REVIEW

Treatment of multiple sclerosis with Anti-CD20 antibodies

Barbara Barun¹, Amit Bar-Or*

Neuroimmunology Unit and Experimental Therapeutics Program, Montreal Neurological Institute and Hospital, McGill University Hospital Centre Montreal, Quebec, Canada

Received 2 February 2011; accepted with revision 8 April 2011

Abstract  The recently successful targeting of B cells in patients with multiple sclerosis (MS) using monoclonal antibodies (mAbs) targeting CD20 has established that it is no longer a question of whether B cells contribute, but how they contribute, to MS disease activity. Here, the focus will be to review results that have emerged over the last few years from clinical trials of different anti-CD20 mAbs in patients with MS. We will also consider the biological basis underlying the apparent therapeutic efficacy of B cell depletion in MS. To this end, we will draw on several instructive observations made in MS patients who were treated with the anti-CD20 mAb rituximab. While the initial application of rituximab to patients with MS was based on the concept that B cell depletion may translate into decreases in potentially pathogenic CNS-autoreactive antibodies, insights from these studies have underscored the importance of non-antibody mediated functions of B cells.

© 2011 Elsevier Inc. All rights reserved.

Contents

1. CD20 and the development of therapeutic anti-CD20 antibodies .................................................. 0
2. Anti-CD20 antibody Clinical Trials in Multiple Sclerosis .......................................................... 0
   2.1. Rituximab ......................................................................................................................... 0
   2.2. Ocrelizumab ............................................................................................................... 0
   2.3. Ofatumumab .............................................................................................................. 0
3. Insights into the therapeutic mode of action of B cell depletion in MS ........................................... 0
4. Conclusion ............................................................................................................................ 0
5. References ........................................................................................................................... 0

⁎ Corresponding author at: Neuroimmunology Unit, Montreal Neurological Institute, 3801 University Street, Suite # 111, Montreal, Quebec, Canada H3A 2B4. Fax: +1 514 398 7371.
E-mail address: amit.bar-or@mcgill.ca (A. Bar-Or).
¹ Current address: Medical Faculty, University of Zagreb, Zagreb, Croatia.

1521-6616/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2011.04.005

1. CD20 and the development of therapeutic anti-CD20 antibodies

The CD20 molecule is a transmembrane protein expressed on a broad range of cells of the human B cell lineage, from pre-B cells through naïve and memory B cells, but not on stem cells, pro-B cells, or differentiated plasma cells [1]. The function of CD20 is not fully elucidated, though the structure predicts major hydrophobic regions and it has been described as having features of a calcium channel with possible roles in B cell activation and differentiation [2, 3]. Rituximab (Rituxan, Genentech and BiogenIdec, RTX) represents the first genetically engineered chimeric anti-CD20 monoclonal antibody (mAb) that was found to target and efficiently deplete circulating CD20+ B cells in humans, and was approved in 1997 for the treatment of non-Hodgkin’s B-cell lymphomas [4]. The CD20 molecule was found particularly suitable for mAb targeting of B cell lymphomas due to its (i) high levels of expression on B cells of lymphoma patients; (ii) its propensity to remain on the cell surface without internalization after interaction with the antibody; and (iii) the apparent absence of free CD20 molecules in the serum which could otherwise compete with anti-CD20 mAb binding [5–7].

A growing interest in the potential benefits of targeting B cells in non-neoplastic conditions has lead to clinical trials of rituximab in several autoimmune disorders, resulting in its 2006 FDA approval, in combination with methotrexate, for rituximab in several autoimmune disorders, resulting in its approval in 1997 for the treatment of non-Hodgkin’s B-cell lymphomas [4]. The CD20 molecule was found particularly suitable for mAb targeting of B cell lymphomas due to its (i) high levels of expression on B cells of lymphoma patients; (ii) its propensity to remain on the cell surface without internalization after interaction with the antibody; and (iii) the apparent absence of free CD20 molecules in the serum which could otherwise compete with anti-CD20 mAb binding [5–7].

Mechanisms that are thought to contribute to how anti-CD20 mAbs deplete CD20-expressing cells include: (i) complement dependent cytotoxicity (CDC); (ii) antibody-dependent cellular cytotoxicity (ADCC); and (iii) induction of B cell apoptosis [8, 9].

