CD20 monoclonal antibodies for the treatment of multiple sclerosis: up-to-date

Abstract

Introduction: Featuring demyelination and axonal degeneration, multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system representing a prominent cause of disability in young adults. The recently established therapeutic targeting of B cells in MS patients using CD20 monoclonal antibodies (CD20-mAbs) not only profoundly suppresses inflammatory disease activity, but also materializes as the first treatment approach against disability accumulation in a subset of patients with primary progressive MS.

Areas covered: We will review current concepts regarding the multifaceted immunopathology of B cells in MS as well as results of clinical trials with CD20-mAbs, from the murine-human chimeras rituximab and ublituximab to their increasingly humanized counterparts ocrelizumab and ofatumumab. We conducted a literature search using the databases PubMed, clinicaltrials.gov and clinicaltrialsregister.eu. Randomized controlled studies emphasized the effectiveness of these mAbs in reducing MS disease progression against an acceptable risk profile. Lastly, we will turn to outstanding questions regarding anti-CD20 therapy in MS.

Expert opinion: CD20-mAbs constitute an attractive option for patients with relapsing or even primary progressive MS. Nevertheless, questions regarding optimal dose, long-term safety, maintenance regimens, B cell response markers, as well as their impact on disease progression, remain.

Keywords: CD20 monoclonal antibody, B-cell therapy, multiple sclerosis, ofatumumab, ocrelizumab, rituximab, ublituximab

1. Introduction

During the last two decades, CD20 monoclonal antibody mediated B cell depletion attracted increasing attention in the field of multiple sclerosis (MS) therapy. Notwithstanding the possibility of inducing experimental autoimmune encephalomyelitis (EAE), the animal analogue of MS, via adoptive transfer of myelin-sensitized T cells in rodents, converging lines of evidence pointed to a pathophysiological role of B cells as well. These began with the discovery of the presence of oligoclonal bands (OCB) and, later, activated B cells and plasmablasts in cerebrospinal fluid (CSF). Moreover, autopsy studies from MS patients revealed complement and antibody deposition within demyelinating lesions[1, 2]. However, the possibly most compelling argument arose from the unequivocal empirical success of CD20-mAb mediated B cell depletion in clinical MS trials.

In order to convey the latest advances regarding CD20-mAb therapy of MS, we conducted a systematic review of completed and ongoing clinical trials for MS, through searches of PubMed and references from selected articles, using “monoclonal antibodies”, “CD20 antibodies” or “B cell therapy” in combination with the term “multiple sclerosis”. We largely selected primary references from the past 5-6 years, but did not exclude consecrated and highly regarded earlier publications.

The first section of the review summarizes the complex contribution of activated B cells on MS pathogenesis, entailing both pro-inflammatory roles, as precursors of antibody-secreting plasma cells, specialized antigen presenting cells (APC) or sources of cytokines, as well as protective influences, as in the case of regulatory B cell subsets. The central part provides a comparative assessment of the current clinical evidence for the CD20-mAbs rituximab, ocrelizumab, ofatumumab and ublituximab, highlighting advantages and disadvantages within existing data. Finally, we emphasize on key future research topics and endeavour to deduce directions of advancement in the field of CD20-directed therapy in MS.

2. Immunopathology of B cells in MS

Systemic B cell responses, entailing aberrant activation of immune cells and their diapedesis across the blood-brain barrier (BBB) underlie the relapsing-remitting aspect of the disease, as recapitulated by EAE[3]. However, CNS-inherent immune responses, originating in germinal centre-like follicular structures within the leptomeninges are likely to underlie the smouldering, progressive aspect of the disease[4]. Hereby implicated autoreactive B cells originate due to a breach of peripheral, not central B cell tolerance, underscoring the importance of T_{reg} cell function[5]. Furthermore, B cells seem to thrive in the CNS, since their clonally expanded successors, CNS-resident plasma cells, persist over years, as reflected in the stability of OCB patterns in MS patients[6].

2.1 B cells and antibody-driven autoimmunity within the CNS

The initial suspicions against B cell involvement in MS arose from the presence of CSF-specific OCB in most MS patients, and autopsy studies revealing, on the one hand, antibodies bound to myelin fragments within phagocytic cells in the CNS parenchyma[7, 8], and, on the other hand, immunoglobulin and complement sedimentation on disintegrated myelin sheaths and demyelinating lesions[1, 2, 9]. In MS, increased numbers of B cells, plasmablasts and plasma cells are present in CSF and their numbers seem to relate to intrathecal immunoglobulin synthesis and CNS inflammation[10, 11]. Immunoglobulins comprised in the OCBs are secreted within the CNS by CSF-circulating or parenchyma-resident B cells, as confirmed by overlapping, somatic-hypermutation-generated Ig transcriptomes and proteomes of oligoclonally expanded B cells in the CSF of MS patients[12]. Nonetheless, antigen-driven affinity maturation preconditions somatic hypermutation in B cells. The ensuing quest for key

epitopes revealed so far intracellular proteins[13] or lipid determinants[14], whereas some of the antibodies seem to bind specifically to glia cells and myelin[15], although the molecular target of these antibodies has not been identified. Similar studies on the specificity of CSF or serum antibodies have not provided conclusive evidence on the targets of autoantibodies in MS, as opposed to AQP4 (aquaporin-4) in neuromyelitis optica (NMO), despite some promising candidates[16, 17, 7, 18]. While the likelihood persists that autoantibody-related mechanisms are not or only partly responsible for the acute effects of $CD20^+$ cell depletion, their long-term contributions remain as yet unclear.

2.2 B cells as antigen-presenting cells modulate T cell dysfunction

The most convincing argument for an antibody-independent role of B cells in MS originated from the prompt and sustained decrease in new relapsing MS activity in the context of CD20-mAb therapy. The onset of substantial benefit as early as 4-8 weeks[19] after infusion is, considering the half-life of plasma cells and secreted antibodies, unlikely due to removal of any putatively pathogenic ones[3]. Conceivably, stagnating the influx of B cells ($CD20^+$ as opposed to plasma cells) across the BBB could interrupt local Ab release, thus enhancing therapeutic effects. B cells function as notoriously efficient antigen-presenting cells (APCs), defined by constitutive expression of MHC class II, and specialized in capturing soluble and, even more efficiently, membrane-tethered antigens. Antigen-specific B cells are 1.000 to 10.000 times more efficient in presenting antigens and activating T cells than non-specific B cells[20]. Given the high and early efficacy of B cell depletion, it is tempting to speculate that B cells play the dominant role in presenting autoantigens in MS. This could be due to the nature of the autoantigen, which might be preferentially processed by B cells, or more generally due to a dominant role of B cells in processing CNS antigens. Nevertheless, the acute effects of B

cell depletion most probably rely on their part as APCs and therefore not only on the directly targeted B cells, but also on the potentially interacting autoantigen-specific T cells.

