacted the overall survival (OS) for younger (age < 60 years; OS 75% vs 47%), but not older (age > 60 years; OS 64% vs 65%) patients [60]. This suggests that inclusion of CD20-directed antibodies to the therapeutic regimen of B-ALL patients may improve therapeutic outcome of at least a proportion of the patients. However, it should be noted that the observed difference could also be based on patient selection, as it has been reported that CD20 expression was absent in the high risk patient group with translocation t(4;11) [56]. In standard-risk patients, CD20 expression was associated with poor survival [61]. As previously described, CD20 expression in CLL (both in percentage of cells expressing CD20 and in number of CD20 molecules expressed per cell) was lower compared to other B-cell lymphomas and normal peripheral B cells [36, 38, 40, 41]. When taking into account that CD20 expression on B cells from lymphoma patients correlates both with response rate and overall survival when applying RTX-containing therapy [62], this would not bode well for CD20-directed immunotherapies. Nevertheless, a clear benefit of including CD20-targeting antibodies to the treatment paradigm of patients with CLL has been observed in various studies. In previously untreated CLL patients, the addition of CD20 antibodies to chlorambu-

cil treatment resulted in a greater efficacy for all tested CD20 antibodies compared to chlorambucil alone [63,64]. This was also found for relapsed/refractory CLL, where OFA + fludarabine (F) + chlorambucil (C) (O-FC) and RTX-FC (R-FC) both improved outcome compared to patients treated with FC alone [65,66].
In DLBCL, high CD20 expression was a prognostic for better overall survival independent of treatment (cyclophosphamide, doxorubicin, vincristine plus prednisone (CHOP) or R-CHOP) [67]. Inclusion of RTX to the CHOP treatment regimen further increased the overall survival compared to CHOP alone, although this was dependent on CD20 expression and heterogeneity thereof [67-70].

The prognostic significance of CD20 expression in Hodgkin's lymphoma was found only when present on the B cells [71], but not when expressed on Reed Sternberg cells (the large multi-nuclear cells of B-cell origin that are a hallmark of Hodgkin's lymphoma and are used for diagnosis [72]), perhaps due to the fact that CD20 expression on the latter cells was much lower compared to that on morphologically normal B lymphocytes [73]. Besides the possible prognostic role of CD20 expression on malignant cells, the presence of circulating CD20 (cCD20) in the sera of NHL patients was shown to negatively correlate with progression-free survival [74,75]. Since the general consensus is that CD20 cannot be shed, this cCD20 likely reflects the remainder of killed $CD20^+$ cells, a fact underscored by the association of cCD20 with tumor burden in CLL [76].
Although the role of CD20 expression (often determined by flow cytometry on samples from previously untreated patients) as a prognostic marker for outcome is well documented, the role of CD20 expression should not be over interpreted as many CD20-directed immune therapies require CD20 positivity, determined by immunohistochemistry (IHC) as one of the inclusion criteria.

TWO TYPES OF CD20-SPECIFIC MONOCLONAL ANTIBODIES

Soon after the discovery of CD20, a dichotomy in the effector activity of CD20 mAbs was observed. Golay *et al.* described that two CD20 mAbs, 1F5 and B1, both inhibited immunoglobulin (Ig) production of activated B cells. However, only 1F5 was capable of activating resting B cells [17]. Deans *et al.* showed that some CD20 mAbs can induce translocation of CD20 into lipid rafts [31], an activity which appeared to be independent of the epitope recognized [16]. Cragg and Glennie proposed a functional classification of CD20 antibodies into categories based on their *in vivo* killing capacity. The type I antibodies RTX and 1F5 were shown to use complement activation to eradicate lymphoma xenografts, whereas the type II antibody B1 did not use complement to eradicate the lymphoma xenografts [77].
The type I/II classification of CD20 antibodies provides insight into the mechanism of action employed. Type I CD20 antibodies translocate CD20 in raft domains and induce complement-dependent cytotoxicity (CDC), whereas type II CD20 antibodies do not, but cause homotypic aggregation (HA), followed by programmed cell death (PCD) to elicit cell death. The ability to recruit effector cells for antibody-depen-

dent cell-mediated cytotoxicity (ADCC) or antibody-dependent cell-mediated phagocytosis (ADCP) is shared by both classes of CD20 antibodies. A molecular characteristic, which may explain the difference was identified by Niederfellner and colleagues, by comparing crystal structures of RTX and obinutuzumab (OBZ, GA101) in complex with CD20-derived peptides [11,78]. They observed a ~70° difference in orientation of Fab fragments of RTX and OBZ binding to a CD20 peptide due to their differential interaction with asparagine residues at positions 171 and 176. It is thought that the angle at which the antibody binds the CD20 strongly impacts its pharmacodynamic activity.

CD20 ANTIBODIES FOR THE TREATMENT OF NHL

In 1997, RTX became the first CD20 antibody approved for clinical use. RTX represents a chimerized variant of the murine anti-human CD20 mAb 2B8 (C2B8) [79]. Since then, RTX has continued to transform the therapeutic landscape of lymphoma and B-cell malignancies, with various large studies showing the undeniable benefit of adding RTX to chemotherapeutic standards of care [80-83]. Originally approved for relapsed, refractory indolent NHL, RTX is currently approved for NHL (R-CHOP (aggressive NHL such as DLBCL) or R-CVP (low grade NHL)), and CLL (R-FC). RTX, as a B cell-depleting agent, displays efficacy beyond lymphoma with approval in the autoimmune diseases rheumatoid arthritis (RA) (in combination with methotrexate) and granulomatosis (in combination with glucocorticoids) (summarized in Table 1).

RTX has a first-line approval for many of these indications.
As many patients eventually relapsed or became refractory to RTX treatment, novel CD20-targeting antibodies have been developed. New antibodies were often selected based on superior efficacy *in vitro* compared to RTX. In Table 1 the approved CD20 antibodies are listed, whereas in Table 2 and Table 3 clinical studies with CD20-directed molecules in the US and Europe, respectively, are listed.

OFA, a next generation fully human CD20 antibody targeting a different epitope than RTX, was selected based on its superior CDC induction and efficacy at lower antigen density compared to RTX [12,84,85]. OBZ, a humanized antibody, was generated from one of the earliest CD20 mAbs (Bly-1), and enhanced in its ability to induce programmed cell death (PCD) and NK cell-mediated lysis by protein engineering and glyco-engineering, respectively [86]. Both mAbs are now FDA- and EMA-approved for the treatment of CLL. Interestingly, despite these completely different *in vitro* modes of action in destroying $CD20^+$ cells, their therapeutic efficacy was quite comparable. In previously untreated CLL patients, treatment with OBZ plus chlorambucil (G-C) resulted in a median progression free survival (PFS) of 26.7 months [63], whereas in a similar study OFA plus chlorambucil (O-C) the median PFS was 22.4 months [64]. The similarity in clinical outcome of CLL treatment with either OFA or OBZ also questions the specific contribution of distinct *in vitro* mechanisms of action to *in vivo* efficacy. Various CD20-targeting antibodies, either novel or altered, have been preclinical-

ly investigated to unravel the respective roles of PCD, ADCC, ADCP and CDC. Table 4 summarizes the antibody based molecules derived of selected CD20 antibodies together with their reported mechanisms of action, a number of these antibodies have been evaluated in clinical settings (Figure 1, Table 2, Table 3).

Interesting novel concepts, such as type I/type II intermediates which combine the mechanisms of action of both type I and type II CD20 antibodies, such as CDC and PCD, were found to be very effective *in vitro* and *in vivo*, even demonstrating superior B cell depletion in the lymph nodes of cynomolgus monkeys compared to RTX. So far, these molecules have not entered clinical studies [87,88]. Other studies were aimed at enhancing specific attributes of antibody functions. Enhancing the affinity (reduced off-rate) [89], ADCC activity (de-fucosylation [90,91], or unknown method) [92], CDC [93,94] or all of them [95,96] resulted in superior *in vitro* efficacy compared to RTX. Of these compounds PRO131921 [97], ocaratuzumab [98] and veltuzumab [91] made it into early clinical development, although none of them have been investigated beyond a phase 1/2 study. This is also the case for CD20 small modular immune-pharmaceuticals (SMIP). SMIPs are IgG1-like molecules that contain a single-chain variable fragment (scFv) as binding region instead of a Fab fragment. 2LM20-4 [99] and TRU-015 [100], both showed superior efficacy in *in vitro* ADCC and CDC compared to RTX. TRU-015 was studied in a phase 1/2 trial, but this trial was terminated before completion. Ublituximab (LFB-R603, TG1101) [101] is a glyco-enhanced type I CD20 antibody that showed promising results in a phase 3 study where ublituximab plus ibrutinib was compared with ibrutinib alone in high-risk CLL patients. The combination resulted in a 78% overall response rate (ORR), compared to an ORR of 45% for ibrutinib alone. No head-to-head comparison with other CD20 antibodies has been done, but it is expected that the FDA will approve this combination for high-risk patients soon, so clinical studies are likely to expand [102]. Ocrelizumab (PRO-70769), another CD20 targeting antibody based on 2H7, was also tested in lymphoma [91], where activity was observed in patients with relapsed/refractory FL after prior RTX therapy, but this therapeutic area was abandoned to pursue multiple sclerosis (MS) and other autoimmune indications.

Additional CD20-targeting antibodies, which display distinct activities *in vitro* and some of which bind to novel epitopes, have been described: 8E4 [103], 5S [104], OUBM [105], 1K1791 [106] and 7D8, 11B8 and 2C6 [12,84,85]. It will be interesting to see how these will compare *in vivo* to the currently approved antibodies. Expiry of the patent protecting RTX has led to a widespread development of RTX biosimilars, some of which have already obtained approval in the EU (Table 1) or are under review by the FDA.

CD20 ANTIBODIES USED FOR TARGETED DELIVERY

Conjugation of CD20 antibodies with cytotoxic payloads has been applied as a means of generating more potent anti-CD20 molecules. Conjugated CD20 antibodies can be divided into three groups. The first group consists of radio-immunoconjugates, aimed at killing tumors by delivery of a radio-

active payload. Antibody-drug conjugates represent a second group, aimed at killing of the tumor cells via intracellular delivery of cytotoxic, chemotherapy-like drugs. These molecules require internalization and processing in lysosomes for the toxic agent to be released. The third group comprises antibody molecules labeled with an agent that is designed to enhance therapeutic efficacy without being cytotoxic, e.g. a cytokine.

