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► To cite this version:

Etienne Crickx, Jean-Claude Weill, Claude-Agnès Reynaud, Matthieu Mahévas. Anti-CD20-mediated B-cell depletion in autoimmune diseases: successes, failures and future perspectives. *Kidney International*, 2020, 97, pp.885 - 893. 10.1016/j.kint.2019.12.025 . hal-03490269

HAL Id: hal-03490269

<https://hal.science/hal-03490269v1>

Submitted on 20 May 2022

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Anti-CD20-mediated B-cell depletion in auto-immune diseases: successes, failures and future perspectives.

Etienne Crickx^{1,2}, Jean-Claude Weill¹, Claude-Agnès Reynaud¹, and Matthieu Mahévas^{1,2}

1- Institut Necker Enfants Malades, INSERM U1151/CNRS UMS 8253, Université Paris Descartes, Sorbonne Paris Cité, 75993 Paris Cedex 14, France

2- Service de Médecine Interne, Centre national de référence des cytopénies auto-immunes de l'adulte, Hôpital Henri Mondor, 51 avenue du Maréchal de Lattre de Tassigny, Assistance Publique Hôpitaux de Paris, Université Paris Est Créteil, 94000 Créteil

Abstract

B-cell depletion with anti-CD20 monoclonal antibodies is widely used for the treatment of auto-immune diseases. In this review, we will discuss mechanisms contributing to success or failure of B-cell depletion therapy in antibody-mediated auto-immune diseases. We will also explain how key informations about disease pathogeny can be provided by the different outcomes observed after B-cell depletion therapy. These findings provide the basis for future innovative therapeutic strategies aiming at an optimized B cell and/or plasma cell depletion to increase long-term disease remission.

Running title: Anti-CD20-mediated B-cell depletion in auto-immune diseases

Address correspondence:

Pr Matthieu Mahévas, Service de médecine interne, Hôpital Henri-Mondor, Assistance Publique-Hôpitaux de Paris (AP-HP), 51 Avenue du Maréchal de Lattre de Tassigny, 94010 Créteil

e-mail: matthieu.mahevas@aphp.fr;

Phone +33149812076

Fax +33149812772

Keywords: cell survival, systemic lupus erythematosus, vasculitis

During the past 2 decades, B cell depletion with anti-CD20 monoclonal antibodies has constituted a major step forward in the treatment of antibody mediated auto-immune diseases. However, the proportion of patients achieving a long-term remission after treatment by anti-CD20 antibody varies greatly according to the disease and clinical context. The exact mechanisms explaining this apparent diversity are still a matter of debate, and intrinsically depends on the type of lymphoid cells involved in the auto-immune process and on the micro-environment and tissues in which autoreactive B-cells are generated. In this review, we will discuss demonstrated and putative mechanisms of resistance towards anti-CD20 therapy and propose future directions to improve long term remission rates.

Generation of autoreactive plasma cells in antibody-mediated auto-immune diseases

The humoral immune protection is conferred by plasma cells (PC) and memory B cells, which are mainly generated during the germinal center (GC) reaction,¹ the differentiation stage during which B cells improve the affinity of their surface immunoglobulin by somatic hypermutation and undergo class-switching. Some PC can also be generated through the extra-follicular pathway but are usually short-lived and low-mutated. T-B cooperation is required for B cell differentiation in both GC and extra-follicular pathways. This implies that autoreactive T cells also contribute to the break of tolerance occurring in auto-immune diseases. For clarity reasons, T cell role in resistance to RTX will not be discussed here in detail.

B cell differentiation in GC and extra-follicular pathways generates PC with variable lifespan, ranging from several days (short-lived PC) to several decades (long-lived PC). PC then migrates toward a CXCL12 gradient to the bone marrow, secondary lymphoid organs, or to inflamed tissues where they continuously secrete antibodies.²

The fate of B cells exposed to antigen in the context of infection or vaccination is reasonably well understood.³ Whether similar conclusions can be drawn in the context of auto-immune responses remains elusive. The direct or indirect pathogenic effect of autoantibodies has been well demonstrated in many auto-immune diseases, such as systemic lupus (SLE), rheumatoid arthritis (RA), immune thrombocytopenia (ITP), auto-immune hemolytic anemia (AIHA), ANCA-associated vasculitis, myasthenia gravis or auto-immune bullous dermatoses. Understanding of where and how pathogenic autoreactive PC are generated and what factors allow their survival is thus of paramount importance to treat antibody mediated diseases. Most autoantibody-secreting cells cloned from patients with such diseases are class-switched and exhibit extensive somatic hypermutation,⁴ suggesting a GC origin. Other studies also proposed that some low-mutated, autoreactive PC are generated through the extra-follicular pathway, particularly in SLE.⁵

Clinical outcomes after B cell depletion may help to delineate the contribution of short-lived and long-lived plasma cells to auto-immunity.