A growing interest in the potential benefits of targeting B cells in non-neoplastic conditions has lead to clinical trials of rituximab in several autoimmune disorders, resulting in its 2006 FDA approval, in combination with methotrexate, for rituximab in several autoimmune disorders, resulting in its approval in 1997 for the treatment of non-Hodgkin’s B-cell lymphomas [4]. The CD20 molecule was found particularly suitable for mAb targeting of B cell lymphomas due to its (i) high levels of expression on B cells of lymphoma patients; (ii) its propensity to remain on the cell surface without internalization after interaction with the antibody; and (iii) the apparent absence of free CD20 molecules in the serum which could otherwise compete with anti-CD20 mAb binding [5–7].

Mechanisms that are thought to contribute to how anti-CD20 mAbs deplete CD20-expressing cells include: (i) complement dependent cytotoxicity (CDC); (ii) antibody-dependent cellular cytotoxicity (ADCC); and (iii) induction of B cell apoptosis [8, 9].

A growing interest in the potential benefits of targeting B cells in non-neoplastic conditions has lead to clinical trials of rituximab in several autoimmune disorders, resulting in its 2006 FDA approval, in combination with methotrexate, for rituximab in several autoimmune disorders, resulting in its approval in 1997 for the treatment of non-Hodgkin’s B-cell lymphomas [4]. The CD20 molecule was found particularly suitable for mAb targeting of B cell lymphomas due to its (i) high levels of expression on B cells of lymphoma patients; (ii) its propensity to remain on the cell surface without internalization after interaction with the antibody; and (iii) the apparent absence of free CD20 molecules in the serum which could otherwise compete with anti-CD20 mAb binding [5–7].

Mechanisms that are thought to contribute to how anti-CD20 mAbs deplete CD20-expressing cells include: (i) complement dependent cytotoxicity (CDC); (ii) antibody-dependent cellular cytotoxicity (ADCC); and (iii) induction of B cell apoptosis [8, 9].
breakthrough disease while on standard approved injectable disease modifying agents [15]. Breakthrough disease was defined by occurrence of a relapse within the 18 months prior to enrollment, while on active treatment with an approved immune modulating agent, and by the presence of at least one Gd-enhancing lesion on any of 3 pre-treatment brain MRIs. Thirty patients were included and received rituximab at a regimen of 375 mg/m$^2$ weekly x 4 doses. The authors reported reduced MRI disease activity, such that 74% of post-treatment MRI scans were free of Gd-enhancing lesions compared with 26% at baseline (p<0.0001). The median number of Gd-enhancing lesions was reduced from 1.0 to 0, and the mean number was reduced from 2.81 per month to 0.33 after treatment (88% reduction). MSFC was noted to be improved (p=0.02), while Expanded Disability Status Scale (EDSS) remained stable. The combination of rituximab with standard injectable therapies in this cohort of patients was overall well-tolerated with no serious adverse events reported [15].

The Phase II clinical trial of rituximab in RRMS was a 48-week, double blind, placebo controlled study that included 104 patients, 69 of whom were randomized to receive rituximab (a single course administered intravenously as 1000 mg on each of days 1 and 15), while 35 patients received placebo [16]. Patients who received rituximab exhibited significantly reduced counts of total new Gd-enhancing lesions, as well as of total new Gd-enhancing lesions at weeks 12, 16, 20, and 24 - and these findings were sustained for 48 weeks. The proportion of patients experiencing relapses in the rituximab-treated group was also significantly reduced compared to patients in the placebo-treated group, at both weeks 24 (14.5% vs. 34.3%, p=0.02) and 48 (20.3% vs. 40.0%, p=0.04). At week 24, no patient in the rituximab-treated group tested positive for HACA, however at week 48, 14 of 58 tested (24.1%) were HACA positive. There was no apparent association between presence of HACA and the type or severity of adverse events, or the efficacy measures throughout the study (at weeks 24, 36, or 48). Infusion associated adverse events (defined as those that happened within 24 hours after the first infusion) were encountered more frequently in the rituximab group than in the placebo group; these were mostly mild-to-moderate in severity and again were found to decrease in frequency and intensity from the first to second infusion. Over the 48 weeks of the study, no differences were observed in the incidence of serious adverse events or infections between rituximab- and placebo-treated groups [16].