2.3 Mechanisms of $CD20^+$ cell depletion

CD20 is a transmembrane, 33-37 kDa, non-glycosylated phosphoprotein with no known natural ligand, which is involved in the generation of T cell-independent antibody responses[21], expressed on the surface of cells of the human B cell lineage from pre-B cells to naïve and memory B cells[22-24] (Fig. 2). The canonical biomarker for monitoring the direct effect of CD20-mAbs is, counter-intuitively, CD19 and not CD20. CD19 represents a broader spectrum of the B cell lineage, including pro-B cells and plasmablasts[25]. However, as CD20-mAbs interfere with flow cytometric analysis of $CD20^+$ cells, $CD19^+$ cells are typically used as a surrogate biomarker[26]. By directly binding CD20 on the surface of B cells, mAbs deplete target cells via induction of four broad categories of mechanisms: (i) antibody-dependent cellular cytotoxicity (ADCC); (ii) complement-dependent cytotoxicity (CDC); (iii) antibody-dependent cellular phagocytosis (ADCP); (iv) induction of $CD20^+$ cell apoptosis[23, 27]. CD20-mAbs currently in use for MS therapy mainly funnel their action through CDC- and ADCC-mechanisms[24, 28]. ADCC relies on the interaction between the Fc region of the CD20-mAb and the Fc gamma receptor (FcγR) on natural killer (NK) cells. Compared to rituximab, the newer mAbs ocrelizumab and ublituximab exhibit enhanced ADCC[29]. CDC, on the other hand, assumes activation of the C1q component of the classical complement pathway by the Fc region of the CD20-mAb[30]. This mechanism is exploited prominently by rituximab and, mostly, ofatumumab from the newer generation of CD20-mAbs in clinical use for MS[30].

After infusion of CD20-mAbs, dose-dependent B cell depletion ensues rapidly (within hours in blood[31]), the nadir being reached approx. 8 weeks after therapy[32]. Undetectable B cell levels in peripheral blood persist for several months post-treatment depending on dose and

nature of the mAb[32, 33]. Without CD20-mAb re-infusion, B cell depletion can persist for as long as 20 months[32], maybe even longer[34].

2.4 Alternative mAbs targeting B cells and their potential for MS therapy

CD20-mAbs currently in use for the treatment of MS are Rituximab (RTX), Ocrelizumab (OCR), Ofatumumab (OFA) and Ublituximab (UTX). Other CD20-mAbs in clinical trials mainly for haematological malignancies, which have not yet been employed in MS therapy, are Veltuzumab, Obinutuzumab, Ibritumomabtiuxetan, Tositumomab and Ocaratuzumab[27, 35-37]. A methodically different targeting of B cells applies in the cases of Alemtuzumab – CD52-mAb depleting both B and T cells, approved for active RRMS[38]; Inebilizumab/MEDI-551 – CD19-mAb, with one completed phase I study in MS[39] and an ongoing late phase study in NMO (NCT02200770); and VAY736 – anti-B cell activating factor (BAFF) with a recently completed, but yet unpublished phase II study in MS (NCT02038049).

3. Clinical trials of CD20-mAbs in MS

3.1 Rituximab

Rituximab (RTX, Rituxan®, MabThera®), a chimeric mouse-human IgG1 mAb[40], unleashes cytolysis mainly through CDC and secondarily through ADCC[27] (Table 1). As a CD20-mAb, it induces cross-linking of CD20 tetramers, followed by translocation of CD20 into large lipid 'rafts' within the plasma membrane upon binding, thus enhancing complement activation and subsequently CDC[41]. In particular, amino acid residues 168-175 of the large extracellular loop of the CD20 molecule appear to represent the critical epitope for RTX binding[41].

RTX was approved for the treatment of B cell-lymphomas (i.e. non-Hodgkin's lymphoma, chronic lymphatic leukaemia)[27, 42] in 1997 (Fig. 3). Nowadays it is being deployed for a multitude of chronic inflammatory diseases apart from MS as well, including rheumatoid arthritis (RA), granulomatosis with polyangiitis and neuromyelitis optica[27, 30].

3.1.1 Randomized controlled trials

Following the index case report on the use of intravenous (i.v.) rituximab (RTX) in a patient with aggressive RRMS[43], one open-label study and two randomized controlled clinical trials were performed.

In an open-label, 72-week, multicentre trial[44], the preliminary safety and tolerability profile for a double course of RTX was established in patients with RRMS aged 18 to 55 years. RTX was administered as 2 infusions of 1 g RTX each on days 1 and 15, followed by an identical course 6 months later. Throughout the follow-up, adverse effects amounted mostly to mild or moderate reactions, seldom reaching severe fatigue, headaches or muscle weakness. More than half of the patients (65.4%) reported IRRs following the first cycle, their proportion decreasing to a minority (8%) through the second cycle, while no serious adverse effects or infections occurred. The study demonstrated a reduction in ARR as compared to the year before starting the study treatment (0.18 on week 72 compared to 1.27 in the year before the study), while the majority of the subjects (80.8%) remained relapse-free. Concomitantly, Gd-enhancing lesions were completely suppressed by week 72, from a mean 1.31 per patient at baseline.

Following, a small, randomized, double-blinded, placebo-controlled and manufacturer-sponsored multicentre phase II clinical trial (HERMES)[19] evaluated the efficacy and safety of

RTX in 104 patients with RRMS over 48 weeks. From these, 69 were randomized to receive a single course of 1g RTX i.v. on days 1 and 15. Even if its short follow-up precluded long-term assessment of treatment safety, the study convincingly attained its primary endpoint by demonstrating a pronounced (91%) relative reduction in the cumulative number of gadolinium (Gd)-enhancing lesions at weeks 12, 16, 20 and 24. Interestingly, patients randomized to the RTX group had even more Gd-enhancing MRI lesions at baseline than those receiving placebo. The clinical impact was reflected in a significantly reduced proportion of patients with relapses at weeks 24 and 48 (20.3% vs 40.0% at week 48). Intriguingly the full effect on preventing new lesions could be observed within 12 weeks after the first dose of RTX, well ahead of any significant depletion of putatively pathogenic antibodies, underscoring antibody-independent roles of B cells. Infusion-related reactions (IRRs), by definition occurring within 24h of infusion, were more frequent (92.6% grade 1 or 2 events vs 40% in the placebo group) as well as more severe (7.4% grade 3 events) in the RTX group and included fever, chills and hypotension – symptoms consistent with a cytokine-release syndrome during B cell lysis[45]. By the time of the second RTX infusion, their incidence did not exceed that in the placebo group. They did not correlate with the development of human anti-chimeric antibodies (HACAs), which could be detected in ca. ¼ of RTX-treated patients by week 48.

Second, a double-blind, placebo-controlled, multicentre and manufacturer-sponsored phase II/III trial[46] investigated the efficacy, safety and tolerability of RTX in 439 patients with PPMS (OLYMPUS). They were randomized 2:1 to receive repeated courses of RTX (2 infusions of 1 g i.v. each, 2 weeks apart) or placebo infusions every 24 weeks through 96 weeks, with safety monitoring through week 122. Enrolment was confined to patients aged 18 to 65 years. The primary endpoint was time-to-confirmed disease progression, sustained for 12 weeks (CDP12). Despite not having delayed the time-to-CDP12, the study's planned subgroup analyses highlighted some interesting aspects. First, a statistically significant delay

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194 in time-to-CDP12 was noticeable in younger patients, aged <51 years, treated with RTX and
195 particularly in those with active inflammatory lesion components, as denoted by Gd-
196 enhancing lesions on cranial MRI. Regarding its safety and tolerability profile, IRR
197 predictably occurred more commonly in the treatment arm, were mostly mild to moderate and
198 clustered during the first RTX course. Serious adverse effects (SAE) were reported by 16.4%
199 of RTX-treated patients and 13.6% of placebo-treated patients while infection-associated SAE
200 prevailed among 4.5% of RTX- and <1% of placebo-treated patients, rates similar to those
201 detected during the HERMES trial.