Rituximab (RTX) is a human/murine chimeric monoclonal antibody (mAb) that specifically targets the transmembrane protein CD20. CD20 acts as a store-operated calcium channel⁶ and is exclusively expressed by B cells, from the pre-B to the memory stages, but not by PC (Figure 1). Upon binding on target cells, RTX induces a spatial reorganization of CD20 molecules in lipid rafts and activates complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC).⁷ RTX achieves rapid and almost complete B-cell depletion in peripheral blood, with minimal numbers of peripheral CD19⁺ cells being detectable in most patients.^{8,9}

RTX efficacy is thought to result mainly from its impact on the generation of short-lived antibody secreting cells (plasmablasts) through depletion of their direct precursors, including activated and GC B cells.¹⁰ Other B cell functions such as antigen presentation and cytokine secretion have also been proposed as additional mechanisms accounting for RTX clinical efficacy.¹¹ However, suppressing the generation of plasmablasts can explain the clinical effect in most B-cell mediated diseases in which RTX is effective, and is further confirmed by the progressive disappearance of pathogenic antibodies observed along clinical remissions such as ANCA or anti-desmoglein antibodies.^{12,13} This contrasts with the general maintenance of stable titers of protective vaccinal antibodies for tetanus or diphtheria after anti-CD20 exposure.^{14,15} These antibodies are thought to be produced by long-lived PC, mainly contained in the CD19-CD138+ fraction of the bone marrow.^{16,17} Moreover, CD19⁻ CD138⁺ CD38⁺ PC are enriched in the bone marrow after B cell depletion with RTX¹⁶ and protective antibody titers are maintained in patients undergoing anti-CD19 chimeric antigen receptor (CAR) T cell therapy for B cell malignancy.¹⁸ Beyond their role in protective humoral immunity, it has been shown that long-lived PC can also contribute to auto-immune responses, thus explaining some RTX failures where complete peripheral B cell depletion contrasts with the persistence of detectable pathogenic autoantibodies.¹⁷

We can speculate that the diversity of clinical responses obtained with RTX reflects the heterogeneity in the production of PC. Understanding why long-lived autoreactive PC are generated or not in different contexts is a fascinating question. Inferring the contributions of short-lived versus long-lived PC in auto-immunity would help to develop innovative strategies to specifically target these cells.

Auto-reactive long-lived plasma cells in spleen and tissues may explain primary failure of RTX.

We analyzed the spleen of ITP and AIHA patients who failed to achieve a response despite a complete peripheral B-cell depletion.¹⁹ We showed that CD19+CD20-CD27highCD38high PC were the main residual CD19+ fraction in the spleen of these patients 1 to 6 months after RTX. More surprisingly, the transcriptomic analysis at bulk and single-cell level of these PC reveals that B-cell depletion had paradoxically induced the settlement of a unique population of splenic long-lived PC, some of them being autoreactive, while such cells were almost absent in the spleen of RTX-untreated patients with ITP or AIHA. The presence of this long-lived PC population could explain the RTX treatment failure for most patients and their subsequent response to splenectomy (Figure 2). We recently demonstrated by using a fate mapping mouse model that B-cell activating factor (BAFF) and CD4+ T-cells played a major role in this process, and that combining anti-CD20 with anti-BAFF or with anti-CD4 treatments reduced the number of splenic PC, opening therapeutic perspectives for ITP.²⁰ It is now well recognized that PC survival is not intrinsic and depends on cytokines and interactions provided by the cellular microenvironment.²¹ BAFF levels immediately rise in all patients treated with RTX and stay elevated during the time of B cell depletion, probably because of an absence of consumption by B cells, and by positive regulation of BAFF transcription²².

Thus, by modifying the PC microenvironment, B-cell depletion therapy induced a particular maturation of PC that contributed to the perpetuation of auto-immunity. B-cell depletion therapy may also create a particular dependence to the cellular and cytokine microenvironment that may explain primary failure of RTX in some patients. To prevent the emergence of such splenic long-lived PC by combining RTX and belimumab appears a very promising strategy and is currently tested in SLE and ITP.

