

# Targeted therapies in rheumatoid arthritis: Focus on rituximab

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**Abstract:** B-cell depletion is a new strategy for treating patients with rheumatoid arthritis (RA). In the past years, several studies have proven the efficacy of anti-CD20 mediated B-cell depletion with rituximab (Mabthera®) in RA patients who failed TNF-blocking therapy. The important role of B-cells in the pathogenesis of RA is deduced from the specific detection of autoantibodies in RA and infiltration of B-cells and plasma cells in inflamed synovium. Pharmacological studies in RA patients treated with rituximab showed that half-life was approximately 3 weeks leading to a 6- to 8-month period of B-cell depletion in peripheral blood. Rituximab treatment led to significant improvements in disease activity of RA patients and the current review summarizes the results from phase III, randomized clinical trials that have been performed. Lastly, data on safety and quality of life are summarized. Although relatively low numbers of RA patients have been treated and long-term data are lacking, current data thus far suggest a relatively good safety profile for rituximab. Future studies will need to focus on predicting responsiveness to rituximab, investigating efficacy of re-treatment with rituximab and extending data on safety and patient-focused outcomes.

**Keywords:** rheumatoid arthritis, B-cell depletion, rituximab, anti-CD20 monoclonal antibodies

The first B-cell depleting agent, rituximab, was approved in 1997 for combination treatment of CD20+ B-cell non-Hodgkin lymphoma (Coiffier et al 2002). In 2001, based on the premise that autoantibodies derived from B-cell-derived antibody-secreting cells were closely associated with disease pathogenesis, the first study was published showing promising effects of rituximab in the treatment of patients with rheumatoid arthritis (RA) (Edwards and Cambridge 2001). Because circulating autoantibodies are a common feature in several other auto-immune diseases as well, B-cell depleting therapies are now widely used in clinical trials assessing the safety and efficacy of this treatment in a variety of systemic and organ-specific auto-immune diseases (Table 1).

Rituximab is a therapeutic agent categorized in the group of “biologicals”. It is a monoclonal antibody directed against the CD20 transmembrane protein present on B-cells. It is generally accepted that the CD20 protein functions as a channel regulator of ion influx (Ernst et al 2005) and that this membrane-bound protein is specifically found on the membrane of B-cells but is not expressed on stem cells nor on terminally-differentiated plasma cells.

The current review addresses the expanding role of B-cell depleting therapy in the treatment of RA patients in rheumatologic practice. In this review, we focus on the role of B-cells in the pathogenesis of RA, on pharmacological aspects of rituximab and on results from clinical trials investigating rituximab in RA patients, including safety, efficacy and quality of life studies.

## Management of RA

Prominent symptoms of RA are symmetrical arthritis of multiple joints, mostly of the hands and feet, typically accompanied by morning stiffness. In the long term, RA leads

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**Table 1** Diseases associated with autoantibodies and the usage of rituximab

Autoimmune diseases	Target organ(s)	Rituximab treatment under investigation?	Inflammatory diseases	Target organ(s)	Rituximab treatment under investigation?
Rheumatoid arthritis	Joints	yes	Henoch-Schonlein purpura	Vasculature, kidney	yes
Systemic lupus erythematosus	Systemic	yes	Chronic graft rejection	Graft	yes
Sjogren's syndrome	Salivary gland	yes	Immunoglobulin A nephropathy	Kidney	no
ANCA-associated vasculitis	Vasculature	yes	Atopic dermatitis	Skin	no
Antiphospholipid syndrome	Vasculature	yes	Asthma	Lung	no
Idiopathic thrombocytopenia	Platelets	yes	Allergy	Skin, lung, gut	no
Autoimmune haemolytic anaemia	Red blood cells	yes			
Guillain-Barre syndrome	Peripheral nervous system	yes	Systemic sclerosis	Connective tissue	no
Autoimmune thyroiditis	Thyroid gland	yes	Multiple sclerosis	Central nervous system	no
Pemphigus vulgaris	Skin, mucous membranes	yes	Lyme neuroborreliosis	Central nervous system	no
Myasthenia gravis	Skeletal muscle	yes	Ulcerative colitis	Large intestine	no
Chronic immune polyneuropathy	Peripheral nervous system	no	Interstitial lung disease	Lung	no
Type I diabetes	Pancreatic islet cells	no			
Addison's disease	Adrenal gland	no			
Membranous glomerulopathy	Kidney	no			
Goodpasture's syndrome	Lung, kidney	no			
Autoimmune gastritis	Stomach	no			
Pernicious anaemia	Stomach	no			
Primary biliary cirrhosis	Liver	no			
Dermatomyositis-polymyositis	Skeletal muscle, skin	no			
Celiac disease	Small intestine	no			