202 Another small, MRI-blinded, investigator-initiated phase II trial[47] for RTX was performed on
203 30 subjects with RRMS who experienced breakthrough disease, i.e. at least one new Gd-
204 enhancing lesion on any pre-treatment brain MRI and a new relapse within 18 months prior to
205 enrolment while on active treatment with standard injectable disease-modifying therapies
206 (DMT), such as interferon ß1a/b (IFN-ß) or glatiramer acetate. The trial evaluated the safety,
207 efficacy and tolerability of add-on RTX administered at a regimen of 4 times 375 mg/m^2 i.v.
208 weekly. Its primary endpoint concerned radiological disease activity, such that 74% of post-
209 treatment MRI scans were free of Gd-enhancing lesions compared with 26% at baseline ($p <$
210 0.0001). Importantly, the combination of RTX with standard DMT was overall well tolerated,
211 with few adverse effects.

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213 **3.1.2 Registry data**

214 The largest (n=822) retrospective, uncontrolled, observational multicentre trial[48] from 3
215 university medical centres in Sweden, yielding a relatively heterogeneous study group with
216 respect to baseline parameters (age, previous treatment regimens, disability index) and disease
217 subtype as well as RTX-treatment regimens (0,5 – 1 g every 6 months), aligned itself to the
218 results of the aforementioned randomized-controlled trials (RCTs), suggesting RTX is safe

and effective in the treatment of MS for at least up to 2 years. The authors observed a remarkably low annualized relapse rate (ARR) of 0.044 for RRMS, but in the absence of a control group this could only hint towards efficacy. The median EDSS remained constant over the follow-up period in RRMS patients, but was sampled at relatively long intervals (every 6-12 months) and was naturally subjected to retrospective measurement error. Regarding SAEs, infections requiring therapy occurred in 14% of RTX-treated patients. No occurrences of progressive multifocal leukoencephalopathy (PML) were detected despite 83.6% of study patients being seropositive for JCV-Ab. However, few patients in the study had been treated for more than 24 months with RTX at the time of sampling. Yet another compelling result emerged from a comparison of RTX vs fingolimod (FGL) following JCV-Ab positivity-induced switching from natalizumab (NTZ) in a multicentre, observational, cohort study of 256 patients at three MS centres in Sweden based upon the Swedish MS register[49]. Adjusting for potentially confounding differences in baseline characteristics (younger RTX patients, shorter median washout times before RTX, a longer period on NTZ for the RTX group) and bearing in mind the nonrandomized design, the authors could illustrate a higher effectiveness-to-tolerability ratio for RTX, which not only reduced Gd-enhancing T1 lesions (1% on RTX vs. 16% on FGL) and new cerebral T2 lesions, as well as clinical relapses compared to FGL (2% of patients relapsing on RTX vs 18% on FGL), but was also associated with less AEs (5% on RTX vs 21% on FGL) and an overall longer drug survival (indicator of tolerability) through week 78. However, the impact of switching to RTX on PML risk and the right timing of the switch were left unanswered by the study.

New insights on the putative cancer-linkage of RTX came from recent data presented at the ECTRIMS Congress 2018 from a nationwide Swedish register-based retrospective cohort study[50] primarily comparing the incidence ratios (IRs) of any malignant cancer or of malignant breast cancer in female patients across 6331 subjects treated with either RTX or

other DMD (disease modifying drugs). After probability weighting, the hazard ratio (HR) of the RTX-treated cohort regarding malignant (breast) cancer risk proved comparable to that of the general population. However, stratification regarding specific forms of cancer needs to be targeted by future studies.

Highly active DMD such as RTX have been associated with an increased risk on infections compared to standard DMD such as interferon-β and glatiramer acetate (IFNβ/GA)[51]. A recent register-based retrospective cohort study[52] of all Swedish MS patients having initiated treatment with rituximab (N=3242) in 2011-2016 indicated similar risks of serious infections under RTX as with natalizumab or fingolimod, but increased ones compared to IFNβ/GA (HR (95% CI) = 0.53 (0.32-0.86)).

Another investigator-initiated US and Swedish retrospective-cohort based study[53] following around 2700 MS-patients from the first RTX infusion between 2011 and 2017 (KPSC and COMBAT-MS cohorts), could not identify any linkage to infusion-related deaths. Among all patients, 15 deaths occurred, none within 2 weeks of the last RTX-infusion. However, the patient cohorts were relatively inhomogeneous, with KPSC-registered patients exhibiting a more event-prone cardiovascular background.

Due to its chimeric character, RTX poses a higher risk than its more modern counterparts for the development of anti-drug antibodies (ADA), in particular human anti-chimeric antibodies (HACA). A recent cross-sectional study[54] in a large cohort of patients from the Swedish MS Registry identified ADA in the serum of 37% of 238 RRMS and in 26% of 101 progressive MS-patients. The authors describe a correlation between ADA presence and titers and incomplete B cell depletion. However, despite the majority of patients terminating RTX during follow-up (non-ignorable exiting), no association between ADA titers and clinical outcomes or infusion reactions could be found.

3.1.3 Safety and tolerability

Considering its profound and lasting impact on cellular and humoral immunity, as well as its chimeric nature, RTX bears an overall acceptable risk profile. The most frequent adverse effects (AEs) amount to IRRs, commencing 30-120 min[42] through the first RTX infusion and diminishing across the following cycles. Precisely, reactions potentially related to mast cell degranulation have been reported[46, 55]: urticaria, angioedema, nausea, chills, pyrexia, dizziness, pharyngolaryngeal pain, pruritus, rashes, flushing, bronchospasm, acute respiratory distress syndrome, hypotension etc. However, isolated reports exist of Kounis syndrome[56] (vasospastic or thromboembolic cardiovascular events complicating anaphylactoid episodes, possibly triggered by mAb specific IgE inducing local or systemic mast cell degranulation) or cardiac arrhythmias due to (systemic inflammatory response syndrome) SIRS-related "cytokine-storms"[57]. Even though the prescription information for RTX warns against serious bacterial, fungal or viral infections during or after treatment with this mAb, infections in general occurred at similar rates in the treatment as in the control arms of existent RCT (69.6% vs 71.4%[19] in the HERMES trial, in more than 10%[46] in both groups in the OLYMPUS trial). Of all infections, urinary tract infections and sinusitis tended to be more common among patients treated with RTX[19, 46]. The RTX prescription information also warns against the increased risk of progressive multifocal leukoencephalopathy (PML)[26], even though up to date no PML case . However, the annual incidence of PML in RTX-treated rheumatological patients is estimated at less than 1:25000, the RTX-attributable portion of risk probably being even lower[36, 58], since in those cases patients exhibited a history of prior or concomitant medication with other immunosuppressive agents or had undergone hematopoietic stem cell transplantation[42, 58-60]. Another concern relates to the reactivation of a latent hepatitis B infection. Especially patients with chronic hepatitis B (HBs-Ag positive) are at a relatively high risk of reactivation (varying reports between 27% and 80%[42]), but also

294 those with resolved HBV infection (HBsAg-negative/cAb-positive)[61]. Prophylactic therapy
295 with lamivudine, entecavir or tenofovir has proved effective in reducing the risk of HBV
296 reactivation and related mortality in HBsAg-positive patients at high risk of reactivation[62].
297 Furthermore, severe mucocutaneous reactions have been observed rarely after treatment with
298 RTX – of note, paraneoplastic pemphigus, Stevens-Johnson syndrome, toxic epidermal
299 necrolysis etc.[42, 55].