Beside the spleen, autoreactive PC may infiltrate target organs such as kidneys,²³ central nervous system,²⁴ synovium,²⁵ or salivary glands.²⁶ We also observed kidney PC with a long-lived program which resisted immunosuppressive therapy in lupus nephritis (Crickx, Eular 2019, manuscript in preparation). These results suggested that modifications of the microenvironment induced by conventional immunosuppressive agents may favor the emergence of long-lived PC in inflamed tissues as well.

Rituximab resistant B cells in peripheral blood and tissues in humans

Two main schedules for RTX administration are being used in auto-immune diseases (either 2 doses of 1000mg or 4 doses of 375 mg/m²), resulting in similar levels of B-cell depletion. The advent of highly sensitive flow cytometry analyses has enabled the detection and the characterization of circulating residual CD19+ cells in peripheral blood in the weeks following RTX administration, particularly in patients with RA.^{27,28} Despite deep analysis of large cohorts, the persistence of residual CD19+ cells in peripheral blood does not correctly predict clinical outcome.²⁹⁻³¹ In RA, these CD19+CD20- cells are mainly CD27+CD38^{high}Ki67+IgA⁺ plasmablasts, originating from resident mucosal B-cells.³² BCR repertoire analysis from total blood of RA patients one month after RTX have shown that circulating CD20- plasmablasts clones are not affected by B-cell depletion therapy.³³ So far, their exact contribution to the pathogeny remains unknown.

Although residual circulating CD19+ B cells remains anecdotal in most auto-immune diseases, incomplete B-cell depletion has been described in SLE and could probably participate to the variable efficacy of RTX in this disease.³⁴⁻³⁶ Non-exclusive explanations for this phenomenon have been proposed, including excess of immune complexes interfering with effector cells, deficiency in the complement pathway, or antibodies directed against RTX.^{34,35,37}

Only few studies have analyzed lymphoid organs or tissues after RTX (Table 1).^{19,38-52} The therapeutic splenectomy performed in ITP and AIHA offers the unique opportunity to study B-cell compartment at different time frames after RTX. Residual CD19⁺ cells represented 0.06 to 1.45% of lymphoid splenocytes in patients splenectomized up to 6 months after 2 g of RTX, and were mainly composed of PC and non-dividing memory B-cells.⁴³ No GC or marginal zone resistant B cells were observed, contrasting with results obtained with several mouse strains.⁵³⁻⁵⁶ These findings highlight that mouse models of anti-CD20 depletion cannot be extrapolated to humans, probably because of different Fc-receptors and effector cells mobilization.⁵⁷

Several groups have described the persistence of CD19+CD27+IgD- memory B cells in lymph nodes from patients obtained one month after a single dose of RTX (500mg) given for prevention of antibody-mediated rejection.^{46,47} A recent analysis of inguinal lymph nodes from RA patients obtained 4 weeks after 1g of RTX showed the persistence of several B-cell follicular structures and documented the persistence of CD27+IgD- memory cells.⁴⁸ Residual B-cells have also been found in the bone marrow and synovium from patients with RA,^{40,51,52} and salivary glands of patients with Sjögren syndrome.^{49,51} Importantly, none of these residual B cells in peripheral blood or tissues had detectable surface expression of CD20, in part due to epitope masking. This could have led to an underestimation of the exact number of residual B cells after RTX, also because other B cell antigens such as CD19 are downregulated after RTX exposure.^{58,59} Antigen modulation in response to RTX binding has been well described and is achieved through CD20 internalization⁶⁰⁻⁶² and trogocytosis.⁶³⁻⁶⁶ Both mechanisms are mediated by FcγR and eventually lead to the lack of CD20 surface expression, thus allowing

B cell survival. However, whether B cell survival through antigen modulation is a stochastic process or favors a particular B cell subset remains an open question.

Steady-state mucosal IgA+ plasmablasts are not abrogated by B-cell depletion, suggesting that residual activated B cells may persist in lamina propria, as well as long-lived resident IgA PCs. No correlation has been demonstrated between rheumatoid arthritis activity and the presence of circulating IgA plasmablasts. A precise assessment of the impact of B cell depletion on mucosal B cell compartment remains challenging because of limited availability of samples in human.^{32,67}

In summary, it has become clear that B-cell depletion is highly effective in peripheral blood and usually last for up to 6 months in most patients. However, residual CD20 negative PC, but also memory B cells in limited numbers remain detectable in lymphoid organs and tissues and could play a role in disease control (Figure 2).