to joint destruction and increased disability (Isenberg et al 2005). Because RA patients have a systemic, chronic and progressive disease, they usually require long-term immunosuppressive treatment. Generally, treatment of RA patients is instituted with a single or combination of Disease Modifying Anti-Rheumatic Drugs (DMARDs), of which methotrexate is currently seen as the anchor drug. Several studies have shown that failure rates for conventional DMARD therapy can accumulate over a follow-up period of 5 years to up to 75% (Maetzel et al 2000; Aletaha and Smolen 2002; van der Kooij et al 2007). In a Dutch treatment strategy study, the so-called BeSt study, which investigated treatment strategies in early RA, approximately 25% of the patients failed on step-up or combination DMARD treatment after 2 years of follow-up (Goekoop-Ruiterman et al 2007). From this perspective, the majority of RA patients will eventually fail conventional DMARD therapy, after which patients are candidates for treatment with biologicals, notably with anti-cytokine treatment directed against TNF (tumor necrosis factor) (Infliximab<sup>®</sup>, Etanercept<sup>®</sup>, Adalimumab<sup>®</sup>). Currently most of the evidence for treating DMARD refractory RA is

derived from treatment with TNF-blocking agents, which were the first biologicals to be approved in this category of patients (Maini et al 1999; Weinblatt et al 1999, 2003; Bathon et al 2000). The success of specifically targeting TNF cytokines in RA patients has augmented the efforts to specifically target other components of the immune system in RA patients. Furthermore, it is estimated that one third of the RA patients eventually fail TNF-blocking agents (Tsokos 2004), which supports the need for new therapies to control disease activity in refractory RA. Newly emerging biologicals include B-cell depleting agents, ie, anti-CD20 monoclonal antibodies (rituximab, HuMax-CD20<sup>®</sup>) and anti-CD22 monoclonal antibodies (Epratuzumab<sup>®</sup>), T-cell activation blockade through monoclonal antibodies against CTLA-4 (Orencia<sup>®</sup>, Abatacept<sup>®</sup>) and IL-6 receptor blockade (Tocilizumab<sup>®</sup>, Actemra<sup>®</sup>). While most of these biologicals are currently investigated in phase II and III trials or have recently been approved, the B-cell depleting agent rituximab has now been approved for treating RA patients refractory to TNF-blockade since 2006 (Approval Rituxan 2007; Mabthera 2007). The success of B-cell depleting therapy in RA

patients has underscored the important role of B-cells in the pathophysiology of RA.

## B-cells are critically involved in the pathogenesis of RA

RA shares a common immune abnormality with other rheumatic diseases, such as systemic lupus erythematoses (SLE), Sjogren's syndrome (SS) and Wegener granulomatosis (WG), namely the production of autoantibodies (Isenberg et al 2005). Since the discovery of autoantibodies more than 50 years ago (Rose et al 1948; Holman and Kunkel 1957), these circulating autoantibodies are the key argument that B-cells play a pivotal role in the pathophysiology of many rheumatic diseases. However, B-cells can contribute in several ways to the development of rheumatic diseases. First, B-cells are precursors of (auto-)antibody-secreting plasma cells. Secondly, they function as (auto-)antigen presenting cells, and, additionally, activated B-cells also produce cytokines (TNF- $\alpha$ , IL-6) that may influence the function of antigen-presenting dendritic cells. Thirdly, activated B-cells express costimulatory molecules, essential in the interaction with effector T-cells (Dorner and Burmester 2003).