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301 **3.1.4 Practical therapy management**

302 Despite compelling clinical data, not only regarding MS, RTX did not proceed to phase III
303 testing, and will not be further developed for MS most likely due to various factors, including
304 licensing issues, economic considerations and company internal decisions[35, 42]. RTX's patent
305 expired in Europe in February 2013 and in the US in September 2016[63]. Interestingly, the
306 therapy costs for RTX in MS even at the highest dose tested (two infusions of 1g 2 weeks
307 apart every 6 months) amount to approx. $30,000 (US) / 12,000 € (Europe) annually,
308 considerably less than the newly approved ocrelizumab, at approx. $65,000 (US) / 33,000 €
309 (Europe) [63], making real-world off-label prescription of RTX an attractive alternative to its
310 licensed counterparts.

311 Practically, before initiating RTX in MS patients in off-label regime, we screen for chronic or
312 active infections (Hepatitis B and C, HIV, VZV, measles). We avoid initiating RTX in
313 patients after the age of 55 (corresponding to the age limits of the study cohorts tested in
314 MS[19, 46]) unless relapse or MRI activity indicate significant inflammatory activity. Switching
315 to RTX after treatment with another DMD such as natalizumab, for example in the case of
316 JCV-Ab positivity, is a routinely encountered clinical decision in MS centres. Depending on
317 the previous therapeutic regimen, we strive a latency of 2-8 weeks (6-8 weeks in the case of
318 natalizumab) before induction with RTX. During RTX therapy, we monitor patients clinically

319 at least every 6 months and radiologically at least yearly. In previous trials for other
320 autoimmune diseases, the duration of B cell depletion with RTX was dose dependent: mean
321 repopulation time was 184 days on the 1000 mg regime and approx. 240 days on the 4 times
322 375mg/m^2 weekly regimen[64]. Considering the latency with which B cell reconstitution ensues
323 in the wake of a RTX therapy cycle, we found controls of CD19$^+$ cell levels 5 months after
324 infusion to be sufficient and manageable by our primary care providers. Furthermore, we
325 monitor serum levels of IgG every 6 months, to adjust the dose of RTX or even substitute
326 immunoglobulins in the case of low levels. Live vaccinations are contraindicated during
327 therapy with RTX, so that necessary vaccines (for instance VZV) should be administered
328 prior to begin of therapy. Current recommendations in the case of females of child-bearing
329 age contraindicate RTX during pregnancy and emphasize contraception during therapy,
330 although a recent systematic review and a case series reported no major complications with
331 RTX use within 6 months of conception[65]. Currently it is recommended to start pregnancy not
332 earlier than 12 months after the last infusion.

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334 **3.1.5 Ongoing and future trials**

335 Currently, several phase III studies are recruiting MS patients and studying the facets of RTX
336 therapy for MS. One of them, a prospective observational study to be completed January
337 2019, will primarily evaluate the tolerability and safety of switching (non-randomly) from
338 RTX to ocrelizumab (ClinicalTrials.gov. ID: NCT02980042). Also, an interventional,
339 randomized, open-label prospective, active comparator phase II/III clinical trial will evaluate
340 the effectiveness of RTX vs glatiramer acetate in active progressive MS (ClinicalTrials.gov
341 Identifier: NCT03315923). An active comparator randomized phase III study of RTX vs
342 dimethyl fumarate (RIFUND-MS) in early RRMS and Clinically Isolated Syndrome (CIS) is
343 due through August 2021 (ClinicalTrials.gov. ID: NCT02746744). A 3-year follow-up

prospective observational 3700-patient cohort study using the Swedish MS registry (COMBAT-MS) is due through December 2021 and will compare effectiveness and safety of RTX against all other DMD used in RRMS (ClinicalTrials.gov. ID: NCT03193866). Further, a phase IV open label, randomized study on the effectiveness of early highly effective DMD (including RTX, Ocrelizumab, Alemtuzumab and Natalizumab) is due to start October 2018 (ClinicalTrials.gov Identifier: NCT03535298). In spite not primarily evaluating the safety and effectiveness of RTX in a randomized-controlled manner, these studies still promise interesting insights on important aspects of treatment decisions in MS.

3.2 Ocrelizumab

In contrast to its predecessor RTX, ocrelizumab (OCR, Ocrevus®) is a humanized IgG1 CD20-Ab that targets an overlapping, but slightly different epitope of CD20[28], which, because of OCR's constant domain's higher affinity for FcγRIII on natural killer (NK) cells, yields enhanced ADCC and a reduced CDC[27, 42, 66-68]. Developed with hindsight towards reduced immunogenicity, i.e. lowering the incidence of HACAs and of infusion reactions, OCR has recently been approved both by the US Food and Drug Administration (March 2017) and the European Medicines Agency (January 2018) for the treatment of RRMS (first approved anti-CD20 mAb) and PPMS patients (first ever approved pharmacotherapy)[69, 70] (Fig. 3, Table 1).

3.2.1. OCR: Randomized controlled trials

A multicentre, randomised, placebo-controlled phase II trial[71] assessing the safety and efficacy of two dose regimens of OCR versus placebo and, as an active comparator, open-label interferon β-1a i.m. in RRMS, provided the premises for subsequent phase III trials. At 24 weeks, gadolinium-enhancing T1-lesions were reduced by 89% and 96% by OCR 600mg

(300mg i.v. on days 1 and 15) and OCR 2000mg (1000mg i.v. on days 1 and 15), respectively, compared to placebo. Underpinning the observations from the RTX trial HERMES, the lasting diminution in incipient radiological disease activity was apparent already 8 weeks into the trial within both OCR treatment groups. Secondarily, annualized relapse rates over the albeit short span of 24 weeks were reduced by 80% and 73% in the 600mg and 2000mg OCR group, respectively. However, as no segregation of effects with two OCR doses could be noted, as well as because of one potentially drug-induced death in the 2000mg group, the lower dosage of 600mg (300mg i.v. on days 1 and 15, followed by 600mg i.v. every 6 months) prevailed during latter trials.