Disease relapses during B-cell reconstitution

Reconstitution of peripheral blood (PB) B cells after B-cell depletion with RTX (RTX) mimics B-cell ontogeny and begins between 6 and 12 months after the last infusion. B-cell reconstitution has been widely described in RA,^{9,68–71} SLE,^{72,73} Sjögren syndrome,⁷⁴ ANCA-associated vasculitis,^{75,76} systemic sclerosis⁷⁷ or idiopathic nephrotic syndrome.⁷⁸ Numerous efforts have been made to correlate B-cell repletion kinetics in peripheral blood and clinical relapses but have largely failed to provide reliable predictive markers, probably because analysis time points in most studies were not enough accurate to capture and analyze the clinical relapse and the peripheral blood repletion at the same time, and because of confounding treatments such as steroid therapy. The main reason could also be that levels of circulating B cells does not closely correlate with the degree of organ repopulation during auto-immune diseases flares/relapses. In this regard, we have very few data regarding the B-cell subpopulations in tissues after B-cell depletion. The spleen of ITP patients who achieved a complete response after RTX but relapsed during B cell reconstitution is the site of intense GC expansion and memory B-cell proliferation (Crickx, manuscript in preparation). It remains unknown if disease relapses after RTX arise from newly generated B cells and/or RTX-resistant “residual” memory B cells (Figure 2). Whatever the scenario, autoreactive memory T-cells that were not directly targeted by RTX probably contribute to the relapse by providing support to activated B cells, thus allowing the generation of new autoreactive PC. In line with this, repeated courses of rituximab (usually every 6 months) have been used in ANCA-associated vasculitis and rheumatoid arthritis^{79,80} to prevent the reemergence of B cells.

Interestingly, increased BAFF levels can influence the threshold of B cell selection, resulting in the survival of newly emergent autoreactive B cells that otherwise would have died by apoptosis. A recent elegant work has demonstrated that BCR ligands delivering a TLR9 agonist (DNA-containing antigens) induced an initial proliferative burst in B cells.⁸¹ This initial phase was followed by apoptotic death and could be rescued by providing BAFF. By analogy, high levels of BAFF induced by B cell depletion could promote an increase in autoreactive B cell clones among the repertoire originating from B cell reconstitution. This hypothesis is supported by studies showing that negative selection of high affinity DNA-reactive B cells was impaired by increased levels of BAFF during B cell depletion in a model of auto-immune mice.^{82,83} Whether this “override” mechanism exists in humans has still to be

demonstrated. These paradoxical effects of RTX provide another rationale to set up combination therapies associating RTX and Belimumab in SLE and auto-immune diseases.⁸⁴

Beside their role in pathogenic humoral immunity, B-cells can produce inflammatory and regulatory cytokines. These regulative properties of B cells have been reviewed elsewhere.⁸⁵ Seminal descriptions have demonstrated a central role for regulatory B cells in controlling experimental auto-immune encephalomyelitis remission through IL-10 production.⁸⁶ Interestingly, some B cells were shown to secrete IL-6 and contributed to disease pathogenesis in the same mouse model,¹¹ suggesting that both regulatory and inflammatory B cells can be targeted by anti-CD20. In mice, CD20- PC can also produce IL-10 and IL-35 and have regulatory properties.⁸⁷ In human, no consensual surface phenotypic marker can identify regulatory B cells or PC. Early descriptions of defective IL-10 producing transitional B cells in SLE⁸⁸ has led many studies to use transitional CD24^{high}CD38^{high} B cells as a marker of regulatory B cells.⁸⁹ However, it has become clear that almost all B cells can secrete IL-10 upon various in vitro stimuli.⁹⁰ Thus, the exact contribution of cytokine-producing B cells in the clinical context of B-cell depletion and repopulation remains to be formally addressed.

New approaches to target resistant B-cells

B-cell and PC depletion can be achieved through several mechanisms, including chimeric or humanized monoclonal antibodies targeting surface molecules (CD19, CD20, CD22, CD38), inhibition of key survival factors (BAFF or APRIL), inhibition of BCR signaling pathway (PI3K, BTK, and SYK inhibitors), or yet disruption of homeostatic mechanisms (proteasome inhibitors) or anti-CD38 MoAbs.