RADIOLABELED CD20 ANTIBODIES

^{90}Y-ibritumomab tiuxetan and ^{131}I-tositumumab are radio-labeled CD20 antibodies and used as part of the FDA-approved radio immunotherapy of respectively Zevalin® and Bexxar® (Table 1) [107]. Zevalin, approved in 2002, was the first radiolabeled anti-CD20 therapy. The therapeutic regimen of Zevalin contains two steps: step 1 includes a first infusion of RTX preceding ^{111}In-ibritumomab tiuxetan to determine bio distribution; step 2 (7-9 days later) consists of a second infusion of RTX followed by ^{90}Y–Zevalin. This was changed in 2011 to two infusions of RTX followed by ^{90}Y–ibritumomab tiuxetan, when the FDA approved the removal of the ^{111}In-ibritumomab tiuxetan imaging step, because the contribution to patient safety was found to be negligible [108]. In a head-to-head comparison between ^{90}Y-ibritumomab tiuxetan and RTX, the former showed a significantly higher ORR than RTX alone (80% vs 56%); the secondary endpoints, duration of response and time to progression, were not significantly different between both arms [109]. However, in a single-arm study of 30 patients who relapsed after, or were refractory to RTX therapy, treatment with Zevalin resulted in a 67% ORR with an 11.8% median duration of response (DR). This led to the approval of Zevalin for patients with relapsed or refractory low grade follicular or transformed B cell non-Hodgkin's lymphoma. Studies in CLL found unacceptable hematologic toxicity [110]. The therapeutic efficacy of Zevalin can be mostly attributed to the radiolabel [111]. After all, ibritumomab represents the mouse antibody parent (2B8) of RTX and the studies above therefore highlight the increase in potency that may result from radiolabeling.

The second approved radiolabeled anti-CD20 therapy was Bexxar. This therapeutic regimen consists of a combination of tositumomab and ^{131}I-tositumomab, which are applied in a dual administration with a dosimetric and a therapeutic part. Davies wrote a comprehensive review on the clinical experience with Bexxar [112]. The Bexxar regimen showed excellent clinical responses, especially in the first-line setting [113], but was also effective in patients who had received prior RTX treatment [114]. As suggested above for Zevalin, the efficacy of Bexxar was also mainly driven by the radiolabeled component [115]. Re-treatment with Bexxar resulted in low efficacy, likely due to the relative high level of human anti-mouse antibodies (HAMA), because of immunogenicity of the mouse antibody in humans [116]. Myelodysplastic syndrome (MDS) has also been reported as adverse event. However, in several studies no correlation was observed between the radiolabel and onset of MDS. The development of MDS in NHL patients was similar for regimens with or without a radiolabel [117]. The

strong potential of Bexxar was highlighted in a follow-up study of a head-to-head comparison of Bexxar and RTX in FL [118]. The addition of Bexxar to the CHOP regimen (CHOP-RIT) resulted in a significantly better 10-year PFS compared to R-CHOP (56% vs 42%). OS was not significantly different between the two treatment arms, neither was the incidence of secondary malignancies or MDS. Bexxar was withdrawn from the market in February 2014, due to the low number of patients being treated.

Some radio-immunotherapy (RIT)-related studies in the pre-clinical setting have been performed with the aim of validating new radiolabels [119, 120] and/or novel CD20-specific antibodies for use as carriers [121-123]. Radiolabeled antibodies have also been employed in mechanistic studies to investigate bio distribution and tumor penetration of CD20 antibodies [120-122, 124, 125]. Radio-conjugates represent useful tools to study tumor penetration *in vivo* and might even be used to determine the tumor burden of patients in the more indolent setting, in the near future. Considering the wide acceptance of unlabeled CD20 antibodies combined with the negative perception of RIT by physicians [126], it seems unlikely, however, that new therapeutic radioactively labeled anti-CD20 antibodies will enter the clinic, despite their strong therapeutic potential.

CD20-SPECIFIC ANTIBODY-DRUG CONJUGATES (ADC)

Although CD20 internalization occurs, this is a relatively slow process, which is dependent on several other antigens. Internalization has been described to be driven by BCR clustering and IgG Fc receptor (FcγR) expression, predominantly through the interaction of type I CD20 antibodies with FcγRIIb [127, 128]. Nevertheless, this has been an area of controversy, as Vervoordeldonk *et al*. [129] demonstrated that the type I CD20 antibody 1F4 did not internalize, irrespective of the isotype tested (IgG1, IgG2a, IgG2b and IgM), whereas the control target CD19 did show antibody-induced internalization. Press *et al*. [130] demonstrated that the type II antibody B1 did not internalize. Despite contradictory studies described in literature, it is now accepted that CD20 internalization occurs, but at a relatively slow rate. Michel and Mattes [123] demonstrated that ^{111}In-labeled anti-CD74 or anti-MHC class II was internalized at a rate of 10^7 antibody molecules per 24 hours, but that ^{111}In-labeled anti-CD20 only reached ~$4x10^6$ intracellular molecules per 48 hours. The fact that CD20 is a relatively slow internalizing target was further demonstrated in a study where CD20 immunotoxin (IT) (CD20 antibody conjugated to the plant toxin saporin S6) was compared to CD22 IT [131]. Even with the higher drug–to-antibody ratio (DAR, i.e. the number of drugs attached to the molecule) for the CD20 IT, the CD22 IT was much more potent as a result of the more efficient internalization.

The use of CD20 as a target for antibody-drug conjugates may seem somewhat counterintuitive regarding its poor ability to internalize. However, because of the highly and selective expression pattern of CD20 and the notion that long-term incubation may still lead to sufficient antibody

payload internalization to induce effective killing, various type I CD20 targeting antibodies, such as 1F5, RTX and OFA were conjugated using a variety of payloads and tested for their ability to induce cell kill. *In vitro* and *in vivo* efficacy of OFA-vcMMAE (the tubulin disrupting agent monomethyl auristatin E (MMAE) linked with a cleavable valine-citruline (vc) linker to OFA) was investigated by Li *et al*. [132] and demonstrated good tumor depletion *in vivo*. Similar results were observed for RTX-vcMMAE [133], although here the drug-linker facilitated internalization, since unlabeled RTX did not accumulate in the cell to the same extent as RTX-vcMMAE or RTX-vcDOX (doxirubicin). This observation was in agreement with a study that compared various B-cell targets for suitability for an ADC approach [134]. Here the choice of linker influenced *in vivo* efficacy, as only CD20-SPP-DM1 was able to delay tumor outgrowth of Granta-519 and CD20-MCC-DM1 was not. This was found to be the case for multiple, but not all, B cell targets. Another study, using a different drug, EPI (a N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-epirubicin conjugate)), demonstrated *in vivo* efficacy that was dependent on the amount of RTX-bound drug [135].
Taken together, there is evidence supporting the *in vivo* suitability of CD20 as an ADC target, albeit dependent on the drug/linker combination. However, due to CD20's slow internalization rate, the number of the payload molecules attached to the CD20 mAb (DAR) needs to be high.

BISPECIFIC ANTIBODIES TARGETING CD20

Bispecific antibodies (bsAbs) combine the specificities of two antibodies in one molecule. A wide variety of bispecific formats exists [136], from bivalent molecules, that bind each target antigen monovalently, to tri- or tetravalent molecules, with an ability for multivalent binding of one or both of the target antigens. BsAbs represent a promising strategy to increase antibody function, as discussed in [137] and depicted in Figure 3.

BsAbs binding to two different epitopes on CD20 potentially have the advantage of combining the MoA of both parental antibodies in one molecule. For instance, combining a type I and type II antibody could lead to a type I/II antibody with superior efficacy by being able to apply more effector mechanisms (higher maximum response) compared to the combination of both parental antibodies or superior potency (lower EC_{50}/IC_{50}) as both antibodies combined. This also applies to targeting two antigens on one cell with a bsAb molecule. Targeting two antigens potentially allows increased specificity, by only targeting cells that express both antigens, or target a more heterogeneous population of cells, by binding to cells expressing one antigen as well as binding to other cells expressing the other antigen. If sufficient binding to one target antigen is obtained via monovalent binding, the other arm can be used for other purposes, such as delivery of a cytotoxic compound or recruitment of effector cells.

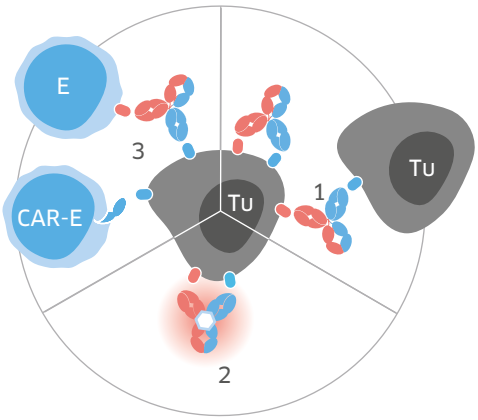

FIGURE 3 Diverse of applications of bsAb against CD20.

BsAb targeting CD20 currently under investigation aim to target two epitopes on CD20 or two different antigens to target two antigens either on the same cell (to increase specificity) or on different cells (to address heterogeneity) **(1)**, or to recruit effecter cells such as NK cells, T cells, macrophages and CAR-Effector (CAR-E) cells **(3)**. Bispecific binding two antigens or two epitopes are also employed to increase specific payload delivery **(2)**.

Many bsAb formats have been described, ranging from fragment-based bsAbs, (genetically fused antibody-binding domains (e.g. domain antibodies or VHH antibodies (also termed nanobodies; Ablynx); bsAb lacking an Fc domain)), to IgG molecules which can be either tetravalent (e.g. Fab_4-IgG) or molecules with a regular IgG architecture (reviewed in [136, 138-140]). Applicability of the specific bsAb format will mainly be driven by the desired effector functions of the compound and choice of half-life. Many of the current bispecific formats in clinical evaluation for CD20 targeting are aimed at recruiting T cells. Here, extension of half-life can be achieved by selection of an IgG-based format that retains/has FcRn binding, such as knob-into-hole [141], DuoBody [142], XmAb [143] and Fc/Fc* (based on differential protein A affinity) [144] . Other platforms circumvent short half-life by continuous infusion, like BiTe [145] or fusion to albumin [146]. IgG-based formats have the potential to recruit Fc-mediated effector components such as complement and FcγR-expressing cells of the innate immune system. Where Fc-mediated effector mechanisms are claimed as part of the potency of the trifunctional bsAb FBTA05 (Lymphomun, bi20), many other formats, such as Fc/Fc*, T cell dependent bispecific antibody (TBD), T cell specific antibodies (TCB), Xmab and DuoBody® can be engineered to selectively render the Fc inert for Fc-driven effector functions, while leaving FcRn binding intact.