Following up on the phase II trial, the efficacy of OCR in RRMS was cemented by two phase III clinical trials, OPERA I and OPERA II[66], conducted as identical, multicentre, randomized, double-blind, double-dummy and manufacturer-sponsored studies randomizing 821 and, respectively, 835 patients with RRMS (McDonald criteria 2010) and similar demography to receive 600mg OCR i.v. (300mg on days 1 and 15, followed by 600mg i.v. every 24 weeks) or 44 µg interferon beta-1a s.c. 3x/week. Employing identical paradigms, both studies lasted through 96 weeks and involved patients aged 18 to 55 years, with an EDSS of 0 to 5.5 as well as recently documented disease activity, paralleled by fitting brain MRI lesions. OCR attained the primary study endpoint by significantly reducing the annualized relapse rate (ARR) from 0.29 (IFN β-1a) to 0.16 per year (46% and 47% lower with OCR, Opera I and Opera II, respectively) through week 96. Additionally, a preplanned pooled analysis of both study groups revealed a 40% reduction in both 12- and 24-week CDP in the OCR group, which also exhibited 94% (Opera I) and 95% (Opera II) fewer Gd-enhancing T1-lesions, as well as fewer new or enlarging hyperintense T2-lesions (77% Opera I; 83% Opera II) on brain MRI scans. The longer follow-up, the larger study cohorts and the more homogenous baseline characteristics, especially concerning previous immunomodulatory treatment, conferred more

validity to the OPERA trials than was the case for the phase II trial. However, the discrepancy between the substantial reduction in radiological disease activity and the less pronounced abatement of disease progression remained unexplained. Possible explanations are pseudo-relapses or a differential impact of OCR on brain and spinal cord, which was not monitored by MRI.

Driven partly by the promising subgroup analyses of the OLYMPUS trial, a phase III randomized, double-blinded, placebo-controlled, manufacturer-sponsored study of the efficacy and safety of OCR in PPMS[72] was conducted (ORATORIO). The trial was undertaken on 732 patients with PPMS aged 18 to 55 years, randomized 2:1 to receive either OCR 600mg i.v. (300mg on days 1 and 15 every 24 weeks) or placebo. Compared to the baseline characteristics of the OLYMPUS cohort, the ORATORIO demography comprised on average younger patients (44 years vs 50 years), a shorter disease duration (6.7 years vs 9 years) and a marginally lower median EDSS at study onset (4.5 vs 5.0). The primary and first secondary endpoints, CDP at 12 and, respectively, 24 weeks were both met, OCR exhibiting a significant, but altogether limited relative risk reduction compared to placebo, of 24% (in absolute 32.9% vs 39.3%) and, respectively, 25% (in absolute 29.6% vs 35.7%). Furthermore, OCR significantly reduced T2-weighted lesion volumes at 120 weeks by 3.4%, and the rate of total brain atrophy by 17.5% compared to placebo. In an FDA requested subgroup analysis it became evident, that the effect of OCR on CDP was also strongly influenced by the age of the patients. CDP in patients 40 years or younger CDP was moderately reduced (HR 0.28-0.36), while patients older than 40 years had almost no effect of the treatment (HR 0.78-0.88)[73].

3.2.2. OCR: Safety and tolerability

In May 2010, the OCR development programme for Rheumatoid Arthritis (RA) was halted not due to a lack of efficacy, but rather to safety concerns, since high dosages of OCR

418 correlated in phase III trials with an increase in severe opportunistic infections. During these
419 RA trials, no increased incidence rate of malignancies or PML cases were recorded[74].
420 However, the concomitant deployment of other immunosuppressive medication and the on
421 average higher age of RA patients implied a higher baseline risk profile, so that OCR studies
422 were only continued in MS. In the phase II MS trial, IRR prevailed in the 600mg OCR
423 (34.5%) and the 2000mg OCR (43.6%) groups compared to IFN β-1a (9.3%), while in the
424 2000mg group one death secondary to a complicated course of systemic inflammatory
425 response syndrome (SIRS) was recorded[71]. As in the case of RTX, IRR clustered during or
426 shortly after the first infusion (25%) and amounted to mostly mild-to-moderate pruritus,
427 rashes, throat irritations and/or flushing. The relatively lower incidence of IRR under OCR
428 compared to the RTX trials (i.e. OLYMPUS) could presumably be due to pre-infusion
429 comedication with antipyretics and antihistaminics. Concerning infections, the statistics were
430 largely replicated in phase III MS trials, of note being slightly more frequent mild-to-
431 moderate infections than in the IFN β-1a group, including upper respiratory tract infections
432 (15.2% vs. 10.5%), nasopharyngitis (14.8% vs. 10.2%) and mild cutaneous herpes infections
433 (5.9% vs. 3.4%)[66]. Notably, treatment with OCR resulted in a considerable decrease of serum
434 IgM levels both in the OPERA and ORATORIO trials[66, 72].

435 Moreover, in the ORATORIO study, significantly more malignancies were signalled in the
436 OCR group than with placebo (2.3% vs. 0.8%), specifically four breast cancers, one
437 endometrial adenocarcinoma, one anaplastic lymphoma, one histiocytoma, one metastatic
438 pancreatic cancer and three basal cell carcinomas[72]. Altogether, the absolute number did not
439 exceed epidemiological expectations, and since the incidence has fallen in open-label
440 extension studies and the diverse malignant entities did not fall into a single pathologic
441 category[75], no clear evidence of causality could be surmised. This is also in line with data
442 from the RA[74] and lymphoma[76] trials with RTX. No cases of PML have yet been reported in

443 the OCR MS trials, but so far (as of February 2019) 7 cases of PML after switch from NTZ or
444 FGL to OCR have occurred, probably in the sense of a "carry-over" effect[77]. Only 0.4% of
445 OCR patients developed drug-Ab during a 96-week treatment course[66], in contrast to the
446 much higher incidence of HACA (28.5%) during RTX trials[44].

447

448 **3.2.3. OCR: Practical therapy management**

449 Preliminary testing for chronic infectious diseases, as well as pregnancy and vaccination
450 recommendations for OCR therapy match those for RTX. Preliminary data presented at the
451 ECTRIMS Congress 2018 revealed no increase of IRR upon switching from RTX to OCR[78].
452 OCR completely depletes $CD20^+$ B cells in serum immediately after the first infusion[71], upon
453 which recovery necessitates 16-32 weeks to reach detectable levels and a median 72 weeks to
454 reach normal lower limits[36]. Interestingly, the therapeutic and radiological effect lasts up to at
455 least 18 months[36], considerably longer than B cell depletion, perhaps pertaining to effects on
456 memory B cells[68]. Even if as yet no head-to-head comparison of OCR vs RTX regarding IRR
457 exists, as CDC activity is believed to underlie the incidence of IRR, OCR should in theory be
458 better tolerable than RTX[67], although during the initial RTX trials no premedication with
459 methylprednisolone was administered[67, 71]. Regarding malignancy prevention, no specific
460 guidelines apply and standard screening measures are recommended.