The exact contribution of anti-CD20 resistant B cells to failures or relapses after B-cell depletion is not well established. It is tempting to postulate that a better depletion would improve clinical outcomes, but this remains to be demonstrated. New generations of humanized (ocrelizumab, veltuzumab, and obinutuzumab) or fully human (ofatumumab) anti-CD20 mabs have been developed to increase complement-dependent cytotoxicity (CDC) and/or ADCC while limiting immunogenicity.⁹¹ These agents are currently in development in various auto-immune diseases (Table 2). Ocrelizumab has demonstrated its efficacy in multiple sclerosis (MS) and was the first approved monoclonal antibody for secondary progressive MS.⁹² Obinutuzumab has been developed to increase ADCC by enhancing binding affinity for the FcγRIII receptor expressed on immune effector cells, and has met its primary endpoint in lupus nephritis in combination with standard of care (NCT02550652).⁹³ Recently, it was announced that two studies comparing Ofatumumab to Teriflunomide met their primary endpoint in MS (NCT02792218 and NCT02792231). These promising new agents are thus probably interesting to achieve a better B cell depletion and immunogenic reactions as seen in SLE. To date, there is no comparative trial of RTX against these newer agents and until now, no data are available regarding the B-cell depletion they induce in tissues.

Anti-CD22 monoclonal antibody epratuzumab was designed to perturb the B cell receptor signaling complex and modulate B cell activity without B cell depletion, but failed to demonstrate efficacy in a randomized trial in SLE.⁹⁴ Targeting CD19 allows for B cell depletion, but could also impact a significant proportion of plasmablasts and PC expressing this marker, unlike CD20. Many anti-CD19 antibodies are currently in development,

including humanized antibodies (such as MEDI 551), “bi-specific T cell engagers”, and antibody-drug conjugates (such as inebilizumab or blinatumomab). Anti-CD19-CAR-T cells are another option that was recently shown to be effective in treating murine lupus,⁹⁵ and a study is being conducted to assess anti-CD19-CAR-T cells safety and efficacy in patients with SLE (NCT03030976). Severe adverse events such as cytokine-release syndrome (CRS) and neurotoxicity limits their indication in non malignant diseases, but CAR T cells could be an attractive option for multirefractory patients.

BAFF and APRIL are two key B-cell cytokines signaling through several receptors. While transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA) are shared by both cytokines, BAFF can also signal through BAFF receptor (BAFF-R), and APRIL can bind to heparan sulfate proteoglycans. TACI and BCMA are mainly expressed on PC and BAFF-R is mainly expressed on immature and naïve B cells. Two anti-BAFF Abs (tabalumab and belimumab) and one BAFF inhibitor (blisibimod) have been developed, and belimumab has been the only approved biological agent for the treatment of SLE to date, demonstrating the importance of this cytokine in the disease pathogeny. Longitudinal analysis of SLE patients treated with belimumab showed a decrease in naïve and transitional B cells, with no impact on switched memory B-cells and circulating plasmablasts, suggesting that belimumab may impact extrafollicular response.^{96,97} As previously stated, promising trials assessing combination therapy of RTX with belimumab with multiple underlying rationales⁹⁸ are under evaluation in SLE (NCT03312907) and in ITP (NCT03154385), after preliminary results obtained in open label studies.⁹⁹

Molecules inhibiting B-cell Receptor signaling (BTK, PI3K, SYK) have been successfully developed for malignant lymphoma and have shown promising effects in experimental models of SLE and RA.^{100,101} Some of them are currently being evaluated in various auto-immunes diseases (SLE, NCT03878303, NCT02537028; RA, NCT02626026). SYK inhibitors target key initiating kinase in the BCR signaling cascade and BAFF-R signaling,¹⁰² but also inhibit macrophages phagocytosis. SYK inhibitor fostamatinib has shown interesting results in ITP,¹⁰³ and is now under development in auto-immune hemolytic anemia (NCT03764618).

Targeting auto-reactive long-lived plasma-cells.

The evidence that non-dividing long-lived CD20- PC contributed to the failure of conventional immunosuppressant including cyclophosphamide has been well emphasized in lupus prone mouse model.¹⁰⁴ Based on these observations, several therapeutic strategies have been developed using bortezomib, an inhibitor of the 20S subunit of the proteasome, approved for the treatment of multiple myeloma, which eliminates long-lived PC by disruption of the unfolded protein response. Treatment with bortezomib has been shown to deplete PC producing antibodies to double-stranded DNA, improved glomerulonephritis and prolonged survival of two mouse strains with lupus-like disease (NZB/W F1, MRL/Lpr mice).¹⁰⁵ The efficacy of this strategy using dexamethasone (Dex) combined with bortezomib (Btz) for targeting autoreactive long-lived PC was then reported in patients with refractory SLE,¹⁰⁶ multirefractory AIHA¹⁰⁷ and refractory thrombotic thrombocytopenic purpura.¹⁰⁸ Hence, the elimination of autoreactive PC by proteasome inhibitors might represent a new treatment

strategy for antibody-mediated diseases, but the delay of action (no response before several weeks of treatment) and the risk of peripheral neuropathy limit its use.

