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"Monoclonal antibodies in the treatment of MS: emergence of B-cell targeted therapies"

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS, and one of the most common causes of disability in young adults. Over the last decade, new disease modifying therapies have rapidly emerged, including monoclonal antibodies (mAbs) that have provided highly targeted therapies with superior efficacy compared to platform therapies. In particular, monoclonal antibodies directed against CD20-positive B cells have shown remarkable results in recent clinical trials, and renewed interest in the mechanism of B-cell depleting therapies to ameliorate relapse activity and progression in MS. In this manuscript, we will review the mechanisms of action and clinical evidence of approved and emerging mAbs, with a focus on B-cell targeted therapies.

Abbreviations

ADCC: antibody-dependent cell-mediated cytotoxicity

APC: antigen presenting cell

ARR: annualised relapse rate

BAFF: B-cell activating factor

CDC: complement-dependent cytotoxicity

CDP: confirmed disability progression

EAE: experimental autoimmune encephalomyelitis

EC: European Commission

EDSS: Expanded Disability Status Scale

EMA: European Medicines Agency

FDA: Food and Drug Administration

Gd: gadolinium

HYP: high yield protein

IFN-β: infereron-beta

JCV: John Cunningham Virus

mAb: monoclonal antibody

MS: multiple sclerosis

NHS: National Health Service

NICE: National Institute for Health and Care Excellence

NK: natural killer

PML: progressive multifocal leukoencephalopathy

PPMS: primary progressive multiple sclerosis

RRMS: relapsing remitting multiple sclerosis

Th: T-helper

Article

1. Multiple Sclerosis

MS can be broadly divided into two, often overlapping clinical courses: that of relapsing MS, characterised by clearly defined attacks of new or worsening neurological symptoms, or

progressive MS where there is worsening neurologic function independent of relapses. Clinical trials over the last 25 years have been productive in discovering an ever increasing list of medications effective in preventing relapses. However the search for therapies to reduce or halt progression in progressive MS has remained elusive until recently, when a new anti-CD20 monoclonal antibody, ocrelizumab, was found to significantly reduce progression in a phase III trial for primary-progressive MS (Montalban et al., 2016). The emergence of monoclonal antibodies in the treatment of MS, in particular those targeting B-cell antigens, will be discussed in further detail.

1.1. Pathology of MS

The pathology of MS is characterised by inflammatory demyelinating lesions, which affect the grey and white matter of the CNS. It is thought that the failure of myelin repair mechanisms, oligodendrocyte loss, and cumulative axonal and neuronal loss, are the main drivers of disability progression in this disease (Lassmann, 2014). Although the triggers for MS remain unknown, it is widely posited that autoreactive CD4+ T-helper (Th) cells, activated in the periphery by molecular mimicry, bystander activation or viral persistence, are central to the initiation of inflammatory demyelinating lesions in MS. This is based mainly on evidence derived from the most commonly used experimental model of MS, experimental autoimmune encephalomyelitis (EAE), where CNS autoimmunity is induced by inoculation with CNS antigens or by the adoptive transfer of myelin specific Th1 or Th17 cells (Robinson et al., 2014; Ben-Nun et al., 1981; Jager et al., 2009). In the EAE model, autoreactive T-cells are necessary for blood brain barrier breakdown and inflammatory CNS lesion development. A role for CD4+ T-cells in MS pathogenesis is also supported by histopathological observations of these cells in MS brain lesions (Traugott et al., 1983); the increased risk of MS in association with genetic variation in the human leukocyte antigen gene region (i.e. the HLADR15 haplotype), which encodes for genes that have a critical role in antigen presentation to T-cells (International Multiple Sclerosis Genetics Consortium, 2005); and functional studies that suggest differences in the frequency and activity of myelin reactive CD4+ T cells in MS cases compared to controls (Pender et al., 2000; Olsen et al., 1992; Zhang et al., 1994). However, there is now increasing evidence to support a role for B-

lymphocyte (B-cells) cells as key effectors in the pathogenesis of MS, principally led by the therapeutic success of antibody-mediated B-cell killing.

1.2. B cells in MS

B-cells develop from haematopoietic stem cells in the bone marrow, and upon entering the circulation, they mature into naïve B-cells. Following exposure to antigen, B-cells undergo a proliferative phase where they develop into antibody producing plasma blasts/plasma cells, or memory B-cells that are primed to respond following re-exposure to the same antigen (reviewed in Claes et al., 2015; summarised in Figure 1). Terminally differentiated B cells can also accumulate and reside in the bone marrow as a lifetime source of stable antibody producing cells.

Antibodies, visualised as intrathecal oligoclonal bands, have been used to diagnose MS for decades (Correale & de los Milagros Bassani Molinas, 2002). Despite the presence of these intrathecal oligoclonal bands in some MS patients, and evidence for antibody deposits in some MS lesions (Lucchinetti et al., 2000), the role of antibodies as a component of the primary pathogenesis of MS, or alternatively, as an epiphenomenon, has remained controversial. Interestingly, elevated antibodies to myelin components including myelin basic protein, myelin proteolipid protein, and myelin associated-glycoprotein (Gorny et al., 1983) as well as various other CNS and non-CNS antigens (reviewed in Fraussen et al., 2014), have been identified in MS patients. However, proof of one or many 'MS autoantigens' is lacking. Antibody production is the function of plasma cells, and many of these cells are long-lived and not targeted by anti-CD20 therapies. However, non-plasma B cells also exert multiple other effector functions including cytokine production and antigen presentation, which could provide an explanation for pathogenic contribution of these cells in MS (Figure 2). Several studies, for instance, suggest that MS could be associated with abnormal B cell cytokine responses, including the production of abnormally high levels of TNF-α and lymphotoxin-α (Duddy et al., 2007, Bar-Or et al., 2010), and an increase in the frequency of granulocyte macrophage-colony stimulating factor (GM-CSF) producing B-cells relative to healthy controls (Li et al., 2015), following B-cell stimulation via CD40 and the B cell receptor. It

has also been shown that B cells from MS patients secrete more pro-inflammatory IL-6 and less regulatory IL-10 compared to controls (Duddy et al., 2007)

As antigen-presenting cells (APCs), B cells could play a role in the presentation of auto-antigens to CD4+ T cells, hence promoting Th1 and Th17 responses. EAE models have demonstrated that while B cells are not the sole APC involved, their activity increases disease severity (Parker Harp et al., 2015). Studies in humans of CD80 and CD86, co-stimulatory molecules that help activate T cells, provide further evidence for a role of B-cells as antigen presenting cells. For example, the GM-CSF expressing B cell subset described by Li et al. (2015) upregulates CD80 and CD86 when activated. Additionally, CD80 and CD86 expression is higher in MS patients than healthy controls, and CD80+ lymphocyte levels increase in MS patients during exacerbations (Aung & Balashov, 2015). Therefore, B cells may be involved in MS not just as sources of cytokines and autoantibodies, but also as APCs that stimulate T cells.

Although the adaptive immune system has not been traditionally viewed as playing a role in progressive MS, descriptions of lymphoid follicle-like structures in the meninges surrounding CNS tissue of secondary progressive MS cases, suggest that B-cells could also play a role in progressive disease (Serafini et al., 2004).

Finally, the most convincing evidence for a role of B-cells in MS pathogenesis is derived from trials with B-cell depleting therapies that have shown efficacy in both relapsing-remitting and primary-progressive forms of the disease. The insights gained from MS therapeutic trials with B-cell depleting monoclonal antibody (mAb) therapies, will be discussed in more detail.

2. Monoclonal antibody therapies for relapsing MS: Efficacy, and Mechanisms of action.

2.1. Approved therapies

2.1.1. Natalizumab

Natalizumab is a humanised IgG4 monoclonal antibody (mAb) directed against the α4 subunit of the α4β1 and α4β7 integrins expressed on the surface of leukocytes (Rice et al.,

2005). Binding to α4β1-integrin (also known as very late antigen-4 or VLA-4) inhibits its interaction with vascular cell adhesion molecule-1 (VCAM-1) expressed on cerebral vascular endothelial cells, and prevents migration of leukocytes through the blood brain barrier (Yednock et al., 1992). Natalizumab may also block binding of α4β1-integrin with other ligands such as fibronectin and osteopontin (Yaldizli & Putzki, 2009), further modulating leukocyte recruitment and activation in the CNS.