461

462 **3.2.4. OCR: Ongoing trials**

463 Several promising trials for OCR are currently underway or in development. Apart from an
464 open-label extension, multicentre, single-arm phase III study evaluating the effectiveness and
465 safety of OCR 600mg i.v. every 24 weeks (ClinicalTrials.gov Identifier: NCT03599245), two
466 new phase IIIb manufacturer-initiated studies for OCR in progressive MS with novel

endpoints have been announced The ORATORIO-HAND multicentre, randomised, placebo-controlled, double-blind study, enrolling approx. 1000 patients with PPMS, will assess the effect of OCR on upper limb function in subgroups of more disabled/older patients (EDSS from 3 to 8) primarily in the Nine-Hole Peg Test 9-HPT (ClinicalTrials.gov Identifier: NCT03562975). The CONSONANCE-trial, designed as a 4-year prospective, single-arm, multicentre phase IIIb study with novel primary composite disability endpoints, including *N*o *E*vidence of *P*rogression (NEP, no progression sustained for at least 24 weeks on CDP, as measured by the EDSS, ≥20% increase in timed 25-foot walk test -T25FWT - and ≥20% increase in 9HPT) and *N*o *E*vidence of *P*rogression of *A*ctive *D*isease (NEPAD, no progression on all of the three components of NEP, no protocol-defined relapse, no enlarging or new T2 lesion and no T1 Gd-enhancing lesion), will investigate the effect of OCR on the entire spectrum of progressive MS, i.e. PPMS and SPMS (ClinicalTrials.gov Identifier: NCT03523858). Another study seeks to underpin the mechanisms of action of OCR and reveal insights into B cell biology in RRMS and PPMS by examining Neurofilament Light (NfL) levels in CSF, $CD19^+$ B cells and CD3+ T cells in CSF (OBOE trial, ClinicalTrials.gov Identifier: NCT02688985). Exploring an area of still significant controversy, whether early treatment with highly effective DMT (OCR among others) can improve the prognosis of RRMS patients, two new studies, DELIVER-MS (ClinicalTrials.gov Identifier: NCT03535298) and TREAT-MS (ClinicalTrials.gov Identifier: NCT03500328) will primarily compare brain volume loss and, respectively, time to sustained disability progression in this condition. Another open-label, multicentre, prospective, single-arm, phase III study, ENSEMBLE (ClinicalTrials.gov Identifier: NCT03085810), will primarily assess the safety and effectiveness of OCR in approx. 600 patients with early stage RRMS.

3.3 Ofatumumab

Ofatumumab (OFA) represents a next-generation, fully humanized IgG1 CD20 mAb[79, 80], designed to boast even less immunogenicity than RTX and OCR[83] (Fig. 3, Table 1). It binds with high affinity to a small membrane loop epitope[81], different from the one of RTX and OCR, even at very low levels of expression of CD20[82, 83]. Consequently, it dissociates from CD20 more slowly than RTX, conferring enhanced CDC to the detriment of ADCC[82, 83]. It is currently approved as therapy against refractory chronic lymphatic leukaemia (CLL, Arzerra®)[84], having demonstrated efficacy also against active RA[85]. Crucially, in addition to the possibility of i.v. administration, it capitalizes on an s.c. formulation, that promises to reduce IRR, eliminate the need for premedication with glucocorticoids and offer a more convenient administration method for patients and health care personnel alike[63].

3.3.1. OFA: Randomized controlled trials

OFA debuted with respect to MS in a small, 48-week, placebo-controlled, 24-week cross-over, randomized, double-blind, phase I/II study in 38 patients with RRMS, designed to assess its safety and preliminary efficacy. The treatment arms administered were OFA 100mg, 300mg or 700mg i.v. 2 weeks apart. The primary endpoint was met, OFA yielding a drastic suppression of new and total number of Gd-enhancing T1-lesions of >99% compared to placebo as well as a significant reduction in new and/or enlarging T2 lesions across all dosages in the first 24 weeks after OFA administration[79]. However, the reduction in relapse rate over the first 24 weeks was all but modest (19% across all dosages vs. 25% on placebo), raising, as in the case of OCR, the question of relevance of brain MRI alone as a prognostic variable. Importantly, none of the patients developed human anti-human Ab (HAHAs) and OFA brought about a profound selective depletion of $CD19^+$ cells, as known from OCR, without affecting serum Ig levels.

Subsequently, a phase II, randomized, placebo-controlled, parallel-group, dose-ranging study was initiated on 232 patients with RRMS (MIRROR trial)[81] treated with 3, 30 or 60 mg OFA s.c. every 12 weeks, 60mg OFA s.c. every 4 weeks or placebo (1:1:1:2:2) for 24 weeks, to evaluate dose-response effects on efficacy and safety outcomes. Again, OFA across all doses led to a 65% (90% when taking effect onset latency into account) reduction in new Gd-enhancing T1 lesions for weeks 4 to 12, but to no significant reduction in relapse rate across the relatively short duration of the study. Interestingly, complete depletion of $CD19^+$ cells was not a prerequisite for the treatment effect.

3.3.2. OFA: Safety and tolerability

During the currently completed trials, the most common adverse effect were infusion/injection-related reactions (IRRs), occurring mostly after the first dose in 41-66% of OFA-treated patients vs. 15% of placebo[81]. Few cases of SAEs have been reported and no occurrence of opportunistic infections. During the haematological trials, neutropenia has been signalled in 3% of patients[86].

3.3.3. OFA: Ongoing trials

Two randomized, double-blind, double-dummy, active comparator-controlled, parallel-group, multicentre phase III clinical trials are currently evaluating the efficacy and safety of OFA 20mg s.c. every 4 weeks over 2.5 years compared to oral teriflunomide in RRMS patients primarily regarding ARR (ASCLEPIOS I and II, ClinicalTrials.gov identifier: NCT02792218/NCT02792231; due through May 2019). Another 24-week, randomized, double-blind, placebo-controlled, parallel-group, multicentre phase II study will compare OFA 20mg s.c. every 4 weeks vs. placebo in 60 patients (randomized 2:1) with RRMS

(ClinicalTrials.gov Identifier: NCT03249714). As a bridging trial towards enhanced patient autonomy, a phase II trial will investigate the pharmacokinetic bioequivalence of OFA injected at the clinical dose of 20mg s.c. via a pre-filled syringe versus an auto-injector device (ClinicalTrials.gov Identifier: NCT03560739).

3.4 Ublituximab

Ublituximab (UTX, TG-1101) is a next-generation chimeric anti-CD20 antibody, targeting a different epitope than its predecessors (Table 1). It is glycoengineered towards low fucose content[37] for higher affinity to all variants of FcγRIIIa receptors, therefore featuring an enhanced ADCC over RTX and OFA, especially in cells with low CD20 expression[36] . Its greater ADCC is hoped to offer a benefit over currently available CD20-mAb because of lower effective doses and shorter infusion times. Ublituximab is primarily evaluated for its efficacy in the treatment of CLL, but has also received FDA orphan drug designation for the treatment of Neuromyelitis optica (Fig. 3).