TARGETING OF TWO RECEPTORS

Li *et al.* [147], produced a tetravalent CD20-targeting molecule, TetraMcAb-4-scFv, containing the binding regions of both C2B8 (RTX) and 2F2 (OFA) with the intention to increase the binding avidity compared to the parental antibodies. Interestingly, it was observed that the ability of this molecule to induce PCD was increased compared to that of the parental antibodies, both of which are type I CD20 antibodies that induce minimal PCD. When including a type II CD20 antibody, that have higher capacity to induce PCD, in the construct (11B8/2F2 dual TetraMcAB) the activity could not be further increased, as shown in *in vitro* and *in vivo* assays [148]. The concern of modest therapeutic efficacy of mAbs, due to heterogeneity of

the tumor and possible antigen-negative relapses, stimulated the research for so called T1xT2 bispecific molecules. HLA-DR and CD20 have been previously linked, as they display similar cell aggregation and antigenic clustering under specific conditions [149] and when administered together showed synergistic anti-lymphoma activity [150]. A bispecific dual-variable domain immunoglobulin (DVD-Ig; a tetravalent IgG1 molecule containing a second variable domain attached to each of the Fab regions) molecule targeting CD20 and HLA-DR induced increased HA, actin rearrangement and apoptotic cell death. Using confocal and light microscopy, it was demonstrated that HA induced by this bispecific molecule resulted in increased cell-cell contact. The DVD-Ig molecule also showed superior cell depletion capacity in a whole blood assay, where it was able to deplete more Raji cells than the parental antibodies alone or a combination thereof [151].

Additional dual-antigen targeting bsAbs have been evaluated in the CD20 space, such as targeting CD20 in combination with CD22. The $F(ab')_2$ fragment of RTX chemically crosslinked to the $F(ab')_2$ fragment of HB22.7 (Bs20x22) displayed similar binding properties as the parental antibodies alone, but showed a greater potency in inducing apoptosis compared to the parental antibodies alone or a combination thereof [152]. Bs20x22 was able to bind simultaneously to both targets, even when expressed on different cells. Due to the absence of an Fc region, Bs20x22 was not capable of inducing CDC, ADCC and ADCP. *In vivo*, the bispecific compound inhibited tumor outgrowth in a Raji xenograft model only slightly better than the combination of IgG1 antibodies. To increase the potency of the Bs20x22, a hexavalent molecule with 2 CD20 targeting arms and 4 CD22 targeting arms or vice versa, also including a functional Fc-region was generated. The addition of an Fc region led to an increase in PCD *in vitro*, but only a modest increase in *in vivo* efficacy, which was shown to depend on the presence of NK cells and/or neutrophils [153, 154]. The *in vivo* activity, as measured by survival of xenograft mice, of a bispecific molecule targeting both CD20 and CD95 was superior to that of an Fc-enhanced CD20 antibody, which in turn was more effective than a chimeric CD20 mAb (all molecules were based on 2H7) [155]. This was even more impressive considering the fact that the bispecific molecule had a shorter *in vivo* half-life than the chimeric and Fc-enhanced molecules (less than 2 hours for the bispecific and 4 hours for the other antibodies, respectively). The shorter half-life was due to the size of the molecule (both binding arms combined in one Fab-arm) and the absence of an FcRn binding site.

DELIVERY OF CYTOTOXIC PAYLOADS USING CD20 BISPECIFIC ANTIBODIES

A second potential application of bsAb is the use as a vehicle to deliver a payload. This was for example achieved by the design of a bsAb molecule containing a CD20 targeting arm (2H7) in combination with an arm targeting a radiolabel (2H7-Fc-C825). This bispecific molecule showed superior *in vivo* bio distribution to the tumor (as detected by analysis of residual radioactiv-

ity in tumor samples and normal organs) compared to a CD20-targeting antibody that recruits a radioactive compound via a streptavidin/biotin linker (1F5-SA) [156]. Furthermore, it showed significantly better tumor-free survival in mice. Although it would have been more accurate to compare 2H7-Fc-C825 with 2H7-SA, this study does show the feasibility of the concept.

Another method to combine an active compound with a bispecific molecule was shown by using the dock-and-lock method, where IFN-alpha (IFNα) was linked to a CD20xHLA-DR bispecific antibody. Derived from the antibodies veltuzumab (CD20) and L243 (HLA-DR), this molecule was termed 20-C2-2b. *In vitro* efficacy of this molecule was shown to be more potent than the parental antibodies or the combination of both parental antibodies. Moreover 20-C2-2b was able to deplete CD20-expressing Daudi cells spiked in whole blood, while sparing other cells such as T cells and endogenous B cells [157]. Whether it is clinically feasible to combine both the CD20 binding arm and an IFNα in one molecule will have to be determined, but CD20 and IFNα is a potent combination [158-160].

RECRUITMENT OF EFFECTOR CELLS

The final and by far the most applied approach for CD20-directed bsAb is the generation of bispecific molecules that can recruit effector cells and direct the tumor antigens for tumor cell killing. The addition of a CD16 targeting Fab fragment to two CD20 targeting scFv fragments (7D8) and an Fc fragment was investigated with a tribody (a Fab fragment and two scFv molecules fused via a CH1 domain) [161]. This molecule showed enhanced ADCC *in vitro* compared to the parental CD20 antibody 7D8 and RTX. This was shown to be mediated by an enhanced interaction with NK cells. Other recruitment strategies mainly focused on neutrophils and macrophages, either by targeting CD89 [162, 163] or CD47 [164, 165]. Fc receptor binding was diminished in these formats/strategies by introducing an N297A mutation in the Fc domain [162] or by using chemically cross-linked $F(ab')_2$ molecules [163]. Stockmeyer *et al.* showed that the concept of recruiting CD89-positive leucocytes worked *in vitro* and even outperformed Fc-receptor targeting bispecific molecules [163]. Li further demonstrated the potential of the bispecific molecule in an *in vivo* setting. Since mice do not express CD89 either human PMNs were added, or CD89 transgenic mice were used. In both models the bispecific molecule showed anti-tumor efficacy however did not outperform RTX.

T cell-recruiting bsAbs artificially link T cells with CD20 molecules expressed on tumor cells and activate T cells upon this cross-linking, to kill the CD20-expressing cells. The bsAb r2820 targets CD28 expression on T cells to induce T cell activation and tumor cell killing [166]. This scFv molecule induces kill of CD20-positive cells by PBMCs. Interestingly, this compound was more effective when combined with a CD3xCD20 bispecific antibody [167]. Targeting CD3, by binding to CD3ε (alone or in combination with CD3δ), is the most widely used method to recruit T cells to the tumor antigen in a bsAb. This has been shown to

induce cytotoxic synapse formation and target cell kill, independent of peptide presentation by MHC on the target cell and TCR specificity of the recruited T cells [168]. Redirecting T cell activity towards tumor antigens can induce dramatic regression of advanced stage malignancy, as was shown for the CD3xCD19 bispecific blinatumomab in heavily pretreated NHL and ALL patients [169].

Various CD3xCD20 bsAb molecules such as an anti-CD20 x anti-CD3 diabody [170], anti-CD20-LHD-scFC anti-CD3 [171], 20-3S [172], BIS20x3 [173] and halfbody CD3xCD20 [174] were mainly designed for concept, platform or clone validation. While these studies were informative from a platform perspective, the majority of data on validity of CD3xCD20 bsAbs comes from the clinical setting. CD3xCD20 bsAbs have been in clinical investigation since 2005. The first to be administered to patients was CD20bi (a heteroconjugate of the CD3 mAb OKT3 and RTX). In a phase 1 study together with *ex vivo* anti-CD3 activated T cells (ATC) and autologous stem cell transplantation (SCT), 9 out of 15 patients were in CR after 90 days, with a median survival of 20.9 months [175]. The role of the bispecific molecule in this study is difficult to assess, due to complexity of the treatment. However, considering that these patients were refractory NHL patients in the high risk group, the results are impressive. The next CD3xCD20 to be investigated in a clinical setting was FBTA05 (Lymphomun, Bi20; a mouse-rat chimeric bsAb created with hybridoma fusion technology) [176]. In a small, dose-escalation study with six patients (three with p53-mutated CLL and three with high-grade NHL), only modest responses of CD20bi in combination with donor lymphocyte infusions (DLI) were observed, with stable disease for four months as best response [177]. In another study with FBTA05 in pediatric patients, 9 out of 10 patients achieved a clinical response with five CRs with a maximum duration of 1424 days [178]. This was especially impressive regarding the heavy pre-treatment, including RTX-based regimens for 5 out of 10 patients. These first generation CD3xCD20 bsAbs contained immunogenic murine sequences, but HAMAs were only sparsely reported. In addition, both have active Fc regions that are expected to induce non-specific T cell activation and are therefore undesired. Next generation CD3xCD20 bsAbs therefore were designed to contain an inert Fc tail (with respect to effector function) or they completely lack an Fc-region. REGN1979, a bsAb containing novel CD3 and CD20 antibodies generated in VelocImmune mice contains an IgG4 Fc-region to minimize Fc effector functions [144]. Clinical investigation of REGN1979 started in a phase 1 dose escalation study in NHL. In 2016, 25 patients have been enrolled of whom it was reported that 16 patients discontinued treatment, most for PD [179]. The low initial efficacy is likely due to the starting dose, as the dose ranged from 0.03-3.0 mg flat dose. In 2017, with dose levels at 5-7 mg flat REGN1979 dose, the ORR was reported to have increased to 45% [180]. Novel CD3xCD20 bsAbs are in development, such as XmAb-13676 [181], RG7828 [141] (both are in phase 1/2 studies with no data reported), RG6026 [182] and GEN3013 (expected to enter clinical studies in 2018). It will be interesting to see wheth-

er the differences in platforms on which these bispecific antibodies are based, will result in differences in clinical efficacy.

CAR EFFECTOR CELLS

An alternative approach to redirect effector cells to the tumor is by gene therapy, in which (patient's) T cells are transfected with a chimeric antigen receptor (CAR). CARs represent scFvs fused to one or multiple co-stimulatory molecules that are expressed on the surface of immune effector cells such as T cells. Evolution of CD20 CARs, like for CD19 CARs, was mainly driven by the signaling domains. In the first generation CARs, the activation was mainly driven by only one signaling domain (CD3ζ). In the second generation CARs, the signaling domain was extended to also include that of CD28 [183], and even including the signaling domain of CD137 in the third generation CARs [184, 185]. This enhanced activation of the CAR-T cells by the inclusion of these co-stimulatory signaling domains in addition to that of CD3 signaling domain enhanced the *in vitro* efficacy of the CARs and led to a better clinical response as described below.