Splenic and bone marrow PC express high levels of CD38. Anti-CD38 monoclonal antibody was shown to be effective for depletion of malignant PC in multiple myeloma, and appears to be an attractive option for patients with refractory auto-immune diseases. To date, no data on the efficacy of this agent in patients with auto-immune diseases are available, except in the particular situation of post-allogenic AIHA.¹⁰⁹ The longevity of long-lived PC is not cell-intrinsic but largely depends on signals provided by the splenic microenvironment, including cellular niche and several key-cytokines such as APRIL, BAFF, IL6, and CXCL12.²¹ Atacicept is a fully human molecule encoding the TACI ligand binding extracellular domain fused to the Fc domain of human IgG, and blocks receptor binding of APRIL and BAFF. This treatment could represent an interesting approach in patients with SLE.¹¹⁰

Conclusion

B cell depletion therapy with RTX represented a major breakthrough in the treatment of many autoantibody mediated diseases. From an immunological perspective, cases of complete responses to anti-CD20 reveal that short-lived antibody secreting cells can be the main contributors to disease pathogeny, while primary failures suggest involvement of long-lived PC in inflamed tissues and/or in bone marrow. T cells resist RTX and play a central role in antibody-mediated diseases. Whether follicular helper T cells are drivers or only accompany relapses occurring after RTX remains to be clarified. Additional work is needed to improve our comprehension of underlining and probably complex mechanisms leading to anti-CD20 treatment failures, to allow the development of personalized and effective therapies.

Disclosure: The author report no conflict of interest.

Table 1 : Presence of residual B cells in various tissues after RTX administration for auto-immune diseases

Table 2. Ongoing clinical trials to target B cells in auto-immune diseases.

Figure 1: B cell differentiation in bone marrow and periphery

Figure 2: Mechanisms of failure and relapses after RTX

RTX depletes peripheral B cells for 6 months, but spares CD20- plasma cells and few B cells in tissues. Some primary failures of RTX can be explained by persistence of plasma cells secreting pathogenic autoantibodies. Most patients responding to RTX eventually relapse during B cell reconstitution, either after antigen re-encounter by some rituximab-resistant memory B cells, or after newly generated B cells reinitiate an auto-immune response favored by a tolerance breakdown occurring during B cell lymphopoiesis.

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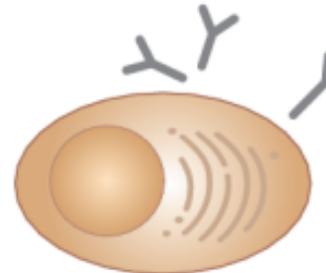
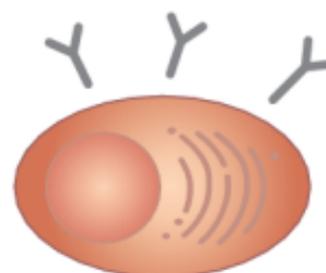
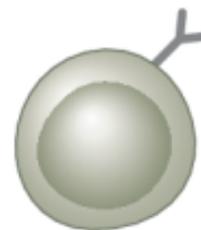
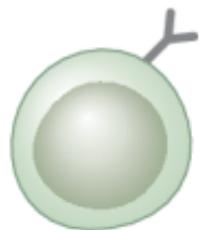
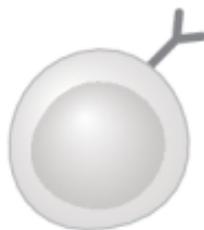
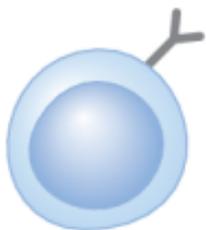
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Bone marrow