These effects on leukocyte entry, and in particular, pathogenic T-cell entry into the CNS; are thought to be the main mechanism by which natalizumab therapy reduces MS disease activity. However, it is important to consider that natalizumab therapy also likely inhibits B-cell entry into the CNS, with reports of reduced numbers of B-cells in CSF, and increased B cell numbers in the peripheral circulation of natalizumab treated MS patients (Stuve et al., 2006; Krumbholz et al., 2008). Furthermore, a disproportionate increase in peripheral pre B-cells has been reported in MS patients following natalizumab therapy (Krumbholz et al., 2008; Saraste et al 2016). Hence, it remains possible that the effects of natalizumab in MS are at least partially mediated through actions on B-cells.

The phase III AFFIRM trial recruited 942 relapsing-remitting MS (RRMS) patients randomised 2:1 to receive monthly intravenous natalizumab 300mg or placebo over 2 years (Polman et al., 2006). In the natalizumab group, the annualised relapse rate (ARR) was reduced by 68% over 2 years compared to placebo, and 3-month confirmed disability progression (CDP) was reduced by 42% (see Table 1 for summary of clinical trial results).

The SENTINEL study was another phase III trial that enrolled 1171 RRMS patients randomised equally to natalizumab combined with weekly intramuscular interferon beta-1a (IFN-β1a) 30μg or IFN-β1a alone (Rudick et al., 2006). Over 2 years, the natalizumab add-on therapy reduced ARR by 55% compared to IFN-β1a alone, and reduced 3-month CDP by 24%.

In 2004, natalizumab was the first mAb to be approved by the US Food and Drug Administration (FDA) for MS. It was subsequently suspended in 2005 after two cases of progressive multifocal leukoencephalopathy (PML) were reported in the SENTINEL trial (Rudick et al., 2006). PML is a serious, often fatal demyelinating disease of the CNS, caused

by infection of oligodendrocytes by the John Cunningham Virus (JCV). It has an increased incidence in those with the following three risk factors: the presence of anti-JCV antibodies, natalizumab treatment for ≥2 years, and prior use of immunosuppressive agents (Bloomgren et al., 2012). Following implementation of a risk-management program for PML, the FDA approved it again in 2006 and as did the European Union in the same year. A 2016 Europeans Medicines Agency (EMA) label update further describes the annualised risk of PML in patients receiving natalizumab segregated by serum anti-JCV antibody level, with patients at lower levels (<0.9) and those found to be repeatedly negative at very low risk (EMA, 2016). The clinical efficacy of natalizumab has been highlighted recently in real-world observational studies. Spelman et al demonstrated superiority of natalizumab over platform therapies when used first-line in treatment-naïve RRMS patients, with a 68% relative reduction in ARR (Spelman et al., 2016). Other studies showed that when natalizumab was used as a second-line agent, it was more effective than fingolimod in terms of ARR reduction and/or short-term disability burden (Kalincik et al., 2015; Lanzillo et al., 2015; Barbin et al., 2016), and superior to alumtuzumab in facilitating disability regression, while comparable in relapse rate and disability progression outcomes in that comparison (Kalincik et al., 2017).

2.1.2. Alemtuzumab

Alemtuzumab is a humanised monoclonal IgG1 antibody targeting CD52, a surface antigen that is expressed at high levels on T and B cells and to a lesser extent on monocytes and macrophages, but not on haematopoietic stem cells (Williams et al., 2013; Jones & Coles, 2014). Alemtuzumab leads to a rapid and profound depletion of CD52+ cells by three mechanisms: antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and induction of apoptosis (Freedman et al., 2013; Ruck et al., 2015); with ADCC being the likely predominant mechanism (Lycke, 2015; Knier et al., 2014). This is followed by repopulation of peripheral T and B lymphocytes with an alteration in the number, proportions and functions of certain lymphocyte subsets, such as increased regulatory T-cell subsets and memory T-cells (Milo, 2016; Hartung et al., 2015).

Changes in the reconstituting B-cell pool have also been reported following alemtuzumab therapy, with a predominance of immature, and later, naïve-memory B-cell subsets observed in association with increased serum levels of the B-cell survival factor, B-cell activating

factor (BAFF) (Thompson et al., 2010). It was also noted that the recovery of memory B cells was incomplete in alemtuzumab treated patients by 12 months. It has been proposed that changes in the proprotions of reconstituting immune cell subsets could be associated with long-term efficacy of alemtuzumab in MS (Havari et al., 2014).

CARE-MS I was a phase III trial of 581 treatment-naive RRMS patients, randomised 2:1 to receive alemtuzumab or subcutaneous IFN-β1a over 2 years (Cohen et al., 2012). Alemtuzumab was given intravenously 12mg daily for 5 days at baseline and for 3 days at 12 months, whilst subcutaneous IFN-β1a 44μg was given three times a week. This study found a significant 55% reduction in ARR in the alemtuzumab group compared to the IFN-β1a control group. There was no significant difference in 6-month CDP.

CARE-MS II was another phase III trial of 840 RRMS patients who had relapsed on platform therapy (Coles et al., 2012). Patients were randomised 1:2:2 to subcutaneous IFN-β1a 44μg, intravenous alemtuzumab 12mg or 24mg, though the 24mg arm was discontinued to aid recruitment. Similar to the CARE-MS I results, the alemtuzumab arm had a significant reduction of 49% in ARR compared to IFN-β1a. However in contrast to CARE-MS I, this study found a significant difference in 6-month CDP, with a 42% improvement in the alemtuzumab arm.

Infusion reactions occurred in over 90% of alemtuzumab patients. Infections were more frequent, in particular herpes infections. Secondary autoimmunity is the major long-term adverse event, with thyroid disease occurring in a third of patients, immune thrombocytopenia in 1% and anti-glomerular basement membrane or other nephropathies in 0.3% (Freedman et al., 2013; Lycke, 2015).

Based on the above phase III randomised control studies, the EMA approved alemtuzumab for the treatment of RRMS in September 2013, followed by the UK National Institute for Health and Care Excellence (NICE) in May 2014 and the US FDA in November 2014.

2.1.3. Daclizumab

Daclizumab is a humanised IgG1 mAb targeting the CD25 (α) subunit of high affinity interleukin-2 (IL-2) receptor on activated T cells (Bielekova et al., 2009). Anti-CD25 mAb decreased T-cell proliferation and differentiation in vitro (Kircher et al., 2003) but failed to

suppress T cell proliferation and cytokine production ex vivo (Bielekova et al., 2006). Instead it was found to strongly induce expansion of immunoregulatory CD56^{bright} natural killer (NK) cells, which can utilise IL-2 via their low affinity IL-2 receptor (Knier et al., 2014). This expansion was associated with response to therapy in MS patients, presumably through NK cell-mediated lysis of autologous activated T-cells (Bielekova et al., 2006). More recent work suggests that daclizumab may have other effects on the innate immune system which include the inhibition of antigen-specific T cell activation by dendritic cells (Wuest et al., 2011), and the inhibition of CD40 ligand expression on T-cells (Snyder et al., 2012). Interestingly, there is also limited evidence to suggest that daclizumab treatment can inhibit intrathecal CXCL13 and immunoglobulin G in MS patients, proposed to be mediated indirectly through effects on innate lymphoid cells (Perry et al., 2012).

Daclizumab was used in an early phase II MS trial (Wynn et al., 2010), whilst daclizumab high yield protein (HYP) has been used in more recent phase II and III trials (Gold et al., 2013; Kappos et al., 2015). Daclizumab HYP has the same amino acid sequence as previous versions, but differs in the glycosylation profile, resulting in less immunogenicity as well as less ADCC (Gold et al., 2013).

The phase II SELECT trial was the first to use daclizumab HYP (Gold et al., 2013). 621 RRMS patients were randomised 1:1:1 to monthly subcutaneous daclizumab HYP high dose 300mg, daclizumab HYP low dose 150mg or placebo and followed up for 52 weeks. There was a reduction in ARR of 54% and 50% in the low dose and high dose groups respectively compared to placebo. There was a significant 57% reduction in 3-month CDP in the low dose group only. In the trial, CD56^{bright} NK cells increased in daclizumab patients from 0.6% of lymphocytes at baseline to 3.6% at the end of treatment, first becoming apparent by week 4. CD4+ and CD8+ T cells decreased by 7-10% at week 52.

The phase III DECIDE trial randomised 1841 RRMS patients equally to monthly subcutaneous daclizumab HYP 150mg or weekly intramuscular IFN-β1a 30ug, followed up to 144 weeks (Kappos et al., 2015). The daclizumab arm had a 45% reduction in ARR compared to IFN-β1a. No significant change in 3-month CDP was observed.

In both the SELECT and DECIDE trials, there was an increased incidence in the daclizumab HYP groups of infections (nasopharyngitis, upper respiratory tract infections), cutaneous events (especially rash and eczema) and raised serum alanine aminotransferase or aspartate transaminase more than 5 times the upper limit of normal (Gold et al., 2013; Kappos et al., 2015). No PML has been reported.