In a clinical phase 1 study performed at the Fred Hutchinson Cancer Research Center (NCT00621452), four patients were treated with cyclophosphamide (to achieve lymphocyte depletion) followed by three infusions (2 to 5 days apart) of autologous CD20-specific CAR-T cells and SC IL-2. CAR-T cells were detectable in circulation, lymph nodes and bone marrow, and a measurable clinical response was obtained. However, both could not be definitively attributed to the activity of the CAR-T cells, partially due to low CAR expression as a result of inefficient gene transfer [185]. Some of these issues have now been resolved with an optimized vector and it will be interesting to see what the clinical results [186]. In another clinical study, a phase 2 trial was conducted evaluating CAR-T-20 in 11 patients with lymphoma (NCT01735604). Here, 11 out of 11 patients obtained a PFS with more than 50% achieving a six-month or longer PFS and one patient had a 27-month continuous CR [187, 188]. Besides being very promising, these clinical studies also revealed drawbacks. It became clear that immune-privileged sites such as the testis, are refractory to CAR-T treatment [187], but also other sites, such as lung and liver, showed outgrowth of $CD20^+$ cells, despite the presence of T cells [188]. Sufficient target expression is crucial, even though efficacy was observed *in vitro* on cell lines expressing low numbers of CD20, or on CLL cells (notorious for low CD20 expression) [189]. In patients treated with CAR-T cells, unexpectedly, peripheral B cells were untouched by the CAR-T cells, most likely due to their relatively low CD20 expression level compared to that on lymphoma cells [185].

The occurrence of delayed adverse events, such a cytokine release syndrome, which may be observed 3-9 weeks after T-cell infusion [188] are also cause of concern. Although often manageable, new strategies to increase safety of CAR-T cell therapy are investigated. One strategy that is being explored is by inclusion of suicide switches, such as caspase 9, which upon activation result in the self-destruction of CAR-expressing cells [190]. Another strategy em-

ployed is by generation of a generic CAR-T construct (a scFv targeting PNE) that is only active in the presence of a Fab fragment targeting CD20, containing a PNE motive [191]. Here, the half-life of the Fab fragment determines the efficacy and safety of the CAR-T cell therapy. CD20 itself may also be used as a suicide system for CAR-T cell treatment. As T cells do not express CD20 naturally, the forced expression of CD20 could provide a way to eliminate CAR-T cells via RTX treatment [192].

Another point of concern is the possible relapse of the lymphoma, due to outgrowth of CD20-negative lymphoma cells. One possible solution is dual targeting with CAR-T cells, such as with bispecific CAR-T cells [193-195], which showed *in vivo* efficacy to kill tumor cells expressing either target alone or both targets together. Another option is combined or sequential treatment with two or more different CAR-T cell therapies for which several clinical studies are now recruiting (NCT03207178, NCT03125577, and NCT02737085).
Last but not least, manufacturing issues of CAR-T cell treatment are being addressed [196, 197]. During the manufacturing process steps such as e.g. T cell isolation, gene transduction, and expansion need to be performed under sterile conditions and as fast as possible to avoid the loss of the patient before start of treatment.

In addition to CAR-T cells, also CAR-NK cells are under (early) pre-clinical investigation [198, 199], but so far only *in vitro* analyses are available.

CD20 TARGETING AND IMMUNOMODULATION

The concept of immunomodulation for CD20 antibodies encompasses the recruitment of immune effector cells as mechanism of action, such as NK cells for ADCC and macrophages for ADCP. Also complement plays a role, as C3a and C5a represent chemo-attractants for immune cells. Pre-clinical and clinical studies further investigated this route to improve the efficacy of CD20 antibodies. The first evidence for immunomodulation beyond a direct interaction of effector cells with RTX was provided by Hilchey and colleagues [200]. They demonstrated that inclusion of RTX resulted in a T cell-directed anti-lymphoma response. Later it was discovered that in mice given anti-CD20 therapy, regulatory T cell (Treg) expansion was reduced and Th1 expanse increased [201]. This was in contrast to the previously reported effect on T cell-dependent immunity [202]. However, currently there is no clear understanding of the role that CD20 mAbs in play in the generation of an adaptive memory response.
The administration of *ex vivo* expanded immune cells, such as NK cells and T cells, has also been studied. Although, especially for NK cells, some increase in therapeutic activity was found, these were eventually abandoned in favor of the pursuit of CD20 CAR-T and CD20 CAR-NK cells. Although the concept of CAR-T cells targeting CD20 has been around for over a decade, the general excitement and status of CD19 CAR-T cells has not been achieved. While the initial anti-tumor efficacy was promising, there are still many hurdles to take. Hurdles recently observed for CD19 CAR-T cells,

where massive endogenous T cell proliferation was observed after administration of CAR-T cells [203], also need to be taken for CD20 CAR-T cells. In addition, the longevity of persistence of CAR-T cells in the patients' needs to be addressed as continuous repression of CD20$^+$ cells may raise new issues. As 10 new phase 1 studies with CD20 CAR-T therapies were initiated in 2017, some of these hurdles will be addressed. It is expected that the inclusion of T cell activity into the treatment of lymphomas will be the direction CD20-based therapies are going, whether this will be with CAR-T cells or bispecifics remains to be seen.

OVERALL CONCLUSION

The road traveled for CD20-targeted immunotherapy has been long, but very fruitful. Strong clinical efficacy of CD20-specific antibodies, together with the lack of serious safety issues associated with depletion of CD20-expressing cells makes CD20 an ideal target, despite the relapses or resistance that occur almost inevitably in any cancer treatment. First, there is a benchmark in place with RTX. Second, as depletion of CD20$^+$ cells is safe, any flags raised during testing of novel compounds can be attributed to the compound/format and is not directly target-related.

In the past decades, many lessons have been learned with mAbs targeting CD20 in lymphoma and leukemias. Initially the focus was on mAbs and radiolabeled antibodies. Pre-clinical data suggested that enhancing singled-out effector functions of these mAbs would result in enhanced therapeutic efficacy. OFA and OBZ showed us that *in vitro* studies do not readily predict therapeutic gain as both were different in MoA employed but obtained comparable clinical outcome in a similar patient population and treatment modality. Furthermore, head-to-head clinical comparison of naked and radiolabeled mAbs showed results in favor of the radiolabeled mAbs. However, concerns of MDS, secondary malignancies and complexities with respect to the supply-chain and administration ultimately led to the demise of radiolabeled antibodies.

Currently, many of the RTX bio-similars are approved and it will be interesting to see whether this will make treatment cheaper and more accessible for RTX responders. Furthermore, the applicability of CD20 targeting antibodies beyond the treatment of cancer will be an exciting development, as it might teach us more on the role of B cells in immunomodulation, something the vaccination-like effects of RTX have hinted at. It is expected that patients that have relapsed or are refractory to CD20 mAb therapy will have new CD20-targeting treatment paradigms to their exposure. CAR-T cells, CD3 bsAbs with or without the combination of checkpoint inhibitors and/ or small molecules will be an exciting field to watch. CAR-T cells are expected to have potential in the future, but still many hurdles to take. It will be intriguing to see whether the field is equipped with the technical challenges associated with CAR-T cells, seeing the lost confidence in radiolabeled mAbs was mostly associated with technical challenges. The near future will be dominated by the CD3 as they have the promise of high therapeutic potential with manageable safety and, in contrast to CAR-T cell

technology, are much easier to produce and control. All in all, current development in CD20 immunotherapy sparks the hope for turning B cell malignancies in a controllable disease.

TABLE 1 FDA/EMA approved CD20 antibodies.

International non-proprietary name (INN)	Brand name	Clone	Variant	First Approval, US or EU	Indication of approval	Ref.
Rituximab (RTX)	Rituxan (US) Mabthera (EU)	C2B8	Chimeric IgG1	1997 (US) 1998 (EU)	- Non-Hodgkin's Lymphoma (NHL). - Chronic Lymphocytic Leukemia (CLL). - Rheumatoid Arthritis (RA) in combination with methotrexate in adult patients with moderately- to severely-active RA who have inadequate response to one or more TNF antagonist therapies. - Granulomatosis with Polyangiitis (GPA) (Wegener's Granulomatosis) and Microscopic Polyangiitis (MPA) in adult patients in combination with glucocorticoids.	[204]
Ofatumumab (OFA)	Arzerra	2F2	IgG1	2009 (US) 2010 (EU)	- Chronic Lymphocytic Leukemia (CLL). In combination with chlorambucil, for the treatment of previously untreated patients with CLL for whom fludarabine based therapy is considered inappropriate. For extended treatment of patients who are in complete or partial response after at least two lines of therapy for recurrent or progressive CLL. For the treatment of patients with CLL refractory to fludarabine and alemtuzumab.	[12,84,205]
Obinutuzumab (OBZ)	Gazyva	Bly-1 GA101	IgG1-Fc optimized	2013 (US) 2014 (EU)	- Chronic Lymphocytic Leukemia (CLL). In combination with chlorambucil, for the treatment of patients with previously untreated chronic lymphocytic leukemia. - Non-Hodgkin's Lymphoma (NHL). In combination with bendamustin followed by GAZYVA monotherapy, for the treatment of patients with follicular lymphoma (FL) who relapsed after, or are refractory to, a rituximab-containing regimen.	[86,206]

International non-proprietary name (INN)	Brand name	Clone	Variant	First Approval, US or EU	Indication of approval	Ref.
tositumomab	Bexxar	B1	Mouse IgG2a	2003 (US)[1]	- Non-Hodgkin's Lymphoma (NHL). CD20 positive, relapsed or refractory, low-grade, follicular, or transformed NHL who have progressed during or after rituximab therapy, including patients with rituximab-refractory NHL.	[207]
Ocrelizumab (OCRE)	Ocrevus	2H7	IgG1-Fc optimized	2017 (US)	- patients with relapsing or primary progressive forms of multiple sclerosis (MS)	[208]
Ibritumomab tiuxetan	Zevalin	2B8	mIgG1	2002 (US) 2004 (EU)	- Non-Hodgkin's Lymphoma (NHL). Relapsed or refractory, low-grade or follicular B-cell NHL. Previously untreated follicular NHL who achieve a partial or complete response to first-line chemotherapy.	[209]
Blitzima		C2B8	RTX biosimilar	2017 (EU)	- Non-Hodgkin's Lymphoma (NHL). - Chronic Lymphocytic Leukemia (CLL). - Granulomatosis with Polyangiitis and Microscopic Polyangiitis.	[210]
Ritemvia		C2B8	RTX biosimilar	2017 (EU)	- Non-Hodgkin's Lymphoma (NHL). - Granulomatosis with Polyangiitis and Microscopic Polyangiitis.	[211]
Rituzena		C2B8	RTX biosimilar	2017 (EU)	- Non-Hodgkin's Lymphoma (NHL). Follicular lymphoma and diffuse large B cell NHL. - Chronic Lymphocytic Leukemia (CLL). - Granulomatosis with Polyangiitis and Microscopic Polyangiitis.	[212]
Rixathon		C2B8	RTX biosimilar	2017 (EU)	- Non-Hodgkin's Lymphoma (NHL). - Chronic Lymphocytic Leukemia (CLL). - Rheumatoid arthritis (RA). - Granulomatosis with Polyangiitis and Microscopic Polyangiitis.	[213]

International non-proprietary name (INN)	Brand name	Clone	Variant	First Approval, US or EU	Indication of approval	Ref.
Riximyo		C2B8	RTX biosimilar	2017 (EU)	- Non-Hodgkin's Lymphoma (NHL). - Rheumatoid arthritis (RA). - Granulomatosis with Polyangiitis and Microscopic Polyangiitis.	[214]
Truxima		C2B8	RTX biosimilar	2017 (EU)	- Non-Hodgkin's Lymphoma (NHL). - Chronic Lymphocytic Leukemia (CLL). - Rheumatoid arthritis (RA). - Granulomatosis with Polyangiitis and Microscopic Polyangiitis.	[215]

1) Oct-2013 FDA approval for Bexxar was withdrawn and Feb-2014 Bexxar was discontinued by the manufacturer

TABLE 2 Clinical investigations in the US targeting Lymphoma and leukemia with CD20 mAbs.