Periphery

Bone marrow



Pro-B cell

Pre-B cell

Transitional
B cell

Naïve
B cell

Germinal center
B cell

Memory
B cell

Plasmablast

Plasma
cell

CD20 expression



CD19 expression



CD22 expression

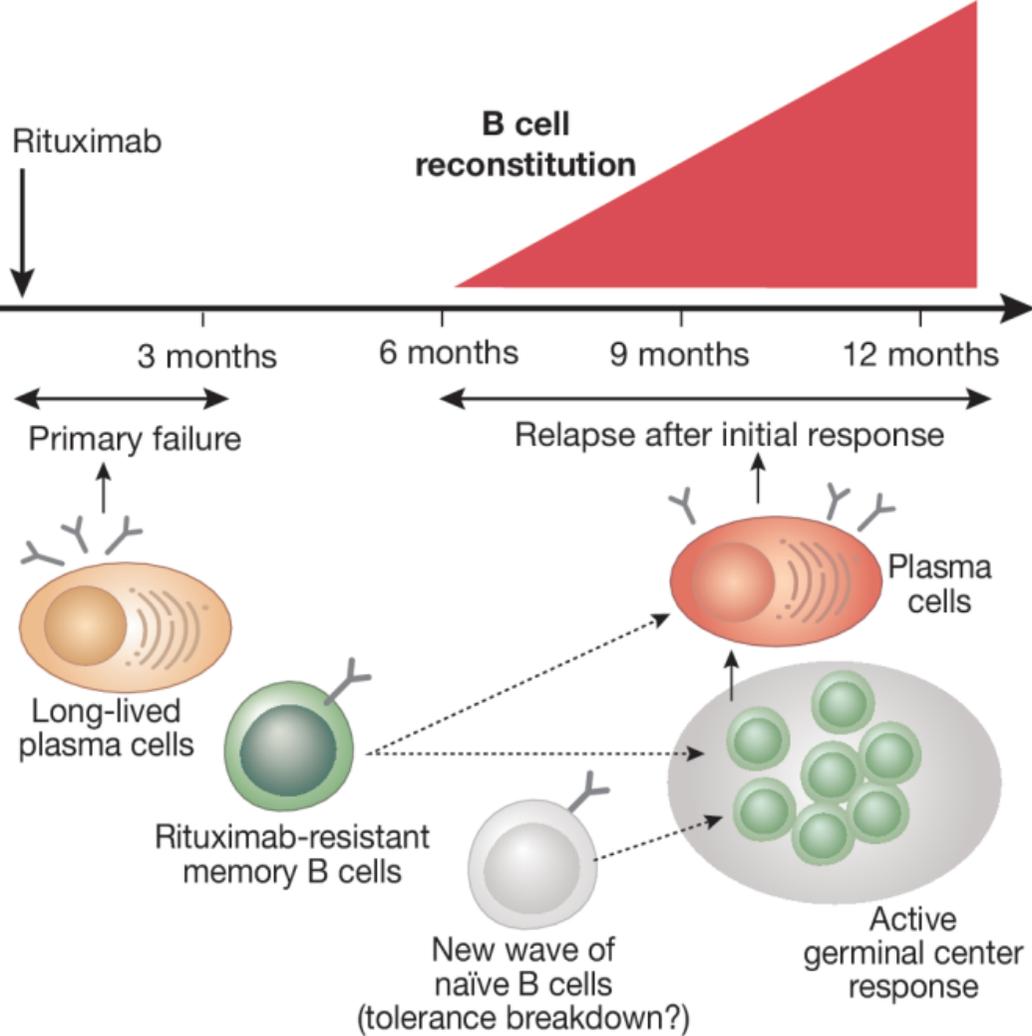


CD27 expression



CD38 expression





Organs	Diseases	RTX regimen	Other therapies	Delay between last RTX infusion and analysis	Number of patients	Residual B cell populations	References
Bone marrow	ITP	4 weekly doses of 375mg/m ²		3 months	1	None	38
	RA	2 doses of 1000mg (2 weeks apart)		3 months	6	Presence of residual CD19+ cells (0.1 to 3.25% in the lymphoid gate), mainly precursors and immature B cells.	39
	RA	2 doses of 1000mg (2 weeks apart)		3 months	25	8 of 25 patients (32%) had residual CD19+ cells (median 2.21%). No CD20+ cells	40
	RA	2 doses of 1000mg (2 weeks apart)		3 months	8	No significant reduction in CD19+ cells numbers	41
	RA	2 doses of 1000mg (2 weeks apart)		1 to 3 months	24	Presence of immature and/or transitional B cells (CD38++CD24++) and CD27+IgD- B cells, while IgD+ cells were completely depleted.	42
Spleen	ITP	4 weekly doses of 375mg/m ²		3 months	1	None	38
	ITP	4 weekly doses of 375mg/m ²	lvlg (4/15)	3 to 6 months	15	CD19+ cells represented 0,06 to 1% of CD45+ cells, including 75% plasma cells. Remaining CD19+ cells were mostly CD27+IgD- memory B cells. No residual germinal center.	19
	AIHA	2 doses of 1000mg (2 weeks apart)	High dose steroids	4 to 5 months	4	CD19+ cells represented 1,45% of CD45+ cells, including 70% plasma cells. Remaining CD19+ cells were mostly CD27+IgD- memory B cells. No residual germinal center.	43
	ITP	4 weekly doses of 375mg/m ² or 2 doses of 1000mg (2 weeks apart)	lvlg (8/10) Steroids (1/10)	3 to 15 months	10	CD19+ cells represented 5,1% of splenocytes, but some patients had already reconstituted their B cells.	44
	Prevention or treatment of antibody-mediated rejection	Single dose of 375 mg/m ²	Tacrolimus, mycophenolate mofetil, steroids, lvlg and rATG	2 to 45 days	7	Presence of few residual CD20+CD79a+ cells in some patients	45
Lymph nodes	Prevention of antibody-mediated rejection	Single dose of 500 mg	Tacrolimus, mycophenolate mofetil, steroids	1 month	5	20% of lymphoid cells were CD19+ (but not CD20+), mainly CD27+IgD- memory B cells	46
	Prevention of antibody-mediated rejection	Single dose of 375 mg/m ²	Tacrolimus, mycophenolate mofetil, steroids, lvlg	1 month	4	35% of lymphoid cells were CD19+ (but not CD20+), mainly CD27+IgD- memory B cells	47