3.4.1. UTX: Randomized controlled trials

At the ECTRIMS Congress 2018 the results of a first phase II multicentre, randomized, cross-over design (from week 24), placebo-controlled, 48-week study of UTX in 48 patients with RRMS focusing on optimal dosing and infusion times were announced[87]. UTX was administered across multiple dosing cohorts (450mg or 600mg over 1-4 hours on days 1 and 15 as well as after 24 weeks) and achieved a substantial B cell depletion in all dosages of over 99%. As its counterparts, it drastically reduced Gd-enhancing T1 lesions (100% reduction at week 24) and decreased T2 lesion volume by 8% and 10% at weeks 24 and, respectively, 48. The ARR in the treatment arms remained low at 0.07. Notably, T cells showed a significant

564 population shift toward naïve and regulatory phenotypes, possibly indicative of interference
565 with the antigen-presenting role of the depleted B cells.

566

567 **3.4.2. UTX: Safety and tolerability**

568 In the phase II trial in MS, no SAE or safety-concerns were reported. The most common
569 adverse effects were mild- to moderate IRR, which showed increase in incidence with
570 decreasing infusion times[87]. Interestingly, UTX could be safely delivered in infusions as fast
571 as one hour[87].

572

573 **3.4.3. UTX: Ongoing trials**

574 Building upon the results of the phase II trial, two new phase III, randomized, multi-centre,
575 double-blinded, active comparator-controlled studies will assess the efficacy (primary
576 endpoint: ARR over 96 weeks) and safety of UTX 450mg i.v. on days 1 and 15, afterwards
577 every 24 weeks, as compared to teriflunomide in approx. 880 patients with RRMS and are due
578 through March 2021 (ULTIMATE 1 and 2, ClinicalTrials.gov identifiers: NCT03277261/
579 NCT03277248).

580

581 ## 4. Conclusion

582 From the traditional interpretation of a T cell-mediated disease, the understanding of MS
583 pathogenesis has evolved to ascribe B cells crucial contributions as well, leading to the
584 emergence of CD20-mAb as therapies with dramatic effect on clinical and radiological
585 measures of inflammation in RRMS. Furthermore, for the first time a partially effective
586 therapy against PPMS has become available. Future translational research paralleled by

587 clinical trials are needed to ascertain the optimal time point of treatment initiation with CD20-
588 mAb, whether more benefits regarding transition to SPMS can be reaped by an earlier therapy
589 start, the optimal dosing, monitoring markers and time points of treatment
590 interruption/cessation, as well as long-term safety including the risk of malignancy or
591 development of PML.

592

593 ## 5. Expert opinion

594 Clinical trials have undoubtedly underpinned the capacity of CD20-mAbs to markedly and
595 sustainably diminish metrics of active inflammation in MS, ushering in benefits in clinical
596 and radiological outcome measures surpassing those of most other approved DMT. So far, all
597 CD20 mAb therapies convincingly reduced relapses and inflammatory MRI-activity,
598 providing a proof-of-concept for the substantial role of B cells in MS pathophysiology.
599 Currently, in hindsight of the risk of overexposing patients to serious side effects by too
600 precocious initiation of anti-CD20 therapy, a “treat-to-target approach” prevails, advocating a
601 gradual escalation to mAb upon on-treatment disease breakthroughs. However, clinical
602 relapses seemingly constitute the “tip of the iceberg” regarding disease progression, while
603 ongoing inflammatory MRI activity exceeds relapse activity, so that relying on on-treatment
604 disease breakthrough as a threshold for escalating therapy might not be sensitive enough. The
605 high level of efficacy of CD20-mAbs coupled with their reasonable safety profile tempts the
606 perspective of employing them as first-line therapies in selected patient cohorts, supposing
607 current and future prognostic markers could validly signal an increased risk of developing
608 SPMS. Clinical trials in this context could shed light on the clinical disease progression upon
609 dissociation from active inflammation. Capitalizing on the lasting suppression of
610 inflammation through singular CD20-mAb infusion cycles even after B cell reconstitution,

611 alternative therapeutic algorithms could rely on a limited CD20-mAb induction therapy
612 followed by either active surveillance, gradual escalation of CD20-mAb dose or less
613 aggressive DMT as a bridging concept between cycles. As yet, uncertainty prevails whether
614 the therapeutic CD20-mAb regimen is optimally guided by $CD19^+$ B cell levels or if rather
615 subclasses of B cells (i.e. memory B cells) would represent a more precise indicator of
616 autoimmunity reconstitution. The recent description of CNS lymphatics[88] and of lymphocyte
617 trafficking to and from the CNS parenchyma and meninges via cervical lymph nodes[89]
618 unveiled potential avenues of escape for $CD20^+$ cells otherwise completely depleted from the
619 peripheral circulation[36].

620 Regarding PPMS, evidence from the ORATORIO trial and the subgroup analysis from the
621 OLYMPUS trial pointed towards a significant, but altogether limited reduction in clinical
622 disability favouring younger patients or those bearing evidence of ongoing (perivascular)
623 inflammatory activity. Burgeoning evidence suggests a dichotomy and coexistence of
624 biological processes underlying progression in MS: one driven by acute inflammation and
625 accumulation of relapses and another characterized by non-relapsing, neurodegenerative
626 worsening. Putatively, the benefits observed in the current PPMS trials might reflect the effect
627 of CD20-mAbs on relapse biology. Precisely how $CD20^+$ B cells might influence non-
628 relapsing progressive disease via direct or cytokine-mediated neurotoxicity as well as via
629 CNS compartments not efficiently reached by CD20-mAbs, such as meningeal B cell-rich
630 follicles[3, 36], remains enigmatic.

631 Summing up, CD20-mAb therapies have the potential to become first-line drugs in selected
632 patients with highly active MS. Innovations such as subcutaneous formulations, as in the case
633 of OFA, or the promise of smart-device-based clinical outcome measures foreshadow more
634 patient autonomy. Still, the question whether and how CD20-mAbs modify the progressive
635 aspects of MS has to be substantiated. Furthermore, future studies will have to evaluate

636 whether dosages and administration regimens currently in use can be optimized, while
637 registry data should shed light on risk versus benefits of prescribing CD20-mAbs on the long
638 term and in the context of immunosenescence occurring in older patients, alongside a
639 potentially increased risk of malignancy.

640 Ultimately, even more specifically targeted therapies against perhaps only subpopulations of
641 $CD20^+$ B cells, sparing protective regulatory B cells, and targeting primarily disease
642 associated B cell clones, could emerge. On the horizon, the prospect of a multifaceted,
643 personalized MS therapy is crystallizing, possibly adjoining CD20-mAb with novel reparative
644 agents based on novel clinical, biochemical and radiological markers of inflammation, de-
645 /remyelination and neuronal degeneration.