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
Bispecific antibodies							
CD20Bi	CD3x-CD20	Barbara Ann Karmanos Cancer Institute	NCT00244946	Immune Consolidation With Activated T Cells Armed With OKT3 x Rituxan (Anti-CD3 x Anti-CD20) Bispecific Antibody (CD20Bi) After Peripheral Blood Stem Cell Transplant for High Risk CD20+ Non-Hodgkin's Lymphomas	Phase I	Completed	2005
CD20Bi	CD3x-CD20	Barbara Ann Karmanos Cancer Institute	NCT00521261	Immune Consolidation With Allogeneic Activated T Cells Armed With OKT3 x Rituxan (Anti-CD3 x Anti-CD20) Bispecific Antibody (CD20Bi) After Allogeneic Peripheral Blood Stem Cell Transplant for High Risk CD20+ Non-Hodgkin's Lymphoma (Phase I)	Phase I	Withdrawn	2007
CD20Bi	CD3x-CD20	Barbara Ann Karmanos Cancer Institute	NCT00938626	Phase I trial is studying the side effects and best way to give treated T cells followed by stem cell transplant in treating patients with multiple myeloma.	Phase I	Completed	2009
FBTA05	CD3x-CD20	Trion pharma	NCT01138579	Phase I/II Dose-escalation Study of the Investigational Trifunctional Bispecific Anti-CD20 x Anti-CD3 Antibody FBTA05 in Combination With Donor Lymphocyte Infusion (DLI) in Patients With CD20 Positive Chronic Lymphocytic Leukemia (CLL), Low and High Grade Non-Hodgkin´s Lymphoma (NHL) After Allogeneic Stem Cell Transplantation	Phase I/II	Terminated	2010
REGN1979	CD3x-CD20	Regeneron	NCT02290951	An Open-Label, Multi-Center Phase 1 Study to Investigate the Safety and Tolerability of REGN1979, an Anti-CD20 x Anti-CD3 Bispecific Monoclonal Antibody, in Patients With CD20+ B-Cell Malignancies Previously Treated With CD20-Directed Antibody Therapy	Phase I	Recruiting	2014

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
XmAb13676	CD3x-CD20	Xencor	NCT02924402	A Phase 1 Multidose Study to Evaluate the Safety and Tolerability of XmAb13676 in Patients With CD20-Expressing Hematologic Malignancies	Phase I	Recruiting	2016
BTCT4465A	CD3x-CD20	Genentech	NCT02500407	An Open-Label, Multicenter, Phase I/IB Trial Evaluating the Safety and Pharmacokinetics of Escalating Doses of BTCT4465A as a Single Agent and Combined With Atezolizumab in Patients With Relapsed or Refractory B-Cell Non-Hodgkin's Lymphoma and Chronic Lymphocytic Leukemia	Phase I	Recruiting	2015
CAR-T cells							
	CD20 CAR-T	Fred Hutchinson Cancer Research Center	NCT00012207	A Phase I Study To Evaluate The Safety Of Cellular Immunotherapy Using Genetically Modified Autologous CD20-Specific Cd8+ T Cell Clones For Patients With Relapsed CD20+ Indolent	Phase I	Completed	2003
	CD20 CAR-T	Fred Hutchinson Cancer Research Center	NCT00621452	A Pilot Study to Evaluate the Safety and Feasibility of Cellular Immunotherapy Using Genetically Modified Autologous CD20-Specific T Cells For Patients With Relapsed or Refractory Mantle Cell and Indolent B Cell Lymphomas	Phase I	Completed	2008
	CD20 CAR-T	Chinese PLA General Hospital	NCT01735604	Pilot Study of Redirected Autologous T Cells Transduced to Express A CD20-Specific Chimeric Immunoreceptor in Patient With Chemotherapy Resistant or Refractory CD20+ Leukemia and Lymphoma	Phase I/II	Recruiting	2012
	CD16 CAR-T + rituximab	Beijing Bio-healthcare Bio-technology Co.,Ltd	NCT02965157	Phase 1 Study of ACTR087, Autologous T Lymphocytes Expressing Antibody Coupled T-cell Receptors (CD16V-41BB-CD3ζ), in Combination With Rituximab, in Subjects With Relapsed or Refractory CD20-Positive B-Cell Lymphoma	Phase I	Recruiting	2016

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
	CD20 CAR-T	Southwest Hospital, China	NCT02710149	A Clinical Research of CD20-Targeted CAR-T in B Cell Malignancies	Phase I	Recruiting	2016
	CD20 CAR-T + CD19 CAR-T	Southwest Hospital, China	NCT02737085	the Sequential Therapy of CD19-targeted and CD20-targeted CAR-T Cell Therapy for Diffuse Large B Cell Lymphoma(DLBCL)	Phase I	Not recruiting	2016
	CD20 CAR-T	Southwest Hospital, China	NCT02846584	A Clinical Research of Sequential CAR-T Bridging Hematopoietic Stem Cell Transplantation in the Treatment of Relapse/ Refractory B-cell Malignancies	Phase II	Not recruiting	2016
	CD20 CAR-T + CD19 CAR-T	Shanghai Longyao Biotechnology Inc., Ltd.	NCT03207178	Sequential Infusion of Anti-CD19 and Anti-CD20 Chimeric Antigen Receptor(CAR) T Cells Against Relapsed and Refractory B-cell Lymphoma	Phase I	Recruiting	2017
	CD20/ CD19 CAR-T	Beijing Doing Biomedical Co., Ltd.	NCT03271515	Phase I Study of T Cells Expressing an Anti-CD19 and Anti-CD20 Bispecific Chimeric Receptor in Patients With B Cell Malignancies	Phase I	Not recruiting	2017
	CD20 CAR-T	Shanghai Unicar-Therapy Bio-medicine Technology Co.,Ltd	NCT03196830	Safety and Efficacy of Chimeric Antigen Receptor T Cell (CAR-T) Treating Relapse/ Refractory CD19/CD20/CD22/CD30 Positive Non-Hodgkin Lymphoma	Phase II	Recruiting	2017
	CD20 CAR-T + CD19 CAR-T	Shenzhen Geno-Immune Medical Institute	NCT03125577	Combination CAR-T Therapy of 4SCAR19 Plus 4SCAR20, 22, 38, and 123 Targeting Hematological Malignancies	Phase I/II	Recruiting	2017
	CD20 CAR-T	Fred Hutchinson Cancer Research Center	NCT03277729	A Phase I/II Study to Evaluate the Safety of Cellular Immunotherapy Using Autologous T Cells Engineered to Express a CD20-Specific Chimeric Antigen Receptor for Patients With Relapsed or Refractory B Cell Non-Hodgkin Lymphomas	Phase I/II	Recruiting	2017
	CD20/ CD19 CAR-T	Chinese PLA General Hospital	NCT03097770	Clinical Study of CD19/CD20 tanCAR T Cells in Relapsed and/or Chemotherapy Refractory B-cell Leukemias and Lymphomas	NA	Recruiting	2017

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
	CD20 CAR-T	Froedtert Hospital & Medical College of Wisconsin	NCT03019055	Phase 1 Study of Redirected Autologous T Cells Engineered to Contain an Anti CD19 and Anti CD20 scFv Coupled to CD3ζ and 4-1BB Signaling Domains in Patients With Relapsed and/or Refractory CD19 or CD20 Positive B Cell Malignancies	Phase I	Recruiting	2017
	CD20 CAR-T	Chinese PLA General Hospital	NCT03185494	Clinical Study of CD19/CD20 Tan CAR T Cells in Relapsed and/or Chemotherapy Refractory B-cell Leukemias and Lymphomas	Phase I/II	Recruiting	2017
Unlabeled antibodies							
B001	mAb	Shanghai Pharmaceuticals Holding Co., Ltd	NCT03332121	Phase Ia Study to Evaluate the Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Recombinant Humanized Anti-CD20 Monoclonal Antibody for Injection(B001)	Phase I	Not recruiting	2017
veltuzumab (hA20)	mAb	Immunomedics, Inc.	NCT00285428	A Phase I Study of Immunotherapy With hA20 Administered Once Weekly for 4 Consecutive Weeks in Patients With CD20+ Non-Hodgkin's Lymphoma	Phase I/II	Completed	2006
	mAb	Immunomedics, Inc.	NCT00546793	Phase I/II Study of Subcutaneously Administered Veltuzumab (hA20) in Patients With CD20+ Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia	Phase I/II	Completed	2007
	mAb	Immunomedics, Inc.	NCT00596804	A Phase I Study of Immunotherapy With hA20 Administered Once Weekly for 4 Consecutive Weeks in Patients With CD20+ Non- Hodgkin's Lymphoma	Phase I/II	Completed	2008
	mAb	Immunomedics, Inc.	NCT00989586	A Phase I/II Study of Veltuzumab (IMMU-106, hA20), a Humanized Anti-CD20 Monoclonal Antibody, Combined With Milatuzumab (IMMU-115, hLL1), a Humanized Anti-CD74 Monoclonal Antibody, in Relapsed and Refractory B-cell Non-Hodgkin's Lymphoma	Phase I/II	Completed	2009