	RA	2 doses of 1000mg (2 weeks apart)		1 month	14	10% of lymphoid cells were CD19+, mainly CD27+IgD- memory B cells	48
Salivary glands	Sjögren syndrom	2 doses of 1000mg (2 weeks apart) then 1000mg every 6 months		6 months	19	No CD20+ B cells. Ectopic germinal centers reduced from 53 to 5%.	49
	Sjögren syndrom	2 weekly doses of 375mg/m ²		4 months	8	No residual B cells	50
Synovial tissue	RA	2 doses of 1000mg (2 weeks apart)		1 month	17	Presence of residual CD22+ B cells	51
	RA	2 doses of 1000mg (2 weeks apart)		2 to 6 months	17	Presence of CD20+ B cells in 2/17 patients (22%) and of CD79a+ B cells in all patients	40
	RA	2 doses of 1000mg (2 weeks apart)		1 and 4 months	16	Presence of residual CD22+ B cells	52

ITP: immune thrombocytopenia, RA: rheumatoid arthritis, RTX: rituximab, TdT: terminal deoxynucleotidyl transferase

Table 1 : Presence of residual B cells in various tissues after RTX administration for auto-immune diseases

Molecule	Target	Condition	ClinicalTrials.gov identifier
Ocrelizumab	Humanized anti-CD20	SLE Multiple sclerosis	NCT00626197 NCT03344094, NCT03523858 NCT04035005
Obinutuzumab	Glycoengineered humanized anti-CD20	SLE	NCT02550652
Ofatumumab	Fully humanized anti-CD20	Multiple sclerosis Pemphigus Vulgaris Rheumatoid arthritis	NCT02792218, NCT02792231 NCT01920477 NCT00611455
Combination therapy with Rituximab and belimumab	Chimeric anti-CD20 and fully humanized anti-BAFF	ITP SLE ANCA Vasculitis Primary membranous nephropathy	NCT03154385 NCT02260934, NCT03312907 NCT03967925 NCT03949855
MEDI 551	Anti-CD19	Neuromyelitis Optica Multiple sclerosis	NCT02200770 NCT01585766
VAY736	Anti-BAFF-receptor	Rheumatoid arthritis SLE Sjogren's Syndrome	NCT03574545 NCT03656562 NCT02962895
Telitacicept (RC18)	TACI-Antibody fusion protein	SLE Sjogren's Syndrome	NCT02885610, NCT04082416 NCT04078386
CAR-T cells	Anti-CD19 CAR T cells	SLE	NCT03030976
Ixazomib	Proteasome inhibitor	Multiple sclerosis SLE	NCT03783416 NCT02176486
Bortezomib	Proteasome inhibitor	ITP AIHA Autoimmune encephalitis Antibody mediated autoimmune diseases	NCT03013114, NCT03443570 NCT04083014 NCT03993262 NCT02102594
Evobrutinib	BTK inhibitor	Multiple sclerosis	NCT04032171
INCB050465	PI3K inhibitor	AIHA	NCT03538041

Table 2. Ongoing clinical trials to target B cells in auto-immune diseases.