646

647 ## 6. Article highlights box

648 - The pivotal role of B cells in the pathogenesis of MS is undisputable, as underlined by
649 the therapeutic effect of CD20-monoclonal antibodies (CD20-mAbs)
650 - CD20-mAbs elicit prompt and sustained decreases in MS disease activity,
651 underpinning antibody-independent roles of B cell in MS
652 - Rituximab (RTX), the first CD20-mAb deployed in MS, has provided compelling
653 proof of effectivity in relapsing MS and the longest follow-up on adverse effects, but
654 has to be prescribed off-label
655 - Ocrelizumab (OCR), is the first CD20-mAb approved for use both in relapsing and
656 progressive disease, yet incurs significantly higher therapy costs
657 - Experience with newer generation CD20-mAbs is limited, but ofatumumab (OFA)
658 could represent an interesting alternative due to its s.c. formulation, while ublituximab
659 (UTX) could promise shorter infusion times

660

661

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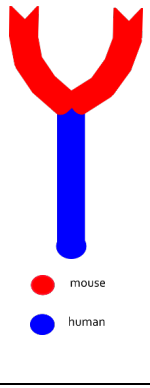
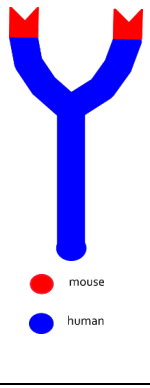
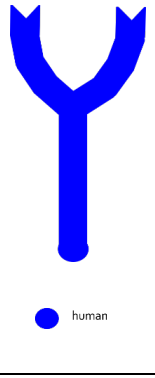
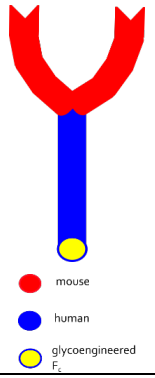
Table 1	Overview of CD20-monoclonal antibodies currently implemented in Multiple Sclerosis							
	Rituximab (RTX)		Ocrelizumab (OCR)		Ofatumumab (OFA)		Ublituximab (UTX)	
Structure		Chimeric IgG1 (65% human)		Humanized IgG1 (>90% human)		Recombinant fully human IgG1		Glycoengineered chimeric IgG1
Regimen		1g i.v. d. 1 & d. 15, followed by 1g every 24w.		300mg i.v. d. 1 & d. 15, followed by 600mg every 24 w.		20mg s.c. every 4 w.		450mg i.v. d. 1 & d. 15, followed by 450 mg i.v. every 24 w.
Primary mechanism of action		CDC		ADCC		CDC		ADCC
Generation		1st		2nd		3rd		3rd
Immunogenicity		+++		++		+		++

Table 2	Relevant completed and ongoing clinical trials for monoclonal CD20-antibodies in Multiple Sclerosis							
MS type	**Trial / Phase (Patients, Duration)**	**Treatment vs. comparator**	**Primary endpoint**	**ARR % reduction (p-value)**	**CDP % reduction (p-value)**	**% red. number Gd-enhancing lesions (p-value)**	**% red. number of new/enlarging T2 lesions (p-value)**	**Status**
Rituximab								
RRMS	HERMES (II): 104 p. / 48 w.	RTX 2x 1g i.v. every 24 w. vs. Placebo	Total count Gd-enhancing lesions	20.3% vs. 40.0% (p = 0.04)	NS	**91% (p < 0.001)**	NS	Hauser *et al.* [2008]
PPMS	OLYMPUS (II/III): 439 p. / 96 w.	RTX 2x 1g i.v. every 24 w. vs. Placebo	Time to CDP	NS	NS	NS	NS	Hawker *et al.* [2009]
Ocrelizumab								
RRMS	OPERA I (III): 821 p. / 96 w.	OCR 600 mg i.v. every 24 w. vs. IFN-β1a s.c.	ARR by week 96	0.16 vs. 0.29 (= 46%) (p < 0.001)	5.9% vs. 9.5% (=38%) (p = 0.03)	94% (p < 0.001)	0.32 vs. 1.41 (=77%) (p < 0.001)	Hauser *et al.* [2017]
RRMS	OPERA II (III): 835 p. / 96 w.	OCR 600mg i.v. every 24 w. vs. IFN-β1a s.c.	ARR by week 96	0.16 vs. 0.29 (= 47%) (p < 0.001)	7.9% vs. 11.5% (=31%) (p = 0.003)	95% (p < 0.001)	0.33 vs. 1.90 (=83%) (p < 0.001)	Hauser *et al.* [2017]
PPMS	ORATORIO (III): 732 p. / 96 w.	OCR 600mg i.v. every 24 w. vs. IFN-β1a s.c.	Time to CDP	NA	29.6% vs. 35.7% (=17%) (p = 0.04)	NA	-3.4% vs. 7.4% (p < 0.001)	Montalban *et al.* [2016]
PPMS	ORATORIO-HAND (III) : 1000 p. / 96 w.	OCR 600mg i.v. every 24 w.	9-HPT	-	-	-	-	Ongoing [NCT03562975]
PPMS, SPMS	CONSONANCE (IIIb) : 600 p. / 192 w.	OCR 600mg i.v. every 24 w.	No Evidence of Progression (NEP)	-	-	-	-	Ongoing [NCT03523858]
RRMS	DELIVER-MS (IV): 800 p. / 144 w.	OCR 600mg i.v. every 24	Loss of brain volume	-	-	-	-	Ongoing [NCT03535298]

		w. / other DMD						
RRMS	TREAT-MS: 900 p. / 192 w.	OCR 600mg i.v. every 24 w. / other DMD	Time to CDP	-	-	-	-	Ongoing [NCT03500328]
Ofatumumab								
RRMS	NCT00640328 (II): 38 p. / 24 w.	OFA 100mg, 300mg, 700mg i.v. 2w. apart vs. Placebo	Safety relative to dosage	NS	NS	>99% ($p < 0.001$)	99% ($p < 0.001$)	Sorensen *et al.* [2014]
RRMS	MIRROR (II): 232 p. / 24 w.	OFA 3mg, 30mg, 60mg s.c. 2w. apart vs. Placebo	Number of Gd-enhancing lesions (p-value)	NS	NS	>90% ($p < 0.001$) (OCR >30mg)	NS	Bar-Or *et al.* [2018]
RRMS	ASCLEPIOS I and II (III) : 929 p. /	OFA 20mg s.c. 4 w. apart vs. Teriflunomide	ARR by week 120	-	-	-	-	Ongoing [NCT02792218/NCT02792231]
Ublituximab								
RRMS	NCT02738775 (II): 48 p. / 48 w.	UTX 450mg, 600mg every 24 w.	Safety relative to dosage	NS	NS	100% ($p=0.003$)	NS	Completed (results published at ECTRIMS 2018)
RRMS	ULTIMATE 1 and 2 (III): 880 p. / 96 w.	UTX 450mg i.v. every 24 w. vs. Teriflunomide	ARR by week 96	-	-	-	-	Ongoing (NCT03277261/ NCT03277248)

Legend. p. = patients, w. = weeks, ARR = annualized relapse rate, NA = not assignable, NS = not significant, CDP = confirmed disease progression

Fig. 1. Overview of B cell pathophysiology in MS.

Legend: APC – antigen-presenting cell; CNS – central nervous system; Ab – antibody; CSF – cerebrospinal fluid, MHC – major histocompatibility complex.

Fig. 2. B cell lineage and surface CD19 and CD20 expression.

Fig. 3. Milestones in CD20-mAb therapy for MS.

Legend: RTX – rituximab, OCR – ocrelizumab, OFA – ofatumumab, UTX – ublituximab.