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
	mAb	Immunomedics, Inc.	NCT01147393	Phase I/II Study of Combination Veltuzumab (Anti-CD20) and Fractionated 90Y- Epratuzumab (Anti-CD22) Radio immunotherapy in Patients With Follicular Lymphoma	Phase I/II	Terminated	2010
	mAb	Takeda	NCT01390545	a Randomized, Double Blind, Placebo Controlled, Multicenter, Multinational Phase II Dose Range Finding Trial in Subjects With Moderate to Severe Rheumatoid Arthritis Insufficiently Controlled With Either Methotrexate Alone or Methotrexate Plus Anti-tumour Necrosis Factor Biological Treatment, Comparing 3 Different Subcutaneous Dosages of Anti-CD20 Monoclonal Antibody Veltuzumab to Placebo as an add-on Therapy to Methotrexate	Phase II	Terminated	2011
	mAb	Immunomedics, Inc.	NCT01279707	Phase I/II Study Combining Humanized Anti-CD20 (Veltuzumab), Anti-CD22 (Epratuzumab) and Both Monoclonal Antibodies With Intensive Chemotherapy in Adults With Recurrent or Refractory B-precursor Acute Lymphoblastic Leukaemia (ALL)	Phase I/II	Not recruiting	2014
	mAb	Immunomedics, Inc.	NCT01101581	Phase I/II Study of Veltuzumab Combined With 90Y-Epratuzumab Tetraxetan in Patients With Relapsed/Refractory, Aggressive Non-Hodgkin's Lymphoma	Phase I/II	Not recruiting	2015
Ocaratuzumab (AME-133v, Y2469298)	mAb	Applied Molecular Evolution	NCT00354926	Open-Label, Multicenter, Phase 1/2 Dose-Escalation Study of AME-133v (LY 2469298), Administered Intravenously in Four Weekly Doses, in Subjects With CD20+ Follicular Relapsed or Refractory Non-Hodgkin's Lymphoma	Phase I	Completed	2006
	mAb	Mentrik Biotech, LLC	NCT01858181	A Phase I Study of Subcutaneous Ocaratuzumab (Fab- and Fc-engineered Anti-CD20 Monoclonal Antibody) in Patients With Previously Treated CD20+ B-Cell Malignancies	Phase I	Not recruiting	2014

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
PRO131921	mAb	Genentech	NCT00452127	An Open-Label, Multicenter, Phase I/II Trial of the Safety of Escalating Doses of PRO131921 in Patients With Relapsed or Refractory Indolent Non-Hodgkin's Lymphoma Who Have Been Treated With a Prior Rituximab-Containing Regimen	Phase I/II	Terminated	2007
Ocrelizumab	mAb	Roche	NCT02723071	A Study of Ocrelizumab in Participants With Follicular Non-Hodgkin's Lymphoma (NHL)	Phase I	Completed	2016
Ublituximab (LFB-R603)	mAb	Laboratoire français de Fractionnement et de Biotechnologies	NCT01098188	This study is designed to evaluate the safety, pharmacokinetics and preliminary efficacy of the anti-CD20 monoclonal antibody LFB-R603 in patients with relapsed or refractory B-cell chronic lymphocytic leukemia who have received at least one prior fludarabine-containing regimen.	Phase I	Completed	2010
	mAb	TG Therapeutics, Inc.	NCT01744912	Phase I/II Study of Ublituximab in Combination With Lenalidomide (Revlimid®) in Patients With B-Cell Lymphoid Malignancies Who Have Relapsed or Are Refractory After CD20 Directed Antibody Therapy	Phase I/II	Completed	2012
	mAb	TG Therapeutics, Inc.	NCT01647971	An Open Label Phase I/II Study of the Efficacy and Safety of Ublituximab in Patients With B-cell Non-Hodgkin Lymphoma Who Have Relapsed or Are Refractory After CD20 Directed Antibody Therapy	Phase I/II	Not recruiting	2012
	mAb	TG Therapeutics, Inc.	NCT02006485	A Phase I/Ib Study Evaluating the Efficacy and Safety of Ublituximab, a Third-Generation Anti-CD20 Monoclonal Antibody, in Combination With TGR-1202, a Novel PI3k Delta Inhibitor; and Ibrutinib or Bendamustin, in Patients With B-cell Malignancies.	Phase I	Recruiting	2013

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
	mAb	TG Therapeutics, Inc.	NCT02535286	Phase I/II Study of Pembrolizumab in Combination With TG-1101 (Ublituximab) and TGR-1202 in Patients With Relapsed-refractory Chronic Lymphocytic Leukemia (CLL) or Richter's Transformation (RT	Phase I/II	Recruiting	2015
	mAb	TG Therapeutics, Inc.	NCT03379051	Venetoclax in Combination With Ublituximab and Umbralisib (TGR-1202) in Patients With Relapsed or Refractory CLL/SLL	Phase I/II	Not recruiting	2017
CHO-H01	mAb	Cho Pharma Inc.	NCT03221348	A Phase I Open-label, Multiple Dose Study of CHO-H01 Administered Intravenously as a Single Agent to Subjects With Refractory or Relapsed Follicular Lymphoma	Phase I	Not recruiting	2017
TL011	RTX-Biosimilar	Teva Pharmaceutical industries Ltd.	NCT01205737	A Phase Ib, Double Blind RCT to Evaluate and Compare the PK, PD and Safety of MabThera® With TL011, in Combination With CHOP, in Subjects With CD20+ DLBCL	Phase I	Completed	2010
CMAB304	RTX-biosimilar	Shanghai CP Guojian Pharmaceutical Co.,Ltd.	NCT01459887	An Open-labeled, Multi-center, Randomized, Prospective Phase III Study Comparing CMAB304 in Combination With CHOP to CHOP Alone With CMAB304 Maintenance in Patients With DLBCL	Phase III	Completed	2011
MK-8808	RTX-biosimilar	Merck Sharp & Dohme Corp.	NCT01370694	An Open-Label, Single Arm Study of MK-8808 in Patients With Advanced CD20-Positive Follicular Lymphoma	Phase I	Terminated	2011
BCD-020	RTX-biosimilar	Biocad	NCT01701232	A Multicenter Open-label Randomized Study of BCD-020 (Rituximab, CJSC BIOCAD, Russia) Efficacy and Safety in Comparison With MabThera (F. Hoffmann-La Roche Ltd., Switzerland) in Monotherapy of CD20-positive Indolent Non-Hodgkin's Lymphoma	Phase III	Completed	2012

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
GP2013	RTX-bio-similar	Novartis Pharma-ceuticals	NCT01933516	Phase I Trial to Assess the Safety and Pharmacokinetics of GP2013 Monotherapy Administered Weekly in Japanese Patients With CD20 Positive Low Tumor Burden Indolent B-cell Non-Hodgkin's Lymphoma	Phase I	Completed	2013
			NCT01419665	A Randomized, Controlled, Double-Blind Phase III Trial to Compare the Efficacy, Safety and Pharmacokinetics of GP2013 vs. MabThera® in Patients With Previously Untreated, Advanced Stage Follicular Lymphoma	Phase III	Not recrui-ting	2011
PF-05280586	RTX-bio-similar	Pfizer	NCT02213263	A Phase 3, Randomized, Double-blind Study Of Pf-05280586 Versus Rituximab For The First-line Treatment Of Patients With Cd20-positive, Low Tumor Burden, Follicular Lymphoma	Phase III	Recruiting	2014
SCT400	RTX-bio-similar	Sinocelltech Ltd.	NCT02206308	A Phase I Dose Escalation Study of the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of SCT400, a Recombinant Chimeric Anti-CD20 Monoclonal Antibody in Patients With CD20+ B-cell Non-Hodgkin's Lymphoma.	Phase I	Completed	2014
			NCT02456207	A Phase II, Multi-center, Randomized and Open Study to Evaluate and Compare the PK, PD and Safety of SCT400 With Rituximab in Patients With CD20+ B-cell Non-Hodgkin's Lymphoma	Phase II	Unknown	2015
			NCT02772822	A Phase III, Multi-center, Randomized, Controlled Study to Compare the Efficiency and Safety of SCT400(Recombinant Chimeric Anti-CD20 Monoclonal Antibody, Experimental Drug) Plus CHOP Versus Rituximab Plus CHOP in Untreated CD20-positive DLBCL Patients	Phase III	Not recrui-ting	2016

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
HLX01	RTX-bio-similar	Shanghai Henlius Biotech	NCT02584920	Randomized, double-blind, parallel group study to compare PK and PD profiles between HLX01 and rituximab (MabThera®) in patients with CD20+ B-cell Lymphoma.	Phase I/II	Completed	2015
			NCT02787239	Multicenter, Randomized, Double-blind, Parallel, Phase III Clinical Study to Compare the Efficacy and Safety of Rituximab Biosimilar HLX01 and MabThera in Combination With CHOP, in Previously Untreated Subjects With CD20+ DLBCL	Phase III	Not recruiting	2016
			NCT03218072	A Phase Ia, Multi-centers, Open-label, Dose-escalation Clinical Study to Evaluate Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of HLX01 (a Potential Rituximab Biosimilar) in Patients With CD20-positive B-cell Lymphomas	Phase I	Completed	2017
IBI301	RTX-bio-similar	Innovent Biologics (Suzhou) Co. Ltd.	NCT02945215	A Multicenter, Randomized, Double-blinded, Parallel Controlled Study to Assess the Pharmacokinetics and Safety of Recombinant Human Murine Chimeric Anti CD20 Monoclonal Antibody Injection (IBI301) Compared to Rituximab Injection in CD20 Positive B Cell Lymphoma Patients	Phase I	Recruiting	2016
			NCT02867566	A Multicenter, Randomized, Double-blind, Controlled, Phase III Study to Evaluate the Efficacy and Safety of IBI301 (Recombinant Chimeric Anti-CD20 Monoclonal Antibody) in Combination With CHOP Regimen Versus Rituximab in Combination With CHOP Regimen in Treatment-naïve Patients With Diffuse Large B-cell Lymphoma (DLBCL)	Phase III	Recruiting	2016
ABT798	RTX-bio-similar	Amgen	NCT02747043	A Randomized, Double-Blind Study Evaluating the Efficacy, Safety and Immunogenicity of ABP 798 Compared With Rituximab in Subjects With CD20 Positive B-Cell Non-Hodgkin Lymphoma (NHL)	Phase III	Recruiting	2016

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
Other							
DI-Leu16-IL2	mAb (cytokine)	City of Hope Medical Center	NCT00720135	phase I trial is studying the side effects and best dose of fusion protein cytokine therapy when given after rituximab in treating patients with B-cell non-Hodgkin lymphoma.	Phase I	Completed	2008
		Alopexx Oncology, LL	NCT01874288	A Phase I/II Study of De-immunized DI-**Leu16**-IL2 Immunocytokine Administered Subcutaneously in Patients With B-cell Non-Hodgkin Lymphoma (NHL)	Phase I/II	Completed	2013
			NCT02151903	An Open-Label Extension Study of De-immunized DI-Leu16-IL2 Immunocytokine Administered in Patients With B-cell Non-Hodgkin Lymphoma (NHL)	Phase I/II	Completed	2014
B9E9-scFv-streptavidin	RIT	Fred Hutchinson Cancer Research Center	NCT02483000	Evaluation of Pretargeted Anti-CD20 Radio immunotherapy Combined With BEAM Chemotherapy and Autologous Stem Cell Transplantation for High-Risk B-Cell Malignancies	Phase I	Not recruiting	2015
ScFV-SLT-IA1	ADC	Molecular Templates, Inc.	NCT02556346	This study is intended to provide investigators and sponsor with the following information regarding the investigational new drug MT-3724 in patients with relapsed/refractory Chronic B-cell Lymphocytic Leukemia or Small Lymphocytic Lymphoma:	Phase I	Withdrawn	2015
pINGmmi-niCD20	Vaccine	Memorial Sloan Kettering Cancer Center	NCT00561756	Phase I Trial to Assess Safety and Immunogenicity of Xenogeneic CD20 DNA Vaccination With Patients With B-Cell Lymphoma	Phase I	Completed	2007