Importantly, the detection of rheumatoid factor autoantibodies (RF) and anti-cyclic citrullinated protein autoantibodies (ACPA) are very specific findings in RA patients (Gao et al 2005; Mimori 2005; van Gaalen et al 2005). RF autoantibodies are directed against the Fc $\gamma$ -tail of immunoglobulins and can form immune complexes. ACPA are directed against citrullinated peptides, which originate from the enzymatically conversion of arginine residues of proteins (Vossenaar and van Venrooij 2004). RA patients with circulating ACPA develop more joint damage over time (Kastbom et al 2004) and respond less favorably to anti-rheumatic therapies (Alesandri et al 2004).

B-cell hyperactivity is considered a hallmark of auto-immune disease, as was shown in SLE and SS (Llorente et al 1994; Lipsky 2001; Hansen et al 2004). Recently, a comprehensive, observational study in blood, bone marrow and synovium of RA patients showed similar characteristics of B-cell hyperactivity (Teng, Hashemi et al 2007). Moreover, infiltration of B-cells and plasma cells are commonly observed in synovial biopsies from RA patients (Kruithof et al 2005; van Oosterhout et al 2005; Vos et al 2007). Still, the exact role of autoantibodies in the pathophysiology of RA remains unclear. Clinical studies from the beginning of 21st century already provided the important insight that eliminating RF autoantibodies by plasmapheresis or absorption by a column that specifically bind IgG (Proisorba) was

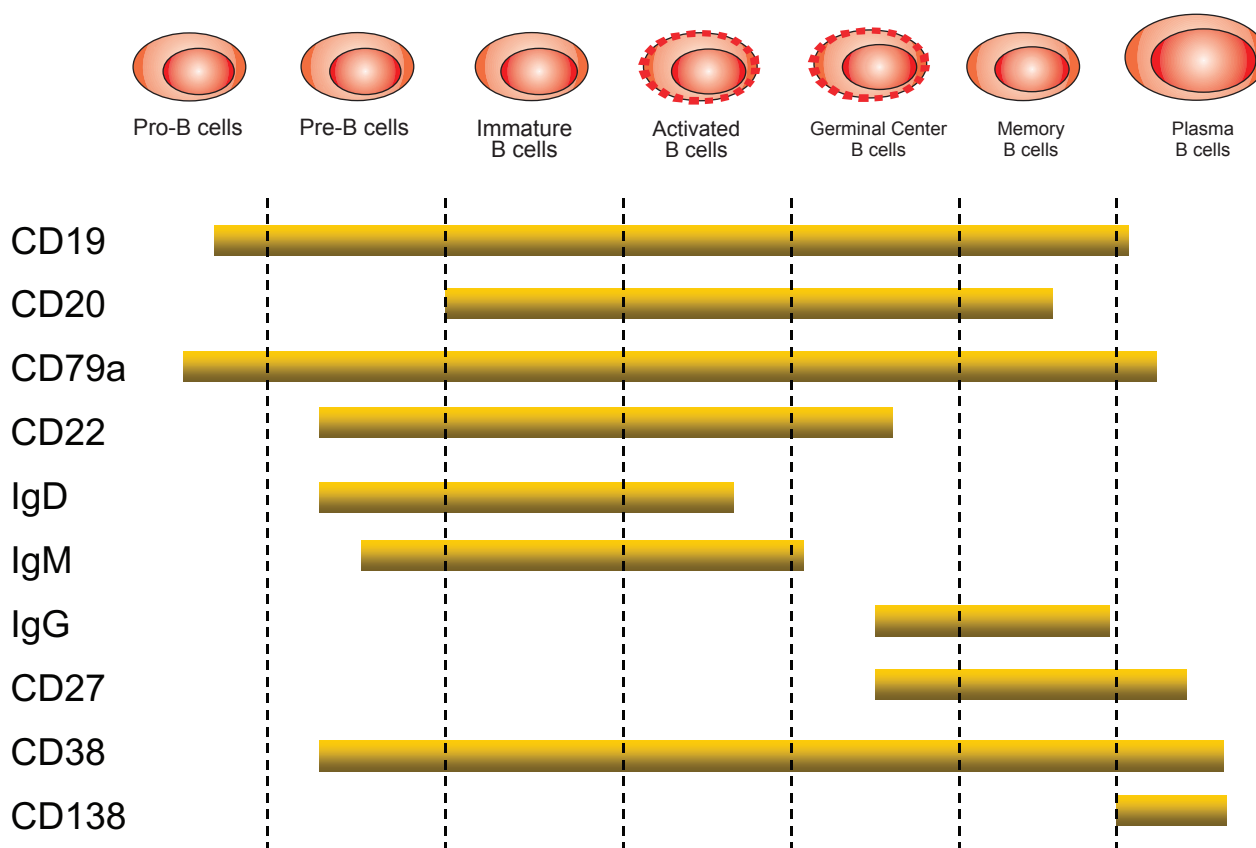
unsuccessful in decreasing disease activity in RA (Gendreau 2001). Therefore, these observations suggest that the efficacy of B-cell depleting strategies in RA is based upon interference with the cellular functions of B-cells and/or the inhibition of differentiation into autoantibody producing cells.