Based on the SELECT and DECIDE trial results, daclizumab HYP was officially approved for RRMS by the FDA in May 2016, and by the European Commission (EC) in July 2016. NICE made an initial decision in September 2016 not to recommend daclizumab be made available on the National Health Service (NHS) in England and Wales, with a final statement expected in 2017.

2.2. Emerging therapies: monoclonal antibodies targeting B-cell antigens

Thus far, three anti-CD20 mAb therapies (rituximab, ocrelizumab and ofatumumab) have been evaluated in phase II and III clinical trials, and have shown high efficacy in relapsing MS. Importantly, ocrelizumab has also shown some efficacy in primary progressive MS (PPMS). The B lymphocyte antigen CD20 (also known as B1) is a 33-35 kD integral membrane spanning protein, belonging to the MSA4 protein family. The CD20 protein is expressed by B cells from the pre B-cell to the mature B-cell stage of development (Stashenko et al., 1980; Milo, 2016) and on a subset of T-cells (Palanichamy et al., 2014). The functions of CD20 are not well characterised, but there is some evidence that it could regulate cell cycle progression (Bubien et al., 1993; Kanzaki et al, 1995) and B-cell activation through effects on calcium mobilisation and signalling (Morsy et al., 2013). As CD20 is ubiquitously expressed on the surface of B-cell lineage cells, except stem cells and plasma cells (Figure 1), it has been demonstrated that anti-CD20 mAb therapies can be used to rapidly deplete peripheral blood B-cells whilst maintaining humoral immune responses (i.e. plasma-cell mediated antibody production).

2.2.1. Rituximab

Rituximab is a chimeric mouse-human IgG1k mAb that binds to the CD20 cell-surface epitope on circulating B cells (Reff et al., 1994). It lyses peripheral B-cell populations through a threefold mechanism of action: a combination of ADCC, CDC and possibly

promotion of apoptosis (Dubey et al., 2013). Rituximab is approved for patients with non-Hodgkin's lymphoma, chronic lymphocytic leukaemia, rheumatoid arthritis, granulomatous polyangiitis and microscopic polyangiitis (Gasperi et al., 2016).

The phase II 48-week HERMES trial randomised 104 RRMS patients to receive rituximab or placebo (Hauser et al., 2008). Rituximab was given intravenously as a single course of 1g on day 1 and 15. The primary endpoint was number of gadolinium (Gd)-enhancing lesions, a radiological marker of recent inflammatory activity, and there was a 91% reduction in total number of Gd-enhancing lesions up to week 24 in the rituximab group relative to placebo. ARR in the rituximab arm was only significantly reduced at week 24, and not at week 48 compared to placebo.

Another Phase II study used rituximab as an add-on to platform therapies in 30 RRMS patients with clinical and radiological activity despite treatment with disease modifying therapy (Naismith et al., 2010). Rituximab was given as four weekly doses of 375mg/m^2 intravenously and patients were followed-up over a year. The rituximab arm had an 88% reduction in Gd-enhancing lesions from pre-treatment to post-treatment scans. Whilst the study was not powered to assess relapse rate reduction, the ARR was reduced at week 52 post-treatment. Secondary endpoints such as the Expanded Disability Status Scale (EDSS) remained stable over 32 weeks.

A Phase II/III placebo-controlled trial in PPMS (OLYMPUS) was conducted on 439 patients, randomised 2:1 to receive rituximab 1g or placebo intravenously every 24 weeks through to 96 weeks and they were followed-up to 122 weeks (Hawker et al., 2009). No significant difference was seen in the 3-month CDP. The rituximab arm had significantly less increase in T2 lesion volume though brain volume change was similar between the groups. Interestingly, subgroup analyses showed that CDP was reduced in rituximab patients who were younger, <51 years and had Gd-enhancing or active inflammatory MRI lesions.

In both the HERMES and OLYMPUS trials, the incidence of adverse events and infections was similar between groups, though more infusion reactions were seen with the first rituximab dose only (Hauser et al., 2008; Hawker et al., 2009). No cases of PML have been reported in the MS trials.

Whilst the above studies show promise for the use of rituximab in MS, no further trials are planned. This is in part due to licensing and patent issues, as well as the emergence of next-generation anti-CD20 antibodies, as discussed below. Although rituximab has not been approved for the treatment of MS, it can be approved for off-label use in certain countries, and recent studies from the Swedish MS register provide insights into the real-world experience of using off-label rituximab. In a heterogenous cohort of 822 patients, Salzer et al demonstrated comparable safety and efficacy of rituximab to that reported in earlier trials (Salzer et al., 2016). Using the same registry, Alping et al found superior efficacy and tolerability of rituximab compared to fingolimod in 256 stable RRMS patients who had switched from natalizumab due to JCV antibody positivity (Alping et al., 2016). The findings of these observational studies further support the utility of this anti-CD20 mAb in MS.

2.2.2. Ocrelizumab

Ocrelizumab is a recombinant humanised IgG1 antibody that binds to a different but overlapping epitope compared with rituximab, and is thought to bind with higher affinity to CD20 (Sorensen & Blinkinberg, 2016). As it is a humanised molecule, it is expected to be less immunogenic with repeated infusions and hence, to have a more favourable benefit-to-risk profile (Menge et al., 2016). Compared to rituximab, ocrelizumab has increased ADCC and reduced CDC due to differences in the Fc portion of the antibody (Kappos et al., 2011). It has previously been trialled in rheumatoid arthritis, systemic lupus erythematosus and non-Hodgkin's lymphoma.

Two identical phase III trials in RRMS patients (OPERA I & II) were recently published (Hauser et al., 2016). A total of 1656 patients were randomised 1:1 to receive ocrelizumab 600mg intravenously every 24 weeks or IFN-β1a 44ug subcutaneously three times a week over a 96 week treatment period. In the ocrelizumab arms, the ARR at 2 years were reduced by 46% and 47% compared to the IFN-β1a arms. A pooled analysis combining both studies showed a relative reduction of 40% in 3-month and 6-month CDP in the ocrelizumab arms. The phase III PPMS study (ORATORIO) results were also recently published (Montalban et al, 2016). 732 patients with PPMS were randomised 2:1 to receive two 300mg infusions of ocrelizumab or placebo 14 days apart, every 24 weeks to 120 weeks. Compared to placebo,

the ocrelizumab arm had a significant 24% reduction in 3-month CDP and 25% reduction in 6-month CDP.

There were more infusion-related events in the ocrelizumab groups, highest after the first infusion and mostly mild-moderate in severity (Kappos et al., 2011; Hauser et al., 2015; Montalban et al., 2015). There were no opportunistic infections and no cases of PML. Interestingly, the clinical development of ocrelizumab in phase III trials of rheumatoid arthritis and systemic lupus erythematosus were suspended due to safety concerns surrounding the high incidence of serious and fatal opportunistic infections (Menge et al., 2016). It remains unclear why ocrelizumab was associated with a less favourable safety profile in these trials. However it has been suggested that differences in patient population (i.e. older age, Asian ethnicity), higher dosing and use of adjunct immunosuppressive therapies may have been contributing factors (Sheridan, 2015; Emery et al., 2014; Sorenson & Blinkenberg, 2016).

The overall incidence of malignancies was higher in patients treated with ocrelizumab, 0.40 per 100 patient-years of exposure, compared with 0.20 per 100 patient-years of exposure in the pooled comparator groups (Hauser et al., 2016; Montalban et al., 2016). Whilst the numbers of cancers seen post ocrelizumab give some cause for concern, longer-term studies will provide a more accurate assessment of these risks.

Based on the phase III RRMS and PPMS studies, the FDA granted ocrelizumab priority review in mid 2016 with a decision expected in December 2016 or early 2017. The EMA received an application for centralised marketing authorisation of ocrelizumab in November 2016.

2.2.3. Ofatumumab

Ofatumumab is a fully human IgG1 anti-CD20 mAb that binds to an epitope distinct from rituximab and ocrelizumab (Sorensen & Blinkenberg, 2016; Knier et al., 2014). It is proposed to have improved efficacy over rituximab associated with a slower dissociation rate and more pronounced CDC activity with relatively less ADCC (Milo, 2016; Reagan & Castillo, 2011). In vitro studies have shown that ofatumumab depletes B-cell lines resistant to rituximab (Wierda et al., 2011; Gupta & Jewell, 2012). As a fully human antibody, it has a very low immunogenic risk profile that could be associated with improved safety. It has demonstrated

efficacy in rheumatoid arthritis and haematological malignancies and is currently approved for treatment of refractory chronic lymphocytic leukaemia (Ostergaard et al., 2010; Gupta & Jewell, 2012).