In the double blind, placebo controlled phase II/III trial of rituximab in PPMS, 439 patients were randomized (2:1) to receive repeated courses of rituximab (2 infusions of 1,000 mg each, two weeks apart), or placebo infusions, every 24 weeks through week 96, with further safety monitoring through week 122, or until restoration of circulating B cell counts [17]. As entry criteria, all patients were required to have a diagnosis of PPMS, documentation of abnormal CSF (elevated IgG index and/or presence of oligoclonal bands), and an EDSS at baseline between 2.0 and 6.5 points with the functional systems (FS) score of $\geq$ 2.0 for the pyramidal system or gait abnormality due to lower extremity deficits. The percentage of male patients was higher in the rituximab-treated group (52.1%) as compared to the placebo-treated group (44.9%) but gender differences did not affect the outcome of the trial. There were no baseline differences between randomized groups with respect to age, disease duration, prior therapy, EDSS or brain MRI findings. Gd-enhancing MRI lesions were found in 24.5% of all patients at baseline. The primary endpoint was 'time to confirmed disease progression (time to CDP)’, defined as an increase in EDSS that was sustained for at least 12 weeks. Secondary endpoints included change in hyper-intense T2 lesion volume and total brain volume on brain MRIs. No differences were found in the primary endpoint of ‘time to CDP’ between rituximab- and placebo-treated groups at completion of the 96-week study (96-week rates: 38.5% placebo, 30.2% rituximab; p=0.14). Changes in brain volume were similar in both groups (p=0.62), though rituximab-treated patients exhibited a lesser increase in T2 lesion volume (p<0.001). While formally a negative study, a pre-planned subgroup analysis showed delays in ‘time to CDP’ in rituximab-treated patients aged $\leq$ 51 years (hazard ratio [HR]=0.52; p=0.010), those with gadolinium-enhancing lesions at baseline (HR=0.41; p=0.007), and those aged $\leq$ 51 years with gadolinium-enhancing lesions at baseline (HR=0.33; p=0.009), compared with placebo-treated patients. These results indicate that, even though the primary end point was not reached, B cell depletion may be beneficial in a subgroup of patients with PPMS, particularly those who are younger and/or exhibit active inflammatory lesions on brain MRI [17]. With respect to the safety and tolerability profile of rituximab in the PPMS study, infusion-associated events, which as expected were more commonly seen in rituximab-treated patients, were predominantly mild-to-moderate and mostly experienced during the first course. These infusion associated events decreased in frequency and intensity with subsequent infusions in the same patients, eventually reaching the same rates of infusion-associated events reported by placebo-treated patients. All other adverse events were comparable between groups. Serious adverse events were reported in 16.1% of rituximab-treated patients and 13.6% of the placebo-treated group while serious infections occurred in 4.5% of rituximab- and 0.7% of placebo-treated patients. Three patients died: one placebo-treated patient developed pneumonia, one of the patients receiving rituximab died from cardiopulmonary failure, and another patient receiving rituximab also succumbed to pneumonia, with the history of brainstem lesions and aspiration. A double blind, phase I/II trial (NCT01212094) was recently initiated testing rituximab versus placebo in patients with ‘low-inflammatory’ secondary progressive multiple sclerosis (SPMS) using a combination of both intravenous and intrathecal injections administered on the same day, in two courses set one year apart. The study involves a 1-year series of pretreatment visits, followed by a 2-year treatment period. The primary outcome measure is progression of brain atrophy. Eligible patients are required to have had no evidence of a clinical MS relapse during the year prior to recruitment, and should have non-remitting/sustained (>3 months) progression of disability with EDSS 3.0 to 7.0, as well as absence of gadolinium enhancing lesions on all MRIs performed within the last 12 months prior to recruitment. This interesting study is expected to enroll 80 participants and to be completed by September 2012. (http://clinicaltrials.gov).
2.2. Ocrelizumab