## B-cell depleting therapies

B-cell depletion can be achieved either by non-specific, high dose immunosuppression (Storek and Saxon 1992; Verburg et al 2001) or by specifically targeting B-cell specific membrane proteins, such as CD20 and CD22 (Goldenberg 2006; Silverman 2006). High dose chemotherapy has been shown to induce significant but temporary improvement of disease activity in RA patients (Snowden et al 2004), which was associated with significant and long-lasting T-cell suppression in peripheral blood (Verburg et al 2001). Therefore, specific targeting of merely B-cells is a less toxic and less rigorous therapy, possibly with similar efficacy in RA. In this respect, membrane proteins specific for B-cells are ideal targets for B-cell depleting therapies. Figure 1 shows several B-cell specific and non-specific membrane-bound proteins during B-cell development. It is obvious that targeting any of the B-cell specific proteins will target a large part but not the complete B-cell population. Currently, three agents, rituximab, HuMax-CD20 and epratuzumab, have been investigated to induce specific B-cell depletion but only the use of rituximab has been reported in RA patients.

## Pharmacological aspects of rituximab treatment in RA

Pharmacodynamic properties of rituximab are related to its specificity for the CD20 membrane protein, found on the surface of B-cells. Although not fully elucidated, the cytotoxic effects of rituximab on CD20+ cells appear to involve complement-dependent cytotoxicity, complement-dependent cellular cytotoxicity, antibody dependent cellular cytotoxicity and induction of apoptosis (Cerny et al 2002; Maloney et al 2002; Smith 2003). The time to recovery of B-cells in peripheral blood is generally between 6 and 9 months (Edwards et al 2004a; Leandro, Cambridge et al 2006; Roll et al 2006). Recently, our group reported on the depleting effects of rituximab in peripheral blood, bone marrow and synovium of RA patients (Teng, Levarht et al 2007). Rituximab led to a rapid and complete depletion of all B-cells in peripheral blood. It was also shown that CD20+ B-cells in bone marrow were completely depleted at 12 weeks after therapy, in keeping with a small study in 5 RA patients by Leandro, Cooper et al (2006). Still, not all CD19+ B-cells were eliminated in



**Figure 1** Expression of B-cell specific and non-specific markers during the differentiation of early progenitor B-cells into mature memory B-cells and/or plasma cells.