In 2014, a phase II RRMS trial randomised 38 patients to receive either placebo or 2 infusions of ofatumumab (100mg, 300mg, 700mg) 14 days apart (Sorensen et al., 2014). Another phase II RRMS placebo-controlled study (MIRROR) was conducted using subcutaneous ofatumumab (Bar-Or et al., 2014). 232 patients were randomised into one of five treatment groups: placebo, ofatumumab 3mg every 12 weeks, 30mg every 12 weeks, 60mg every 12 weeks or 60mg every 4 weeks. At week 12, placebo patients received a single 3mg ofatumumab dose. The MRI results of both studies show profound reductions on new Gd-enhancing lesions and/or T2 hyperintense lesions (Table 1).

In these phase II trials, there were no unexpected safety signals or dose-related safety concerns. Infusion-related reactions were more common in the ofatumumab arm for both studies. No opportunistic infections were reported (Bar-Or et al., 2014; Sorensen et al., 2014).

Two randomised, double-blind, double-dummy phase III clinical trials are currently underway to evaluate the efficacy and safety of ofatumumab compared to teriflunomide in RRMS (ASCLEPIOS I and II) [ClinicalTrials.gov identifier: NCT02792218/NCT02792231]. Patients will be randomised to receive either subcutaneous ofatumumab every 4 weeks or teriflunomide orally once daily. Recruitment started in September 2016 and is expected to be complete by July 2019.

2.2.1.1. Immune effects of CD20 monoclonal antibody therapies

Trials with CD20 mAb therapies commonly report a rapid depletion of peripheral CD19+ B-cells, followed by a recovery phase that occurs several weeks after the final infusion. In the HERMES trial, after one course of rituximab, there was rapid depletion of CD19+ peripheral B-cells from 2 weeks post treatment until week 24 (>95% reduction from baseline). By week 48, CD19+ cells returned to 31% of baseline (Hauser et al., 2008). The OLYMPUS trial saw a similar depletion of CD19+ B cells following rituximab therapy, with 35% of patients recovering peripheral B-cell counts to within normal limits by week 122 (Hawker et al., 2009). Phase II trials of ocrelizumab and ofatumumab in RRMS, suggest that these therapies could be associated with a similarly rapid, but more complete depletion of CD19+ peripheral

B cells relative to rituximab therapy (99-100%), though head-to-head comparisons are lacking (Kappos et al., 2011; Sorenson et al., 2014).

Importantly, as limited changes in immunoglobulin concentrations were reported with CD20 mAb therapies in clinical trials (Hauser et al., 2008; Kappos et al., 2011; Sorensen et al., 2014), it was suggested that the unchanged immunoglobulin levels and early effects of rituximab therapy make it unlikely that the beneficial outcomes in the studies are due to depletion of pathogenic autoantibodies from the peripheral circulation. Rather, it was proposed that CD20 mAb therapies are more likely to target B-cell cytokine production and antigen presenting functions as part of their mechanisms in MS.

Detailed analyses of the recovered B-cell subsets following a single cycle of rituximab therapy in MS patients suggest that rituximab therapy could, at least in the short term, promote reduced activity in the B-cell compartment even as B-cell numbers recover. In their study, Palanichamy et al (2014) demonstrated that replenishing B-cells are largely comprised of the naïve (IgD+/CD27-) and transitional B-cell subsets, possibly derived from pro B-cells that do not express CD20. The repletion of memory B-cell subsets was more delayed, occurring from around 37-52 weeks. It remains unclear how these changes in the B-cell compartment associate with disease activity in MS; and it is also unclear if these effects are sustained over-time. Nonetheless, the capacity for memory B-cell numbers to recover over time, suggests that some maintenance therapy may be required to achieve sustained therapeutic benefit with CD20 mAb therapies.

Although no significant effects on CD3+ T-lymphocyte cells were reported in the HERMES and OLYMPUS trials for rituximab in MS, there is some evidence to suggest that rituximab therapy could deplete a small subset of CD3+CD20dim T-cells (<10% of total $CD3^+$ cells) as part of its actions in MS (Palanichamy et al., 2014). Recent work suggests that CD20+ T-cells may show enhanced cytokine expression with stimulation in vitro (Schuh et al., 2016), however, it is not known if these cells contribute to MS pathogenesis or if their depletion is part of the mechanisms of rituximab therapy in MS. It is possible therefore, that CD20 mAb therapies may directly target both the B-cell and T-cell functions as part of their mechanisms in MS.

2.3. Other B-cell therapies in development for MS

In addition to the CD20 mAb therapies, several other biologicals targeting B-cell surface antigens or B-cell cytokine signalling molecules have also been trialled for MS. Importantly, the use of targeted therapeutics to modify B-cell functions has already begun to provide novel and often unexpected insights into the functions of B-cells in MS pathogenesis, suggesting that they are important contributors to immune regulation in MS.

2.3.1. CD19 monoclonal antibody therapies

The CD19 antigen is expressed throughout B-cell development, and in contrast to the CD20 antigen, is also present on plasmablasts/plasma cells (Levesque et al., 2008). Monoclonal antibodies against CD19 may therefore show improved efficacy over CD20 mAbs for MS, as they may deplete both circulating B-cells and pathogenic autoantibody levels (Tedder, 2009). In animal models, the anti-CD19 mAb, MEDI-551, is associated with prolonged depletion of most B-cells, including pre B-cells and antibody producing cells (Herbst et al., 2010; Yazawa et al., 2005). It remains unclear if CD19 mAb therapies will show efficacy for MS; however, a phase I randomised, blinded, placebo controlled dose-escalation study to assess the safety and tolerability of MEDI-551 in relapsing MS patients was recently completed (ClinicalTrials.gov identifier: NCT01585766). Future trials with these therapies are keenly anticipated as they may clarify the role of antibodies; and also CD3+CD20dim T-cells, which do not express CD19, in MS pathology.

2.3.2.Therapies targeting B-cell cytokine signalling molecules

The survival of B-cells has been shown to be dependent on both B-lymphocyte stimulating factor (BLyS/BAFF) and a proliferation inducing ligand (APRIL), endogenous proteins produced by activated T-cells, monocytes and neutrophils; these molecules and their receptors represent potential therapeutic targets for auto-immune diseases with a likely B-cell component (Schneider et al., 1999; Hahne et al., 1998).

Atacicept, a fusion protein comprising the extracellular domain of the BAFF/APRIL receptor TACI (Transmembrane activator and CAML interactor and human immunoglobulin FC domain), was developed as a biologic therapy with binding activity for both BAFF and ARPIL. In a phase II placebo controlled double blind trial of 255 RRMS cases, atacicept therapy was associated with reduction in circulating mature B-cells of up to 70% percent; and

dose dependent reduction in serum IgM, IgG and IgA levels, suggesting an effect on antibody producing plasma cells (Kappos et al., 2014). Unexpectedly however, atacicept therapy was associated with an increase in the frequency of relapses, suggesting that some B-cell/plasma cell functions are important for reducing autoimmune attacks in MS. An increase in the proportion of patients converting to clinically definite MS was also reported in trials with atacicept for optic neuritis (Sergott et al., 2015). It remains unclear why targeting of BAFF/APRIL is associated with pro-inflammatory effects in MS and optic neuritis, however these effects could be partially explained by the types of B-cell subsets that are depleted in association with the neutralisation of BAFF/APRIL (Lühder & Gold, 2014). Interestingly, studies in mice suggest that the survival of memory B-cells is independent of BAFF/APRIL signalling, and hence, atacicept may not efficiently deplete B-cells that are primed to respond to autoantigens (Benson et al., 2008). It was also suggested that APRIL and/or BAFF may be associated with the induction of IL-10 producing regulatory B-cell types (Yang et al., 2010; Hua, 2016), and hence, atacicept therapy may disrupt regulatory B-cell functions to shift immune balance towards a pro-inflammatory state (Lühder & Gold, 2014). Finally, as atacicept reduced immunoglobulin levels, these effects on plasma cells could be, in themselves, deleterious in MS, potentially by removal of protective auto-antibodies.

2.4. Future challenges for B cell therapies for MS

Monoclonal antibody therapies that are largely designed to deplete circulating lymphocytes or to prevent their migration into the CNS compartment, have been successfully used to modulate the immune response in MS. Although it has long been assumed that T-cells play an important role in the pathogenesis of this disease, targeted B-cell depleting therapies have also shown surprisingly good efficacy in clinical trials for MS, suggesting that B-cell functions may also contribute to the pathogenesis of this disease.