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
combination	Vaccine	Genitope Corporation	NCT00071955	A Phase II Study to Evaluate Safety and Efficacy of Specific Immunotherapy, Recombinant Idiotype Conjugated to KLH and GM-CSF Following the Anti-CD20 Antibody, Rituximab, in Previously Treated Patients With Follicular Non-Hodgkin's Lymphoma	Phase II	Completed	2003
		Dana-Farber Cancer Institute	NCT03361852	A Pilot Study of a Personalized Neoantigen Cancer Vaccine Following Front-Line Rituximab in Follicular Lymphoma	Phase I	Not recruiting	2017
		NantKwest, Inc.	NCT03169790	NANT Non-Hodgkin Lymphoma (NHL) Vaccine: Combination Immunotherapy in Subjects With Relapsed CD20-positive NHL	Phase I/II	Not recruiting	2017
		Mayo Clinic	NCT00438880	A Phase I/II Trial of CpG 7909, Rituximab Immunotherapy, and Y-90 Zevalin Radio immunotherapy for Patients With Previously Treated CD20+ Non-Hodgkin Lymphoma	Phase I/II	Completed	2007

CD20 compound	Format	Sponsor	Clinical study #	Clinical study design	Highest level of clinical investigation	Recruitment status	First date
RTX + NK cells	Ab + effector cells	Masonic Cancer Center, University of Minnesota	NCT00625729	MT2007-12 Allogeneic Natural Killer Cells With Rituximab in Patients With CD20 Positive Relapsed Non-Hodgkin Lymphoma or Chronic Lymphocytic Leukemia. Strategies to Increase Sensitivity of CLL Tumor Cells to Natural Killer Cell-Immune-Mediated Cytolysis	Phase I/II	Terminated	2008
		M.D. Anderson Cancer Center	NCT01619761	Natural Killer Cells In Allogeneic Cord Blood Transplantation	Phase I	Recruiting	2012
		M.D. Anderson Cancer Center	NCT02727803	Personalized Natural Killer (NK) Cell Therapy in Cord Blood Transplantation	Phase II	Recruiting	2016
		Masonic Cancer Center, University of Minnesota	NCT03019666	A Phase I Trial Testing NAM Expanded Haploidentical or Mismatched Related Donor Natural Killer (NK) Cells Followed by a Short Course of IL-2 for the Treatment of Relapsed/ Refractory Multiple Myeloma and Relapsed/ Refractory CD20+ Non-Hodgkin Lymphoma	Phase I	Recruiting	2017
RTX + T cells		PLA General Hospital	NCT01828008	The safety and efficacy of CD20 antibody usage followed by CIK transfusion in refractory and/or chemo resistant lymphomas.	Phase I/II	Unknown	2013
		National University Hospital, Singapore	NCT02315118	Pilot Study of Autologous T Lymphocytes With Antibody-Dependent Cell Cytotoxicity in Patients With CD20-Positive B-Cell Malignancies	Phase I/II	Recruiting	2014
		Unum Therapeutics Inc.	NCT03189836	Phase 1 Study of ACTR707, an Autologous T Cell Product, in Combination With Rituximab, in Subjects With Relapsed or Refractory CD20+ B Cell Lymphoma	Phase I	Recruiting	2017
RTX coated nano particles		Mayo Clinic	NCT03003546	A Phase I Trial of AR160 (Abraxane/Rituximab 160nm Nanoparticle) in Relapsed/Refractory B Cell Lymphomas Including Transformed Follicular Lymphoma	Phase I	Recruiting	2016

TABLE 3 Clinical investigations in Europe directed at Lymphoma and leukemia with CD20 mAb.

CD20 compound	Format	Sponsor	EudraCT Number:	Clinical study design	Phase	Recruitment status	First date
Bispecific antibodies							
FBTA05	CD3x-CD20	Medizinische Fakultaet der Technischen Universitaet Muenchen	2009-014641-88	Phase I/II dose-escalation study of the investigational trifunctional bispecific anti-CD20 x anti-CD3 antibody FBTA05 in combination with donor lymphocyte infusion (DLI) in patients with CD20 positive chronic lymphocytic leukemia (CLL), low and high grade non-Hodgkin´s lymphoma (NHL) after allogeneic stem cell transplantation.	Phase I	Prematurely Ended	2010
FBTA05	CD3x-CD20	Fresenius Biotech GmbH	2006-006694-24	Phase I/II dose-escalation study of the investigational trifunctional bispecific anti-CD20 x anti-CD3 antibody FBTA05 in relapsed or refractory chronic lymphocytic leukemia	Phase I/II	Prematurely Ended	2009
REGN1979	CD3x-CD20	Regeneron Pharmaceuticals, Inc	2015-001697-17	A Phase 1 Study to Assess Safety and Tolerability of REGN1979, an anti-CD20 x anti-CD3 bispecific monoclonal antibody, and REGN2810, an anti-programmed death-1 (PD-1) monoclonal antibody, in Patients with B-cell Malignancies	Phase I	ongoing	2015
Ublituximab	mAb	LFB BIOTECHNOLOGIES	2008-002601-40	Open, non-controlled, multicenter, first-in-man study using escalating doses of LFB-R603 in patients with advanced stage B-Chronic lymphocytic leukemia	Phase II	ongoing	2008

CD20 compound	Format	Sponsor	EudraCT Number:	Clinical study design	Phase	Recruitment status	First date
Naked antibodies							
Veltuzumab	mAb	Immunomedics, Inc.	2008-000434-47	A Phase I/II Study of Immunotherapy with Subcutaneous Administered Veltuzumab (hA20) in Patients with CD20+ Non-Hodgkin's Lymphoma or Chronic Lymphocytic Leukemia	Phase I/II	ongoing	2008
	mAb	Queen Mary Medical University of London	2008-002286-32	Phase I/II Study combining humanized anti-CD20 (veltuzumab), anti-CD22 (epratuzumab) and both monoclonal antibodies with chemotherapy in adults with recurrent B precursor acute lymphoblastic leukaemia (ALL)- MARALL	Phase I/II	completed	2009
2H7	mAb	Roche Products Limited	2004-004110-17	An open-label, multicenter, dose-escalating phase I/II trial of 3-weekly rhuMAb 2H7 in patients with follicular non-Hodgkin's lymphoma	Phase I/II	completed	2005
GP2013	RTX-Biosimilar	Hexal AG	2010-021184-32	A randomized, double-blind, controlled study to evaluate pharmacokinetics, pharmacodynamics, safety and efficacy of GP2013 and rituximab in patients with rheumatoid arthritis refractory or intolerant to standard DMARDs and one or up to three anti-TNF therapies	Phase III	completed	2010

CD20 compound	Format	Sponsor	EudraCT Number:	Clinical study design	Phase	Recruitment status	First date
CT-P10	RTX-Biosimilar	CELLTRION, Inc	2011-002813-12	A Phase 3, Randomized, Parallel-Group, Active-Controlled, Double-Blind Study to Compare the Efficacy and Safety of CT-P10 With MabThera, Each Administered in Combination With Cyclophosphamide, Vincristine, and Prednisone (CVP) in Patients With Advanced Follicular Lymphoma.	Phase III	Prematurely ended	2011
	RTX-Biosimilar	CELLTRION, Inc.	2013-004493-96	A Phase 1/3, Randomized, Parallel-Group, Active-Controlled, Double-Blind Study to Demonstrate Equivalence of Pharmacokinetics and Noninferiority of Efficacy for CT-P10 in Comparison With Rituxan, Each Administered in Combination With Cyclophosphamide, Vincristine, and Prednisone (CVP) in Patients With Advanced Follicular Lymphoma	Phase I/III	ongoing	2014
	RTX-Biosimilar	CELLTRION, Inc.	2014-005324-10	A Phase 3, Randomized, Parallel-Group, Active-Controlled, Double-Blind Study to Compare Efficacy and Safety between CT-P10 and Rituxan in Patients with Low Tumour Burden Follicular Lymphoma	Phase III	ongoing	2015
BI 695500	RTX-Biosimilar	Boehringer Ingelheim International mbH	2011-002908-33	A randomized, double-blind, multi-center, multi-national Phase III trial to compare efficacy and safety of BI 695500 plus chemotherapy versus rituximab plus chemotherapy in patients with untreated follicular non-Hodgkin's lymphoma.	Phase III	Terminated	2013
MabionCD20	RTX-Biosimilar	Mabion	2013-005506-56	Randomized, Parallel-group, Double-blind, Comparative Bioequivalence Trial of MabionCD20 (Mabion SA) Compared to MabThera (rituximab by Hoffman-La Roche) in Patients with Diffuse Large B-cell Lymphoma	Phase III	ongoing	2014

CD20 compound	Format	Sponsor	EudraCT Number:	Clinical study design	Phase	Recruitment status	First date
PF-05280586	RTX-Biosimilar	Pfizer Inc	2014-000132-41	A PHASE 3, RANDOMIZED, DOUBLE-BLIND STUDY OF PF-05280586 VERSUS RITUXIMAB FOR THE FIRST-LINE TREATMENT OF PATIENTS WITH CD20-POSITIVE, LOW TUMOR BURDEN, FOLLICULAR LYMPHOMA	Phase III	ongoing	2014
ABP 798	RTX-Biosimilar	Amgen Inc.	2013-005542-11	A Randomized, Double-Blind Study Evaluating the Efficacy, Safety and Immunogenicity of ABP 798 Compared with Rituximab in Subjects with CD20 Positive B-Cell Non-Hodgkin Lymphoma (NHL)	Phase III	ongoing	2014
BI 695500	RTX-Biosimilar	Boehringer Ingelheim International mbH	2014-004544-36	A Phase III, randomized, double-blind, multi-center, multi-national trial to evaluate efficacy and safety of BI 695500 versus rituximab as a first-line immunotherapy treatment in patients with low tumor burden follicular lymphoma	Phase III	completed	2015
SAIT101	RTX-Biosimilar	Archigen Biotech Limited	2016-001966-27	A Randomized, Double-blind, Multi-center, Multi-national Trial to Evaluate the Efficacy, Safety, and Immunogenicity of SAIT101 Versus Rituximab as a First-line Immunotherapy Treatment in Patients with Low Tumor Burden Follicular Lymphoma	Phase III	ongoing	2016
Other							
RTX	vaccine	Biogen Idec Inc	2004-004774-85	A Multicenter Study to Evaluate the Effect of Rituximab (IDEC-102) on Primary Humoral Response, Recall Response, and Maintenance of Acquired Immunity to Specific Antigens	unknown	completed	2005
RTX+L19Il2		Philogen S.p.A.	2014-001949-25	A Phase I/II study of the tumor-targeting human L19IL2 monoclonal antibody-cytokine fusion protein in combination with Rituximab in relapsed or refractory Diffuse Large B-cell Lymphoma (DLBCL)	phase I/II	ongoing	2015
RTX	RIT	Institut Jules Bordet	2011-005474-38	89Zr-Rituximab PET/CT-Imaging and Dosimetry and 90Y-Rituximab Radio immunotherapy in CD20+ B-Cell lymphoma	unknown	Ongoing	2012

TABLE 4 CD20 targeting antibodies in pre-clinical research.