bone marrow as pre-B-cells and CD20<sup>-</sup> plasma cells were not targeted. Lastly, we showed for the first time that also in synovium all CD20<sup>+</sup> B-cells, but not all CD79a<sup>+</sup> B-cells, were eradicated (Teng, Levarht et al 2007). The latter is in line with the findings of a previous study showing incomplete depletion of CD22<sup>+</sup> B-cells in synovium (Vos et al 2007). In summary, it can be concluded that one treatment course consisting of 2 infusions of rituximab is able to completely deplete the subset of CD20<sup>+</sup> B-cells, but not other B-cell lineage cells such as plasma cells in bone marrow and synovium. Of note, data on B-cell counts after rituximab treatment should be carefully interpreted because recent studies have shown that rituximab can mask the CD20 epitope for other diagnostic anti-CD20 monoclonal antibodies used to identify B-cells (Teeling et al 2006; Teng, Ioan-Facsinay et al 2007). Therefore, it is crucial that other pan-B-cell markers, eg, CD19 or CD79a, are also reported.

One study has reported on the pharmacokinetics of 2 dosages of 1g rituximab in 107 RA patients, of whom 37 patients also received intravenous cyclophosphamide and 36 patients oral methotrexate (Ng et al 2005). This study showed rituximab had a distribution volume of 45 mL/kg with an average

clearance rate of 276 mL/day. The half-life of rituximab after the first infusion was 2.4 days and after the second infusion 19.7 days. This difference in half-lives can be explained by differences in the distribution of rituximab from the intravascular to the extravascular compartment and to some extent by the different rate of elimination of the rituximab after binding to the CD20 membrane protein. The latter contrasted to data from lymphoma patients, in whom baseline B-cell values did affect clearance rate and distribution volume of rituximab. Importantly, this study also showed that clearance rate and distribution of rituximab were related to patients' body surface area (BSA), but that BSA could only explain 20% of the variability in clearance rate between patients. Furthermore, a study on the effects of BSA-adjusted dosage of rituximab in these patients showed only modest differences in exposure, indicating that BSA-adjusted dosage schemes for RA patients probably do not improve pharmacokinetic characteristics of rituximab (Ng et al 2005).

## Efficacy of rituximab in RA

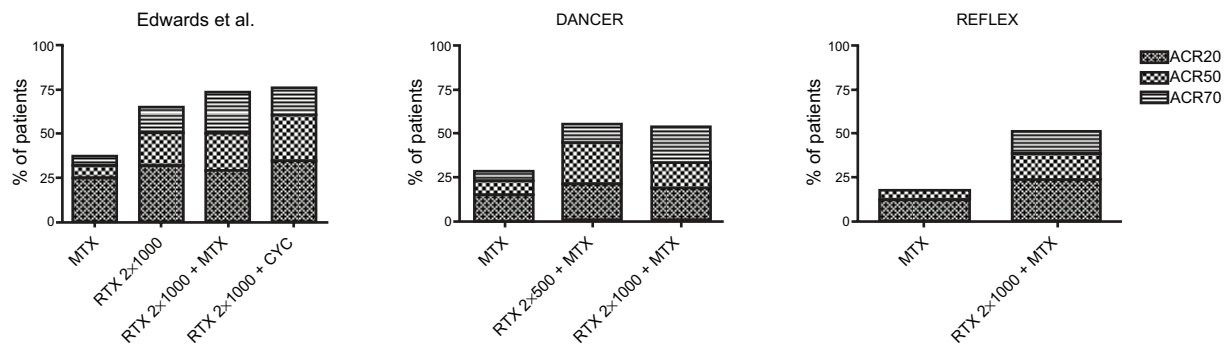
Following several open-label studies of rituximab treatment showing promising improvements in patients with

RA (Edwards and Cambridge 2001; Leandro et al 2002), Edwards et al conducted a randomized, placebo-controlled, multicenter trial to assess the efficacy of rituximab as a single agent or in combination therapy with methotrexate or cyclophosphamide. The study assessed 161 patients with RA, and compared the rituximab regimens with methotrexate alone (Edwards et al 2004b). The primary endpoint was defined as an ACR50 response (ie, a 50% or greater improvement in the signs and symptoms of RA, as defined by the American College of Rheumatology [ACR]) within 24 weeks. In this pivotal trial, 33% of the patients treated with rituximab alone achieved an ACR50 response, compared with 13% in the methotrexate group. The differences were even larger when combination therapy was used: 41% and 43% of patients achieved an ACR50 response when treated with rituximab in combination with methotrexate and cyclophosphamide, respectively. Naturally, more patients achieved an ACR20 response (20% improvement) in this trial, the main results of which are summarized in Figure 2. Treatment with rituximab, particularly when combined with methotrexate, remained more efficacious than methotrexate alone for at least 1 year. In addition, extension studies showed sustained