Importantly, despite promising results with CD20 monoclonal antibody therapies in trials for MS, it is important to note the multi-dimensional role of B cells as antigen presenting cells, antibody secreting cells, as well as potent sources of both pro-inflammatory and anti-inflammatory cytokines suggest that untargeted therapeutic manipulation of the B-cell compartment in MS could be associated with long-term deleterious effects. The most obvious

concern is long-term depletion of the slowly replenishing plasma cell compartment from the repeatedly targeted plasma cell pools, which could lead to potentially prolonged hypogammaglobulinemia.

Animal studies have highlighted the ability of particular B cell subsets to negatively regulate immune responses through the production of anti-inflammatory cytokines, which modulate pathogenic CD4+ T cell subtypes and innate immune cell responses, as well as via interactions with regulatory T cells. Reduced regulatory B cell outputs (i.e. IL-10) in MS patients and the severe exacerbation of disease in patients treated with atacicept, provide some perspective on the consequences of targeting the majority of B cell populations as a therapeutic strategy. Future studies will require a focus on the specific consequences of the various B cell therapies, with the ultimate goal being a balance between removal of the drivers of pathogenic immune responses versus maintenance of the beneficial immune regulatory functions of B cell subsets in MS.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

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References

Alexander SP, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E *et al.* (2015). The concise guide to PHARMACOLOGY 2015/16: Overview. Br J Pharmacol 172: 5729–5743.

Alping P, Frisell T, Novakova L, Islam-Jakobsson P, Salzer J, Bjorck A, *et al.* (2016). Rituximab versus fingolimod after natalizumab in multiple sclerosis patients. Ann Neurol 79**:** 950-958.

Aung LL, Balashov KE. (2015) Decreased Dicer expression is linked to increased expression of co-stimulatory molecule CD80 on B cells in multiple sclerosis. Mult Scler. 21(9):1131-8.

Baker D, Marta M, Pryce G, Giovannoni G, Schmierer K. (2017). Memory B Cells are Major Targets for Effective Immunotheraoy in Relapsing Multiple Sclerosis. EBioMedicine, http://dx.doi.org/10.1016/j.ebiom.2017.01.042.

Barbin L, Rousseau C, Jousset N, Casey R, Debouverie M, Vukusic S, *et al.* (2016). Comparative efficacy of fingolimod vs natalizumab: A French multicenter observational study. Neurology 86**:** 771-778.

Bar-Or A, Grove R, Austin D, Tolson J, Vanmeter S, Lewis E, *et al.* (2014). The MIRROR Study: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Dose-Ranging Study to Investigate the Safety and MRI Efficacy of Subcutaneous Ofatumumab in Subjects with Relapsing-Remitting Multiple Sclerosis (RRMS). Neurology 82(10 Suppl)**:** I7–1.

Bar-Or A, Fawaz L, Fan B, Darlington PJ, Rieger A, Ghorayeb C, Calabresi PA, Waubant E, Hauser SL, Zhang J, Smith CH. (2010). Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? Ann Neurol. 67(4):452-461.

Ben-Nun A, Wekerle H, Cohen IR. (1981). The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur J Immunol 11: 195-199

Benson MJ, Dillon SR, Castigli E, Geha RS, Xu S, Lam K-P, Noelle RJ. (2008). Cutting Edge: The Dependence of Plasma Cells and Independence of Memory B Cells on BAFF and APRIL. J Immunol 180(6): 3655-3659.

Bielekova B, Catalfamo M, Reichert-Scrivner S, Packer A, Cerna M, Waldmann TA, *et al.* (2006). Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. Proc Natl Acad Sci U S A 103**:** 5941-5946.

Bielekova B, Howard T, Packer AN, Richert N, Blevins G, Ohayon J, *et al.* (2009). Effect of anti-CD25 antibody daclizumab in the inhibition of inflammation and stabilization of disease progression in multiple sclerosis. Arch Neurol 66**:** 483-489.

Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, *et al.* (2012). Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med 366**:** 1870-1880.

Bubien JK, Zhou LJ, Bell PD, Frizzell RA, & Tedder TF (1993). Transfection of the CD20 cell surface molecule into ectopic cell types generates a Ca2+ conductance found constitutively in B lymphocytes. J Cell Biol 121**:** 1121-1132.

Claes N, Fraussen J, Stinissen P, Hupperts R, Somers V (2015). B Cells Are Multifunctional Players in Multiple Sclerosis Pathogenesis: Insights from Therapeutic Interventions. Front Immunol 6:642.

Cohen JA, Coles AJ, Arnold DL, Confavreux C, Fox EJ, Hartung HP, *et al.* (2012). Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. Lancet 380**:** 1819-1828.
Coles AJ, Twyman CL, Arnold DL, Cohen JA, Confavreux C, Fox EJ, *et al.* (2012). Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. Lancet 380**:** 1829-1839.
Correale J, de los Milagros Bassani Molinas M (2002). Oligoclonal bands and antibody responses in multiple sclerosis. J Neurol 249(4):375-89.
Duddy M, Niino M, Adatia F, Hebert S, Freedman M, Atkins H, Kim HJ, Bar-Or A. (2007). Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. J Immunol. 15;178(10):6092-9.
EMA (2016). EMA confirms recommendations to minimise risk of brain infection PML with Tysabri: European Medicines Agency.
Emery P, Rigby W, Tak PP, Dorner T, Olech E, Martin C, *et al.* (2014). Safety with ocrelizumab in rheumatoid arthritis: results from the ocrelizumab phase III program. PLoS One 9**:** e87379.
Fraussen J, Claes N, de Bock L, Somers V. (2014). Targets of the humoral autoimmune response in multiple sclerosis. Autoimmun Rev. 13(11):1126-37.
Freedman MS, Kaplan JM, & Markovic-Plese S (2013). Insights into the Mechanisms of the Therapeutic Efficacy of Alemtuzumab in Multiple Sclerosis. J Clin Cell Immunol 4.
Gasperi C, Stuve O, & Hemmer B (2016). B cell-directed therapies in multiple sclerosis. Neurodegener Dis Manag 6**:** 37-47.
Gold R, Giovannoni G, Selmaj K, Havrdova E, Montalban X, Radue EW, *et al.* (2013). Daclizumab high-yield process in relapsing-remitting multiple sclerosis (SELECT): a randomised, double-blind, placebo-controlled trial. Lancet 381**:** 2167-2175.
Górny MK, Wróblewska Z, Pleasure D, Miller SL, Wajgt A, Koprowski H. (1983). CSF antibodies to myelin basic protein and oligodendrocytes in multiple sclerosis and other neurological diseases. Acta Neurol Scand. 67(6):338-47.
Gupta IV, & Jewell RC (2012). Ofatumumab, the first human anti-CD20 monoclonal antibody for the treatment of B cell hematologic malignancies. Ann N Y Acad Sci 1263**:** 43-56.
Hahne M, Kataoka T, Schröter M, Hofmann K, Irmler M, Bodmer J-L, *et al.* (1998). APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. J Exp Med 188 (6): 1185-1190.
Hartung HP, Aktas O, & Boyko AN (2015). Alemtuzumab: a new therapy for active relapsing-remitting multiple sclerosis. Mult Scler 21**:** 22-34.
Hauser SL, Bar-Or A, Comi G, Giovannoni G, Hartung HP, Hemmer B, *et al.* (2016). Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N Engl J Med. DOI: 10.1056/NEJMoa1601277.
Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, *et al.* (2008). B-cell depletion with rituximab in relapsing-remitting multiple sclerosis N Engl J Med 358**:** 676-688.

Havari E, Turner MJ, Campos-Rivera J, Shankara S, Nguyen TH, Roberts B, Siders W, Kaplan JM. (2014). Impact of alemtuzumab treatmet on the survival and function of human refulatory T cells in vitro. Immunology 141(1): 123-131.
Hawker K, O'Connor P, Freedman MS, Calabresi PA, Antel J, Simon J, *et al.* (2009). Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. Ann Neurol 66**:** 460-471.
Herbst R, Wang Y, Gallagher S, Mittereder N, Kuta E, Damschroder M, *et al*. (2010). B-cell depletion in vitro and in vivo with an afucosylated anti-CD19 antibody. J Pharmacol Exp Ther 335(1):213-22.
Hua C, Audo R, Yeremenko N, Baeten D, Hahne M, Combe B, Morel J, Daïen C. (2016). A proliferation inducing ligand (APRIL) promotes IL-10 production and regulatory functions of human B cells. J Autoimmun 73:64-72.
International Multiple Sclerosis Genetics Consortium (2005). A high density screen for linkage in Multiple Sclerosis. Am J Hum Genet 77(3): 454-67.
Jäger A, Dardalhon V, Sobel RA, Bettelli E, Kuchroo VK. (2009). Th1, Th17, and Th9 Effector Cells Induce Experimental Autoimmune Encephalomyelitis with Different Pathological Phenotypes. The J Immunol 183 (11): 7169-7177.
Jones JL, & Coles AJ (2014). Mode of action and clinical studies with alemtuzumab. Exp Neurol 262 Pt A**:** 37-43.
Kalincik T, Brown JW, Robertson N, Willis M, Scolding N, Rice CM, *et al.* (2017). Treatment effectiveness of alemtuzumab compared with natalizumab, fingolimod, and interferon beta in relapsing-remitting multiple sclerosis: a cohort study. Lancet Neurol. DOI: 10.1016/S1474-4422(17)30007-8.