Ocrelizumab is a humanized anti-CD20 mAb that binds to a different but overlapping epitope compared with rituximab and appears to deplete B cells primarily through antibody-dependent cellular cytotoxicity (ADCC), rather than by a complement-dependent mechanism. This has been considered to potentially offer an improved profile of efficacy with lesser infusion related reactions [19]. The recently completed 24-week, placebo controlled and active comparator, multi-center Phase II study of ocrelizumab in RRMS, randomized 220 patients to one of four arms, with patients receiving either ocrelizumab at total doses of 600 mg or 2000 mg over 2 infusions (at days 1 and 15); placebo; or interferon beta-1a (IFNβ1a), 30 μg i.m. weekly – the latter as an open-label arm. The primary outcome was the total number of Gd-enhancing T1 lesions at wks 12, 16, 20 and 24. Secondary endpoints included annualized relapse rate (ARR), new or persisting Gd-enhancing T1 lesions, change in T2 lesion volume from baseline, adverse events (AEs), and tolerability. Results demonstrated [20] highly significant differences in the primary endpoint of total number of Gd-enhancing T1 lesions, for both ocrelizumab doses vs placebo (p<0.0001 for both), with relative reductions of 89% for the 600 mg arm, and 96% for the 2000 mg arm. Annualized relapse rate at week 24 was significantly reduced with ocrelizumab treatment (0.125 for the 600 mg arm, p=0.0005; 0.169 for the 2000 mg arm, p=0.0014) compared to placebo (0.637), representing relative reductions in relapse rates of 80% and 73% for the two doses. The total number of new and persisting Gd-enhancing T1 lesions was also lower in both ocrelizumab arms (p<0.0001). In an exploratory analysis, both ocrelizumab treated arms were superior to treatment with weekly IFNβ1a, for the primary endpoint. There was no clear ocrelizumab dose separation on key efficacy endpoints. Serious AEs occurred in 1.8% of patients receiving ocrelizumab 600 mg, 5.5% of patients receiving ocrelizumab 2000 mg, 1.9% of placebo-treated patients, and in 3.7% of patients receiving IFNβ1a. Frequencies of serious infections were similar across all treatment groups. Infusion-related events during first infusion, predominantly mild to moderate, were more common with ocrelizumab treatment (34.5% and 43.6% for the 600 mg and 2000 mg doses) compared to placebo (9.3%), but decreased to rates comparable to placebo with the second infusion. One patient in the group receiving ocrelizumab 2000 mg died in the course of acute-onset thrombotic microangiopathy that occurred 12 wks after initiation of ocrelizumab treatment.

It is noteworthy that in spite of demonstrating efficacy in clinical trials of patients with rheumatoid arthritis (RA), ocrelizumab will not be pursued further in RA, as the risk/benefit profile in these patients was not deemed to be sufficiently better than rituximab which is already approved for RA. An imbalance in serious infection rates in the RA studies was observed consistently with the higher (1000 mg) dose only. The systemic and often multi-organ nature of RA, and the concurrent use of immunosuppressants, likely contributed to increased susceptibility to infectious adverse events in these patients. In addition to the expectation of lower morbidity in patients with MS compared to RA, as well as the use of ocrelizumab as monotherapy in MS rather than add-on therapy in RA, the phase III clinical trials of ocrelizumab in patients with MS aim to mitigate safety concerns observed with the ocrelizumab 1000 mg dose in RA, by reducing the dose of medication in the Phase III programs of MS in RRMS and PPMS.

The phase III study of ocrelizumab in RRMS is designed as a randomized, double-blind (with respect to ocrelizumab dose) and rater-blind (versus active comparator), parallel-group study that will evaluate the safety and efficacy of ocrelizumab compared to high dose interferon beta-1a. Patients with RRMS will be randomized to receive either ocrelizumab 600 mg or 400 mg intravenously every 24 weeks, or interferon beta-1a 44mcg subcutaneously three times weekly. Anticipated time on study treatment is 96 weeks. The primary outcome measure will be annualized relapse rate at 96 weeks. In an extension phase, all eligible patients will have the option to receive open label ocrelizumab.

In the Phase III, multicentre, randomized, parallel-group, double-blind, placebo controlled study to evaluate the efficacy and safety of ocrelizumab in PPMS, patients are randomized 2:1 to receive either ocrelizumab (300 mg intravenously on Days 1 and 15 of the first treatment cycle, followed by 600 mg iv every 24 weeks) or placebo. The blinded treatment period will be at least 120 weeks, followed by open label treatment for patients in both groups. Anticipated time on study is up to 5.5 years. (http://clinicaltrials.gov).