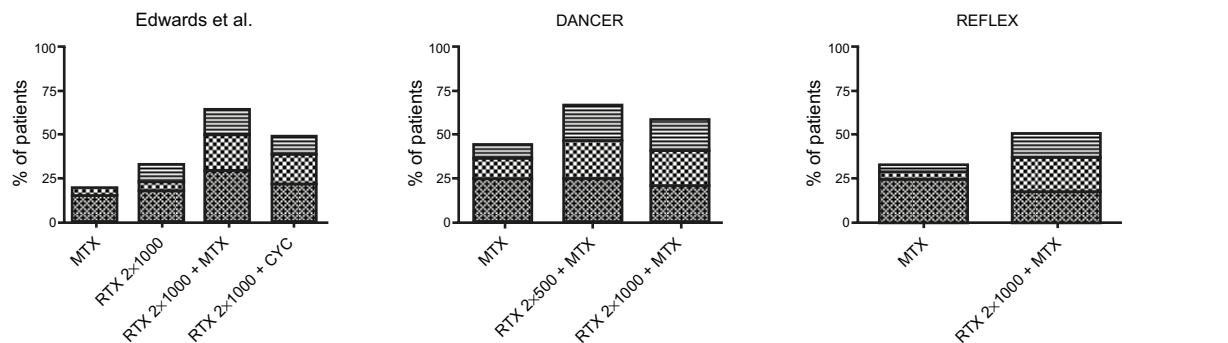
benefit from a single course of rituximab for up to 2 years (Strand et al 2005).

Further evidence for the efficacy of rituximab in RA comes from the Dose-Ranging Assessment International Clinical Evaluation of rituximab in Rheumatoid Arthritis (DANCER) trial, which examined the efficacy of different doses of rituximab (500 mg bd vs 1000 mg bd on days 1 and 15) and glucocorticoids in combination with stable doses of methotrexate (Emery et al 2006) (Figure 2). This trial confirmed the previous positive effects of rituximab on RA disease activity. There was no difference in primary outcomes (ACR20 and ACR50 responses) between medium (500 mg) and high (1000 mg) doses of rituximab. However, using more stringent outcome measures (eg, ACR70 response or remission defined by disease activity score) a trend in favor of high doses was observed. Furthermore, more patients developed circulating human anti-chimeric antibodies (HACA) when treated with medium doses (4.9% vs 2.7%). The DANCER study also demonstrated that corticosteroids administered during the first 15 days of therapy (around the doses of rituximab) did not contribute to efficacy. However, intravenous methylprednisolone

## 24 weeks



## 48 weeks



**Figure 2** Overview of the percentage of rheumatoid arthritis patients achieving an ACR20, ACR50, or ACR70 after rituximab treatment in three pivotal clinical trials: Edwards et al (2004b) (N = 160), DANCER trial (N = 465) (Emery et al 2006) and REFLEX-trial (N = 520) (Cohen et al 2006).



(100 mg iv before rituximab infusions) significantly reduced the frequency of acute infusions reactions at the 1st infusion (35% in the glucocorticoid-placebo group versus 25% in the glucocorticoid group), but not of the 2nd infusion at which time significantly fewer infusions reactions occurred in general (5.6% in both groups).