Kalincik T, Horakova D, Spelman T, Jokubaitis V, Trojano M, Lugaresi A, *et al.* (2015). Switch to natalizumab versus fingolimod in active relapsing-remitting multiple sclerosis. Ann Neurol 77**:** 425-435.

Kanzaki M, Shibata H, Mogami H, & Kojima I (1995). Expression of calcium-permeable cation channel CD20 accelerates progression through the G1 phase in Balb/c 3T3 cells. J Biol Chem 270**:** 13099-13104
Kappos L, Hartung H-P, Freedman MS, Boyko A, Radü EW, Mikol DD *et al* for the ATAMS Study Group. (2014). Atacicept in multiple sclerosis (ATAMS): a randomised, placebo-controlled, double-blind, phase 2 trial. Lancet Neurol 13: 353–63
Kappos L, Li D, Calabresi PA, O'Connor P, Bar-Or A, Barkhof F, *et al.* (2011). Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. Lancet 378**:** 1779-1787.
Kappos L, Wiendl H, Selmaj K, Arnold DL, Havrdova E, Boyko A, *et al.* (2015). Daclizumab HYP versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N Engl J Med 373**:** 1418-1428.
Kircher B, Latzer K, Gastl G, & Nachbaur D (2003). Comparative in vitro study of the immunomodulatory activity of humanized and chimeric anti-CD25 monoclonal antibodies. Clin Exp Immunol 134**:** 426-430.

Knier B, Hemmer B, & Korn T (2014). Novel monoclonal antibodies for therapy of multiple sclerosis. Expert Opin Biol Ther 14**:** 503-513.
Krumbholz M, Meinl I, Kumpfel T, Hohlfeld R, Meinl E (2008). Natalizumab disproportionately increased circulating pre-B and B cells in multiple sclerosis. Neurology. 71: 1350-1354.
Lanzillo R, Carotenuto A, Moccia M, Sacca F, Russo CV, Massarelli M, et al. (2016). A longitudinal real-life comparison study of natalizumab and fingolimod. Acta Neurol Scand. doi: 10.1111/ane.12718.
Lassmann H (2014). Multiple sclerosis: Lessons from molecular neuropathology. Exp Neurol 262 (Part A): 2–7.
Levesque MC, St Clair EW (2008). B cell-directed therapies for autoimmune disease and correlates of disease response and relapse. J Allergy Clin Immunol 121:13–21. 2–3
Li R, Rezk A, Miyazaki Y, Hilgenberg E, Touil H, Shen P, Moore CS, Michel L, Althekair F, Rajasekharan S, Gommerman JL, Prat A, Fillatreau S, Bar-Or A; Canadian B cells in MS Team. (2015). Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. Sci Transl Med. 7(310):310
Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H (2000). Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. Ann Neurol 47:707–717
Lühder F, Gold R (2014). Trial and error in clinical studies: lessons from ATAMS. Lancet Neurol, 13: 340–341.
Menge T, Dubey D, Warnke C, Hartung HP, & Stuve O (2016). Ocrelizumab for the treatment of relapsing-remitting multiple sclerosis. Expert Rev Neurother 16**:** 1131-1139.
Milo R (2016). Therapeutic strategies targeting B-cells in multiple sclerosis. Autoimmun Rev 15**:** 714-718.
Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, *et al.* (2016). Ocrelizumab versus Placebo in Primary Progressive Multiple Sclerosis. N Engl J Med. DOI: 10.1056/NEJMoa1606468.
Morsy DE, Sanyal R, Zaiss AK, Deo R, Muruve DA, & Deans JP (2013). Reduced T-dependent humoral immunity in CD20-deficient mice. J Immunol 191**:** 3112-3118.
Naismith RT, Piccio L, Lyons JA, Lauber J, Tutlam NT, Parks BJ, *et al.* (2010). Rituximab add-on therapy for breakthrough relapsing multiple sclerosis: a 52-week phase II trial. Neurology 74**:** 1860-1867.
Olsson T, Sun J, Hillert J, Höjeberg B, Ekre H -P, Anderson O, Olerup Increased numbers of T cells recognizing multiple myelin basic protein epitopes in multiple sclerosis. Eur J Immunol 22 (4): 1083-1087.
Ostergaard M, Baslund B, Rigby W, Rojkovich B, Jorgensen C, Dawes PT, *et al.* (2010). Ofatumumab, a human anti-CD20 monoclonal antibody, for treatment of rheumatoid arthritis with an inadequate response to one or more disease-modifying antirheumatic drugs: results of a randomized, double-blind, placebo-controlled, phase I/II study. Arthritis Rheum 62**:** 2227-2238.
Palanichamy A., Jahn S., Nickles D., Derstine M., Abounasr A., Hauser SL., Baranzini SE., Leppert D., von Budingen H-C. 2014. Rituximab efficiently depleted increased CD20-Expressing T-cells in Multiple Sclerosis Patients. The Journal of Immunology. 193: 580-586.

Parker Harp CR, Archambault AS, Sim J, Ferris ST, Mikesell RJ, Koni PA, Shimoda M, Linington C, Russell JH, Wu GF. (2015) B cell antigen presentation is sufficient to drive neuroinflammation in an animal model of multiple sclerosis. J Immunol. 194(11):5077-84.
Pender MP, Csurhes PA, Greer JM, Mowat PD, Henderson RD, Cameron KD, Purdie DM, McCombe PA, Good MF (2000). Surges of increased T cell reactivity to an encephalitogenic region of myelin proteolipid protein occur more often in patients with multiple sclerosis than in healthy subjects. J Immunol 165(9): 5322-5331.
Perry JS, Han S, Xu Q, Herman ML, Kennedy LB, Csako G, Bielekova B (2012). Inhibition of LTi cell development by CD25 blockade is associated with decreased intrathecal inflammation in multiple sclerosis. Sci Transl Med 4(145): 10.1126/scitranslmed.3004140.
Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, *et al.* (2006). A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 354**:** 899-910.
Reagan JL, & Castillo JJ (2011). Ofatumumab for newly diagnosed and relapsed/refractory chronic lymphocytic leukemia. Expert Rev Anticancer Ther 11**:** 151-160.
Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, *et al.* (1994). Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 83**:** 435-445.
Rice GP, Hartung HP, & Calabresi PA (2005). Anti-alpha4 integrin therapy for multiple sclerosis: mechanisms and rationale. Neurology 64**:** 1336-1342.
Robinson AP, Harp CT, Norohana ., Miller SD (2014). The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. Handb Clinl Neurol 122: 173-89.
Ruck T, Bittner S, Wiendl H, & Meuth SG (2015). Alemtuzumab in Multiple Sclerosis: Mechanism of Action and Beyond. Int J Mol Sci 16**:** 16414-16439.
Rudick RA, Stuart WH, Calabresi PA, Confavreux C, Galetta SL, Radue EW, *et al.* (2006). Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. N Engl J Med 354**:** 911-923.
Salzer J, Svenningsson R, Alping P, Novakova L, Bjorck A, Fink K, *et al.* (2016). Rituximab in multiple sclerosis: A retrospective observational study on safety and efficacy. Neurology 87**:** 2074-2081.
Saraste M, Penttilä T-L, Airas L. (2016). Natalizumab treatment leads to an increase in circulating CXCR3-expressing B cells. Neurol Neuroimmunol Neuroinflamm 3(6): e292.
Schneider P, Mackay F, Steiner V, Hofmann K, Bodmer J-L, Holler N, *et al.* (1999). BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. J Exp Med 189 (11): 1747-1756.
Schuh E, Berer K, Mulazzani M, Feil K, Meinl I, Lahm H, *et al.* (2016). Features of Human CD3+CD20+ T Cells. J Immunol 197 (4): 1111-1117.
Serafini B, Rosicarelli B, Magliozzi R, Stiglian E, Aloisi F (2004). Detection of ectopic b-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 14: 164-174.
Sergott RC, Bennett JL, Rieckmann P, Montalban X, Mikol D, Freudensprung U, *et al.* ATON Trial Group (2015). ATON: results from a Phase II randomized trial of the B-cell-targeting agent atacicept in patients with optic neuritis. J Neurol Sci 351(1-2):174-8.