2.3. Ofatumumab

Ofatumumab is a fully human anti-CD20 mAb [21] which, compared to rituximab, binds to a completely distinct epitope [22], appears to dissociate more slowly from the CD20 antigen [23], and reportedly exhibits pronounced CDC activity and relatively decreased ADCC [24]. Ofatumumab was approved by the FDA in October 2009 for the treatment of patients with chronic lymphocytic leukemia refractory to fludarabine and ALEM [25] and has been successfully tested in a phase I/II trial in patients with RA, where it appeared to demonstrate clinical efficacy without an increase in the frequency of opportunistic infections [26]. In RRMS, ofatumumab has been studied in a small multicenter, randomized, placebo controlled phase I/II trial. The study investigated three doses of ofatumumab (100, 300 and 700 mg IV) compared to placebo, given in 2 courses 6 months apart. Reported results [27] indicated that all doses of ofatumumab lead to efficient peripheral B cell depletion. The mean cumulative number of new gadolinium-enhancing lesions from weeks 8 to 24 was 0.04 in the combined ofatumumab group, compared with 9.69 in the combined placebo group. The estimated relative reduction was 99.8% (90% confidence interval, 94.7 – 100; p<0.001). Similar reductions were reported for the cumulative number of total gadolinium-enhancing lesions and new and enlarging T2 lesions.

3. Insights into the therapeutic mode of action of B cell depletion in MS

Detailed pharmacokinetic and pharmacodynamic studies carried out in context of the Phase I re-treatment trial and the Phase II placebo-controlled trial of rituximab in RRMS, confirmed a near-complete (~98%) depletion of circulating CD19+B cells within two weeks of initial infusion [14,18].
While the initial expectation was that any success of B cell depletion in MS would reflect removal of pathogenic autoantibodies, several studies have revealed that treatment with rituximab has essentially minimal or no impact on the abnormal CSF IgG levels or oligoclonal IgG banding pattern identified prior to treatment [14,18,28–31].

Taken together with the demonstration that treatment in the rituximab trials resulted in rapid and substantial benefit in terms of reducing new brain lesions and clinical relapses, this indicates that antibody-independent pro-inflammatory functions of B cells must have contributed to relapsing MS biology prior to B cell depletion. Indeed, accumulating evidence from both human and animal model studies now points to several antibody-independent B cell functions that are likely relevant to both normal and pathologic immune responses (Table 1). These include the capacities of B cells to: (i) function as antigen presenting cells (APC) and contribute to T cell activation; (ii) produce effector cytokines that may modulate the local immune environment; (iii) function at the innate-adaptive interface; and (iv) play a role in formation and maintenance of new lymphoid foci, including at ectopic sites such as the inflamed CNS.

It is noteworthy that, in addition to peripheral B cell depletion, rituximab treatment also results in partial decreases in the absolute numbers of both B cells and T cells within the CSF of treated patients [29]. This suggests that B cells contribute to presence of T cells in the MS CNS, which may reflect CNS B cells attracting T cells into the CNS [31], and/or the capacity for B cells to activate these T cells. The latter could occur within the CNS (resulting in local T cell expansion) and/or in the periphery (resulting in activated T cells that can then more efficiently traffic into the CNS). In keeping with the latter mechanism, studies in MS patients participating in the clinical trials with rituximab have demonstrated that in vivo B cell depletion results in significantly diminished proliferative and cytokine responses of both CD4 and CD8 T cells, including Th1 (IFN-γ) and Th17 (IL-17) T cell responses [18]. The extent to which anti-CD20 mAb therapy may impact B cells within meningeal structures in the CNS of MS patients is also of considerable interest [32].

In experimental autoimmune encephalomyelitis (EAE), a commonly used animal model of MS, depletion of B cells was also been associated with decreased CNS autoreactive CD4+ T cell activation as well as differential T cell polarization, attributed in part to loss of B cell APC functions [33–35]. Interestingly, both the timing of B cell depletion in EAE, as well as the immunization paradigm used, influenced whether B cell depletion was beneficial or detrimental [34,35]. These latter findings underscore the emerging theme that B cells can exhibit both pro-inflammatory and anti-inflammatory (Breg) functions, which may have opposing roles on disease activity. Of potential interest in this regard, though too early to confirm, is that the decrease in new inflammatory disease activity seen following B cell depletion in RRMS patients may be sustained (at least in some patients) even as B cells begin