CD20 compound	Clone(s)	Format	Highest level of investigation	Mechanism of Action reported	Summary	Reference
Monoclonal antibodies						
rituximab	C2B8	Chimeric IgG1	approved	CDC, ADCC, ADCP	Type I	[204]
ofatumumab	2F2	Human IgG1	approved	CDC, ADCC, ADCP	Type I	[84]
obinutuzumab	Bly1	Humanized IgG1-Fc optimized	approved	ADCC, ADCP, PCD	Type II, ADCC enhanced through glycol engineering, modified elbow angle for increased PCD	[86]
tositumomab	B1	Murine IgG2	Pre-clinical investigations	ADCC, ADCP, PCD	Type II, backbone of Bexxar	[31]
1.5.3	1.5.3	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	Type I/II, overlapping epitope with rituximab	[87]
BM-ca	BM-ca	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	Type I/II	[88]
Veltuzumab	hA20 2	IgG1	Phase 2	CDC, ADCC, ADCP	Type I, CDR grafting of RTX with 1aa difference in CDR3	[89]
BLX-300	C2B8	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP	ADCC enhanced through glycan engineering,	[90]
Ocaratuzumab (AME-133v, LY2469298)	2B8	IgG1-Fc optimized (P247I/E332I/A339Q)	Phase 1	CDC, ADCC, ADCP	ADCC enhanced through protein engineering	[116]
TGLA	TGLA	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP	Increased ADCC	[92]
113F	C2B8	IgG1/IgG3 chimer	Pre-clinical investigations	CDC, ADCC, ADCP	CDC enhanced	[93]
2H7-EFT +AE (PRO131921)	2H7	IgG1-S267E/H268F/ S324T + G326A/ I322E	Pre-clinical investigations	CDC, ADCC, ADCP	ADCC, ADCP and CDC enhanced through protein engineering	[94]
Triple	C2B8	IgG1-H57DE/ H102YK/L93NR	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	Enhanced CDC, ADCC through protein engineering	[95]

CD20 compound	Clone(s)	Format	Highest level of investigation	Mechanism of Action reported	Summary	Reference
RTX-EFTAE	C2B8	IgG1	Pre-clinical investigations	enhanced for both CDC and ADCC	ADCC enhanced through glycan engineering, CDC enhanced through protein engineering	[96]
2LM20-4	2H7	IgG1-variant (SMIP)	Pre-clinical investigations	CDC, ADCC, ADCP	IgG like molecule	[99]
TRU-015	2H7	IgG1 variant (SMIP)	Phase 1 studies	CDC, ADCC, ADCP	IgG like molecule	[100]
Ublituximab (TG-1101, LFB-R603, EMAB-6)	CAT-13.6E12	IgG1-Fc optimized	Phase 3	CDC, ADCC, ADCP, PCD	Type I	[216]
Ocrelizumab (PRO70769)	2H7	IgG1	Phase 3	CDC, ADCC, ADCP	Type I	[118]
Hu8E4	8E4	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	Increased CDC	[103]
5S	1-28	scFv-IgG	Pre-clinical investigations	CDC	cloned from IgM to IgG	[104]
hOUMB6 hOUMB3	OUMB6 OUMB3	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	overlapping epitope with ofatumumab	[105]
1K1791	1K1791	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	unique germline	[106]
HuMab-7D8	7D8	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP	Type I	[84]
HuMab-11B8	11B8	IgG1	Pre-clinical investigations	ADCC, ADCP, PCD	Type II	[84]
HuMab-2C6	2C6	IgG1	Pre-clinical investigations	CDC, ADCC, ADCP	Type I	[84]
Antibody conjugates						
Tositumumab-I131	B1	mIgG2a-I131	approved*	Radiolabel induced cell death + ADCC, APCD and PCD	Bexxar®	[217]
Ibritumomab-Y90	2B8	mIgG1-Y90	approved*	Radiolabel induced cell death + rituximab MoA	Zevalin®	[122]

CD20 compound	Clone(s)	Format	Highest level of investigation	Mechanism of Action reported	Summary	Reference
1F5-111In	1F5	mIgG2a-111In	Pre-clinical investigations	Radiolabel induced cell death		[123]
[211At]1F5-B10	1F5	mIgG2a-At211	Pre-clinical investigations	Radiolabel induced cell death		[119]
90Y-DOTA-RTX	C2B8	IgG1	Pre-clinical investigations	Radiolabel induced cell death + rituximab MoA		[122]
177Lu-CHX-A-DTPA-RTX	C2B8	IgG1	Pre-clinical investigations	Radiolabel induced cell death + rituximab MoA		[120]
89ZR-obinutuzumab 89ZR-ofatumumab 89ZR-rituximab 89ZR-tositumumomab	Bly-1, 2F2, C2B8, B1	IgG1,IgG1,IgG1, mIgG2	Pre-clinical investigations	Radiolabel induced cell death + antibody MoA		[121]
Technetium-99m rituxi-mab	C2B8	IgG1-99mTc	Pre-clinical investigations	Imaging only		[124]
CY7-obinutuzumab	Bly-1	IgG1	Pre-clinical investigations	Imaging only		[125]
CD20 IT	C2B8	IgG1-saporin-S6 IT	Pre-clinical investigations	conjugate induced cell death		[131]
OFA-MMAE	2F2	IgG1-MMAE	Pre-clinical investigations	conjugate induced cell death		[132]
RTX-MMAE	C2B8	IgG1-MMAE	Pre-clinical investigations	conjugate induced cell death		[133]
RTX-DOX	C2B8	IgG1-DOX	Pre-clinical investigations	conjugate induced cell death		[133]
CD20-SPP-DM1 CD20-MCC-DM1	C2B8	IgG1-DM1	Pre-clinical investigations	conjugate induced cell death		[134]
RTX-P-EPI	C2B8	IgG1-HPMA-epiru-bicin	Pre-clinical investigations	conjugate induced cell death		[135]
Bispecific antibodies						
C2B8(ScFvHL)4 2F2(ScTvHL)4	C2B8, 2F2	TetraMcAb	Pre-clinical	CDC, ADCC, ADCP, PCD		[147]

CD20 compound	Clone(s)	Format	Highest level of investigation	Mechanism of Action reported	Summary	Reference
11B8/2F2(ScFvHL)4-FC	11B8, 2F2	11B8/2F2(ScF-vHL)4-Fc	Pre-clinical investigations	CDC, ADCC, ADCP, PCD		[148]
CD20-HLA-DR DVD Ig	C2B8, HLA-DR	DVD Ig	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	DVD-Ig	[151]
Bs20x22	Rituximab/ HB22.7	$F(ab')_2xF(ab')_2$	Pre-clinical investigations	PCD	Chemical crosslinking	[152]
20-22	hA20 epra-tuzumab	HexAb	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	Hexavalent	[153]
20-20	hA20	HexAb	Pre-clinical investigations	CDC, ADCC, ADCP, PCD	Hexavalent	[153]
CD20xCD95	2H7, CD95	BS9520	Pre-clinical investigations	ADCC	Bivalent Fab arm	[155]
CD20xDOTA	2H7, C825	2H7-Fc-C825	Pre-clinical investigations			[156]
20-2b	hA20	IgG1-4x(IFN$\alpha 2\beta$)	Pre-clinical investigations			[160]
[(CD20)2xCD16]	7D8	Tribody	Pre-clinical investigations	T-cell directed kill		[161]
FcαR1xCD20	1F5, CD89	$F(ab')2xF(ab')_2$	Pre-clinical investigations		Chemical crosslinking	[163]
CD20-CD47	2B8, B6H12	DVD-Ig	Pre-clinical investigations	ADCP	DVD-Ig	[159]
r2820	2H7, 9.3	scFv	Pre-clinical investigations	T-cell directed kill	Bispecific single chain Fv	[166]
CD20Bi	RTX x OKT3	IgG1+mIgG2	phase I	T-cell directed kill + Fc mediated effector functions	heteroconjugation	[218]
FBTA05, Lymphomun, Bi20	TPA10 x 26II6	mIgG2a x rIgG2b	phase I/II	T-cell directed kill + Fc mediated effector functions	Quadroma	[176]

CD20 compound	Clone(s)	Format	Highest level of investigation	Mechanism of Action reported	Summary	Reference
REGN1979	REG30 x REG32	IgG1/4 chimeric	phase I/II	T-cell directed kill		[144]
XmAb-13676	unknown x sp34	Triple-F	phase I/II	T-cell directed kill		[198]
RG7828	2H7 x UCHT1	BsAb IgG1 generated using knob-into-holes technology	Phase I	T-cell directed kill		[141]
RG6026	Bly-1 x SP34	CD20 TCB	Pre-clinical investigations	T-cell directed kill		[158]
GEN3013	7D8 x SP34	DuoBody	Pre-clinical investigations	T-cell directed kill		
anti-CD20 x Anti-CD3 diabody	HIT3a x HI47	Diabody	Pre-clinical investigations	T-cell directed kill		[170]
CD3xCD20 bsAb	unknown x OKT3	IgG1-LHD-scFv fusion	Pre-clinical investigations	T-cell directed kill		[171]
20-3S	ha20 x OKT3	F(ab')2 x ScFv	Pre-clinical investigations	T-cell directed kill		[172]
BIS20x3	Bly-1 x CLB-T3/4	IgG1	Pre-clinical investigations	T-cell directed kill	Quadroma	[150]
halfbody	unknown x OKT3	half DVD-Ig	Pre-clinical investigations	T-cell directed kill	half DVD-Ig	[174]

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