Sheridan C (2015). Anti-CD20 antibody wows in multiple sclerosis. Nat Biotechnol 33**:** 1215-1216.
Snyder JT, Shen J, Azmi H, Hou J, Fowler DH, Ragheb JA (2011). Direct inhibition of CD40L expression can contribute to the clinical efficacy of daclizumab independently of its effects on cell division and Th1/Th2 cytokine production. Blood109(12): 5399–5406.
Sorensen PS, & Blinkenberg M (2016). The potential role for ocrelizumab in the treatment of multiple sclerosis: current evidence and future prospects. Ther Adv Neurol Disord 9**:** 44-52.
Sorensen PS, Lisby S, Grove R, Derosier F, Shackelford S, Havrdova E, *et al.* (2014). Safety and efficacy of ofatumumab in relapsing-remitting multiple sclerosis: a phase 2 study. Neurology 82**:** 573-581.
Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SP et al. (2016) The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. Nucl Acids Res 44:D1054-1068.
Spelman T, Kalincik T, Jokubaitis V, Zhang A, Pellegrini F, Wiendl H, *et al.* (2016). Comparative efficacy of first-line natalizumab vs IFN-beta or glatiramer acetate in relapsing MS. Neurol Clin Pract 6**:** 102-115.
Stashenko P, Nadler LM, Hardy R, & Schlossman SF (1980). Characterization of a human B lymphocyte-specific antigen. J Immunol 125**:** 1678-1685.
Stüve O, Marra CM, Jerome KR, Cook L, Cravens PD, Cepok S, Frohman EM, Phillips JT, Arendt G, Hemmer B, Monson NL, Racke MK. (2006). Immune surveillance in multiple sclerosis patients treated with natalizumab. Ann Neurol 59(5):743-7.
Tedder TF (2009) CD19: a promising B cell target for rheumatoid arthritis. Nat Rev Rheumatol 5:572–577.
Thompson SA, Jones JL., Cox AL, Compton DAS., Coles AJ. (2010). B-Cell Reconsitution and BAFF After Alemtuzumab (Campath-1H) Treatment of Multiple Sclerosis. J Clin Immunol 30: 99-105.
Traugott U, Reinherz EL, Raine CS (1983). Multiple sclerosis. Distribution of T cells, T cell subsets and Ia-positive macrophages in lesions of different ages. J Neuroimmunol 4(3):201-21.
Wierda WG, Padmanabhan S, Chan GW, Gupta IV, Lisby S, & Osterborg A (2011). Ofatumumab is active in patients with fludarabine-refractory CLL irrespective of prior rituximab: results from the phase 2 international study. Blood 118**:** 5126-5129.
Williams T, Coles A, & Azzopardi L (2013). The outlook for alemtuzumab in multiple sclerosis. BioDrugs 27**:** 181-189.
Wuest SC, Edwan J, Martin JF, Han S, Perry JA, Cartagena CM, *et al.* (2011). A vital role for IL-2 trans-presentation in DC-mediated T cell activation in humans as revealed by daclizumab therapy. Nat Med 17(5): 604–609.
Wynn D, Kaufman M, Montalban X, Vollmer T, Simon J, Elkins J, *et al.* (2010). Daclizumab in active relapsing multiple sclerosis (CHOICE study): a phase 2, randomised, double-blind, placebo-controlled, add-on trial with interferon beta. Lancet Neurol 9**:** 381-390.
Yaldizli O, & Putzki N (2009). Natalizumab in the treatment of multiple sclerosis. Ther Adv Neurol Disord 2**:** 115-128.

Yang M, Sun L, Wang S, Ko KH, Xu H, Zheng BJ, Cao X, Lu L (2010). Novel function of B cell-activating factor in the induction of IL-10-producing regulatory B cells. J Immunol 184(7):3321-5.
Yazawa N, Hamaguchi Y, Poe JC, Tedder TF. Immunotherapy using unconjugated cd19 monoclonal antibodies in animal models for b lymphocyte malignancies and autoimmune disease. Proc Natl Acad Sci U S A. 2005; 102:15178–15183.
Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, & Karin N (1992). Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature 356**:** 63-66.
Zhang J, Markovic-Plese S, Lacet B, Raus J, Weiner HL, Hafler DA (1994). Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 179: 973–984.

Figure Legends

Figure 1.

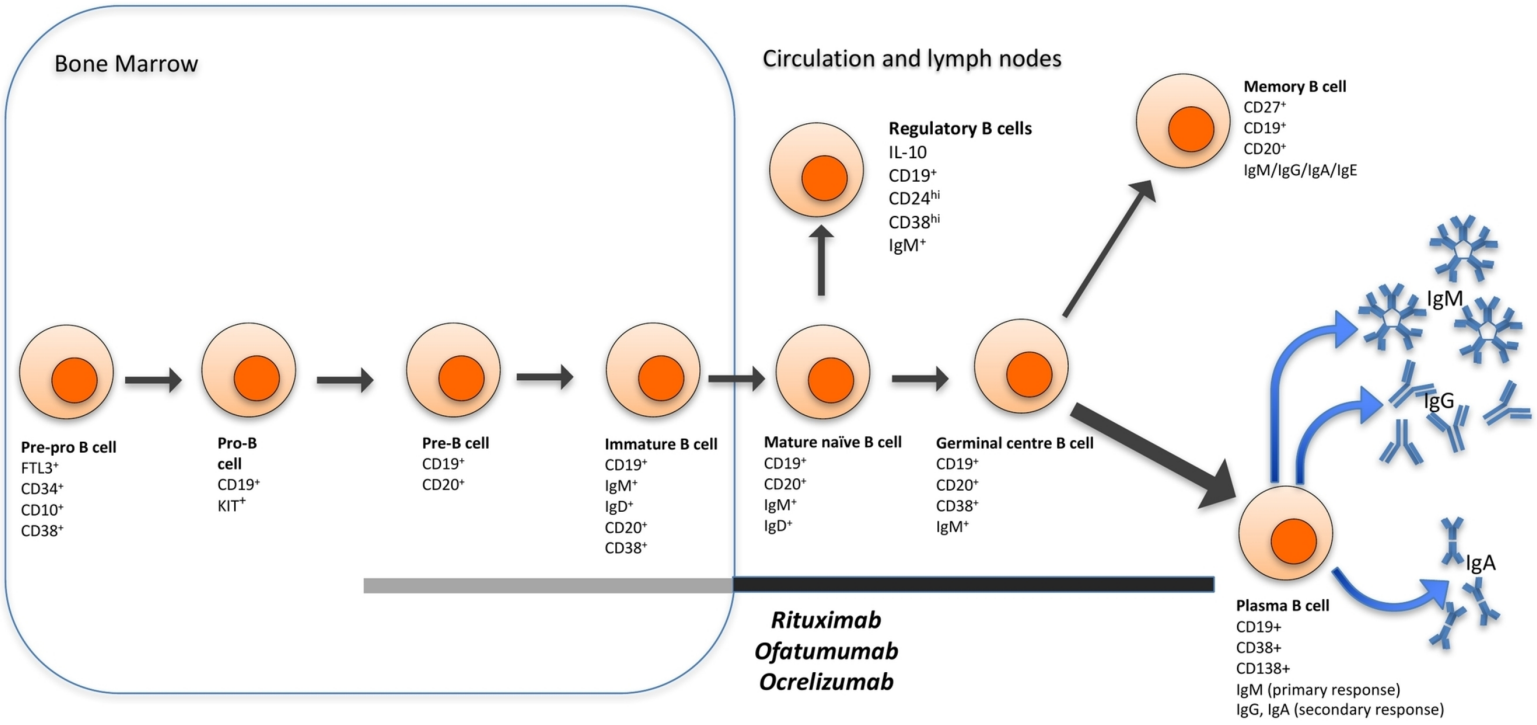
As B cells develop in the bone marrow, they express various combinations of surface molecules during the transition from pre-pro B cell to immature B cell. After leaving the bone marrow and becoming a mature naïve B cell, a small subset will become IL-10 secreting regulatory B cells. Others, upon activation, develop into plasma B cells or memory B cells. Initially, plasma B cells secrete IgM, but after class-switch recombination may secrete IgG, IgA, or IgE.