### Table 1

<table>
<thead>
<tr>
<th>B cell Function</th>
<th>In Health</th>
<th>In Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody (Ab) production&lt;br&gt;(by plasmablasts; plasma cells)</td>
<td>Essential for humoral immunity; Abs activate complement, neutralize and opsonize pathogens</td>
<td>Production of auto-Abs targeting self antigens</td>
</tr>
<tr>
<td>Antigen (Ag) presentation</td>
<td>Ag-specific T cell stimulation through Ag/MHC:TCR and costimulatory molecules, particularly robust B cell APC function for cognate antigens</td>
<td>Autoreactive B cells can function as effective APCs, and solicit T cell help thereby promoting disease</td>
</tr>
<tr>
<td>Regulation (Bregs)</td>
<td>Regulatory B cells (Breg) help to maintain homeostasis and protect from autoimmunity. Several subsets described; IL-10 implicated in regulation</td>
<td>Deficient Breg function contributes to unchecked autoimmune responses</td>
</tr>
<tr>
<td>Bystander activation (antigen-independent)</td>
<td>Activated B cell cytokines and chemokines promote local T cell and myeloid cell responses, which need not require cognate T cell: B cell Ag recognition</td>
<td>Promote autoimmunity by secreting proinflammatory cytokines, such as LT and TNFa</td>
</tr>
<tr>
<td>Lymphogenesis</td>
<td>B cell cytokines and chemokines contribute to generation and maintenance of germinal centers in lymph follicles; essential to adaptive responses</td>
<td>Ectopic follicle-like structures form within target organs; may promote ongoing local immune injury</td>
</tr>
</tbody>
</table>

to reconstitute [14,18], suggesting that the re-emerging B cells are not as pro-inflammatory as the B cells that were present prior to depletion. Indeed, functional studies have shown that B cells reconstituting in MS patients following rituximab treatment (largely comprising CD27- naïve B cells) produce significantly higher levels of the immune regulatory cytokine IL-10 in the face of lower levels of pro-inflammatory cytokines including LT and TNFα [36].

Regulating the balance between pro-inflammatory and anti-inflammatory B cell cytokine responses is emerging as another important antibody-independent mechanism by which B cells can differentially modulate the local immune response in health and disease [18,36,37]. Compared to B cells of normal individuals, B cells of patients with MS have recently been shown to respond to certain activating stimuli, including pathogen-associated molecules, with an exaggerated pro-inflammatory cytokine response profile [18]. Such abnormal B cell responses may contribute through ‘bystander activation’ to aberrant T cell activation, providing another plausible mechanism to explain why anti-CD20 mAb mediated depletion of B cells results in diminished pro-inflammatory T cell responses, and decreased disease activity in patients with MS.

4. Conclusion

Clinical trial outcomes of anti-CD20 mAb treatment in MS, together with studies of the impact of B cell depletion on immune responses in patients, now underscore the important contribution of B cells to relapsing disease biology and have implicated a number of novel B cell mediated pathogenic mechanisms that are only recently being recapitulated in animal studies. Anti-CD20 mAbs substantially reduce new brain MRI lesions and clinical disease relapses in patients with RRMS and there may be a subset of patients with PPMS who will also stand to benefit from this therapeutic approach. Treatment is generally very well tolerated with infusion-related reactions representing the most common adverse event and tending to become milder and less frequent with subsequent exposure. Insights into the risks of long-term B cell depletion in humans remain limited. The rapid onset of beneficial effects of anti-CD20 mAb treatment on MRI and clinical outcome measures in RRMS studies with rituximab, ocrelizumab and ofatumumab, imply an important antibody-independent contribution of B cells to new MS disease activity. Indeed, B cells are being recognized as having multiple effector functions that may be relevant to MS. As these effector mechanisms are elucidated, future work will take into consideration the capacities of distinct B cell subsets to exert pro- or anti-inflammatory functions, and how particular subsets or responses may be targeted so as to optimize therapeutic efficacy while maintaining the most favorable long-term safety profile.

References

Ocrelizumab in Patients with Relapsing-Remitting Multiple Sclerosis: Results of a Phase II Randomized Placebo-Controlled Multicenter Trial. ECTRIMS, 13–16 OCTOBER 2010, GÖTEBORG, SWEDEN; manuscript submitted.