Rituximab, ofatumumab, and ocrelizumab are monoclonal antibodies that bind CD20, killing CD20+ cells through antibody-dependent and complement-dependent cell cytotoxicity. CD20 is expressed on pre-B cells, immature B cells, mature naïve B cells, germinal centre B cells, and memory B cells, rendering them vulnerable to these drugs. The black line indicates B cell subsets present in the periphery that are known to be affected by rituximab, ofatumumab, and ocrelizumab. The grey line indicates CD20+ cells in the bone marrow - because of their location, it is unclear whether these cells are affected by the treatments.

Figure 2.

B cell involvement in multiple sclerosis (MS). B cells contribute to MS pathogenesis by a) secreting pro- and anti-inflammatory cytokines, b) releasing antibodies including various autoantibodies, and c) acting as antigen presenting cells which present auto-antigens to CD4+ T cells, which together with cytokines promote Th1 and Th17 responses.

Figure 1



BPH_13780_f1.jpg

Figure 2

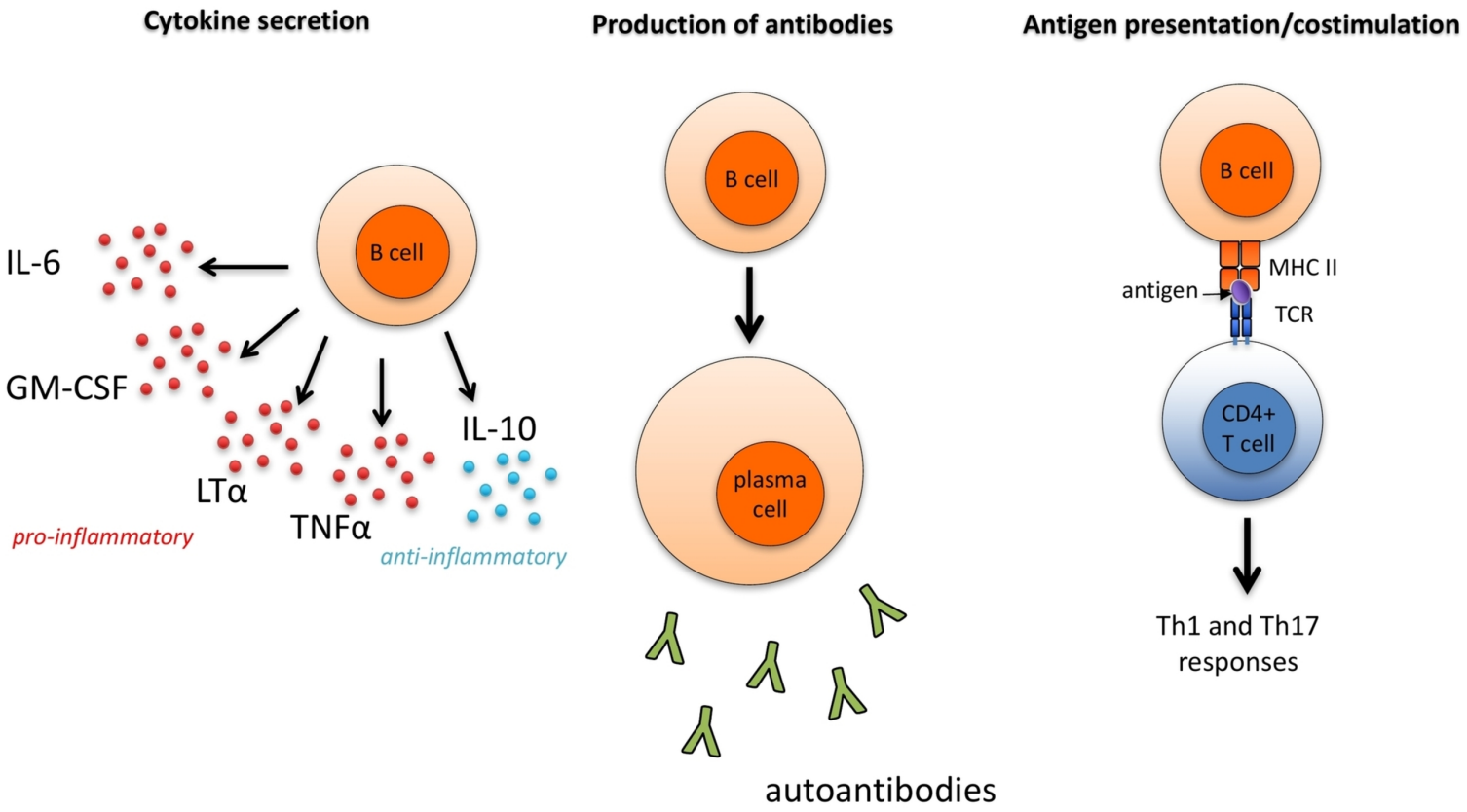


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Table 1. Clinical trials of monoclonal antibodies in multiple sclerosis (MS)

Active drug & MS subtype	Trial/study (Phase)	Sample size	Treatment/control arms	Duration (months)	ARR (percent reduction) (p-value)	Confirmed Disability Progression percent reduction (p-value)	Percent reduction in number of Gd-enhancing lesions (p-value)	Percent reduction in number of new/enlarging T2 hyperintense lesions (p-value)
Natalizumab								
RRMS	AFFIRM (Phase III)	942	Natalizumab i.v. Placebo	24	0.23 (68%) (p<0.001) 0.73	42% (p<0.001)*	92% (p<0.001)	83% (p<0.001)
RRMS	SENTINEL (Phase III)	1171	IFN-β1a i.m. + natalizumab IFN-β1a i.m. + placebo	24	0.34 (55%) (p<0.001) 0.75	24% (p=0.02)*	89% (p<0.001)	83% (p<0.001)
Alemtuzumab								
RRMS	CARE-MS I (Phase III)	581	Alemtuzumab i.v. IFN-β1a s.c.	24	0.18 (55%) (p<0.001) 0.39	30% (p=0.22)†	Reduction^{NS} (p<0.0001)	Reduction^{NS} (p=0.04)
RRMS	CARE-MS II (Phase III)	840	Alemtuzumab i.v. IFN-β1a s.c.	24	0.26 (49%) (p<0.001) 0.52	42% (p=0.008)†	Reduction^{NS} (p<0.0001)	Reduction^{NS} (p<0.0001)
Daclizumab								
RRMS	SELECT (Phase II)	621	Daclizumumab HYP s.c. 150mg Daclizumumab HYP s.c. 300mg Placebo	12	0.21 (54%) (p<0.001) 0.23 (50%) (p<0.001) 0.46	57% (p=0.02)* 43% (p=0.09)*	79% (p<0.0001) 86% (p<0.0001)	70% (p<0.0001) 79% (p<0.0001)
RRMS	DECIDE (Phase III)	1841	Daclizumumab HYP s.c. 150mg IFN-β1a i.m.	24	0.22 (45%) (p<0.001) 0.39	20% (p=0.16)*	60% (p<0.001)	54% (p<0.001)
Rituximab								
RRMS	HERMES (Phase II)	104	Rituximab i.v. Placebo	12	0.37 (49%) (p=0.08) 0.72	NS	91% (p>0.001)	NS
PPMS	OLYMPUS (Phase II/III)	439	Rituximab i.v Placebo	24	NS	22% (p=0.14)*	NS	NS
Ocrelizumab								
RRMS	OPERA I (Phase III)	821	Ocrelizumab i.v. IFN-β1a s.c.	24	0.16 (46%) (p<0.001) 0.29	(Combined OPERA I & II) 40% (p<0.001)*	94% (p<0.001) 95% (p<0.001)	77% (p<0.001) 83% (p<0.001)
RRMS	OPERA II (Phase III)	835	Ocrelizumab i.v. IFN-β1a s.c.	24	0.16 (47%) (p<0.001) 0.29	40% (p=0.003)†		
PPMS	ORATORIO (Phase III)	732	Ocrelizumab i.v. Placebo	24	NS	24% (p=0.03)* 25% (p=0.04)†	NS	NS
Ofatumumab								
RRMS	Sorensen et al. (Phase II)	38	Ofatumumab i.v. 100mg, 300mg, 700mg Placebo	6	NS	NS	>99% (p<0.001)	99% (p<0.001)

RRMS	MIRROR (Phase II)	232	Ofatumumab s.c. 3mg, 30mg, 60mg Placebo	6	NS	NS	≥90% ($p<0.001$) for doses ≥30mg	NS

ARR: annualised relapse rate; Gd: gadolinium; RRMS: relapsing-remitting MS; PPMS: primary progressive MS; HYP: high yield process; NS: not specified

* 3-month

† 6-month