More recently, the Randomized Evaluation of Long-term Efficacy of rituximab (REFLEX) study also showed rituximab to be highly effective in patients with RA who had experienced an inadequate response to one or more TNF-blocking agents (Cohen et al 2006). In this trial 520 refractory RA patients were randomized to methotrexate alone or the combination of methotrexate with rituximab. Although this study defined its primary endpoint as an ACR20 improvement (summarized in Figure 2), the significantly larger proportion of RA patients that achieved an ACR50 improvement with rituximab and methotrexate (27%) as compared to methotrexate alone (5%) is more illustrative for comparison and confirms the efficacy achieved in the first trial of Edwards et al. Moreover, this study was able for the first time to measure a trend towards slower radiographic progression within 24 weeks in patients treated with rituximab (change in total Genant-modified Sharp score of  $0.6 \pm 1.9$ ) in comparison to the methotrexate alone group (change in total Genant-modified Sharp score of  $1.2 \pm 3.3$ ;  $p = 0.17$ ). This was further corroborated after 1 year, when progression was significantly lower in the rituximab-treated group (change in radiographic score: 2.31 vs 0.99;  $p = 0.004$ ) (Keystone, Emery et al 2006).

Interestingly, preliminary data indicate that RA patients can be successfully retreated with rituximab. The efficacy of repeated courses of rituximab did not seem to differ from the first treatment course (Pavelka et al 2005; Keystone, Fleischmann et al 2006).

## Safety and tolerability

Rituximab has been used in the general hematological practice for more than 10 years. Long-term safety is well

established in these patients: no increased incidence of infections has been observed and most infections were typical of those common in normal hosts (McLaughlin 2001). Safety data for rheumatologic patients are thus far limited and conclusions can only be drawn for the short-term adverse events. Most of the side effects are seen during the intravenous administration and consist of mild symptoms (nausea, fever, headache, myalgia). In the DANCER phase-II trial reported 38% (73 of 192 patients) of the RA patients experienced infusion-related side effects while in the REFLEX-trial this was 23% (72 of 308 patients) using 1000 mg rituximab. Significantly fewer side effects were observed at the second infusion: in DANCER 10% (19 of 192 patients) and in REFLEX 8% (26 of 308 patients). This can be explained by desensitization due to the fact that rituximab can still be measured in serum at low concentrations when the second infusion is administered. Furthermore, a study evaluating the concomitant administration of corticosteroids before the infusion of rituximab showed that corticosteroids did not affect the outcome of patients, but did decrease the incidence of infusion-related side effects in one third of the patients (Fleischmann et al 2005).

With respect to infectious complications (Table 2), a non-significant increased incidence of 35% was reported in rituximab treated group versus 28% in the placebo group from the DANCER study. The type and severity of infections were similar in both groups, mostly being respiratory tract infections (7%), urinary tract infections (3%) and nasopharyngitis (6%). Serious infections were not common and occurred in 1%–2% of the patients. When accumulating data from the REFLEX-trial, an incidence of infections of 41% was observed as compared to 38% in the placebo group. The calculated rate of infection per 100 patient years was actually lower in the rituximab treated group (154.6 in the placebo group versus 138.2 infections per 100 yrs in the rituximab treated group). Regarding serious infections there was a trend to a higher incidence in patients treated with

**Table 2** Rate of infectious events in two pivotal, double-blinded, randomized trials assessing efficacy of rituximab in RA patients

<b>DANCER trial (N = 209 vs 308)</b>	<b>Placebo (N = 149)</b>	<b>RTX 2 × 500 mg (N = 124)</b>	<b>RTX 2 × 1000 mg (N = 192)</b>
Total patient years	n.a.	n.a.	n.a.
Incidence of infections	28%	35%	35%
Infections per 100 patient yrs	n.a.	n.a.	n.a.
Serious infections per 100 patient yrs	3.19	0	4.74
<b>REFLEX trial</b>	<b>Placebo (N = 209)</b>		<b>RTX 2 × 1000 mg (N = 308)</b>
Total patient years	82.1		134.6
Incidence of infections	38%		41%
Infections per 100 patient yrs	154.6		138.2
Serious infections per 100 patient yrs	3.7		5.2

rituximab (3.7 in the placebo group versus 5.2 infections per 100 years in the rituximab treated group). Collectively, these short-term data suggest that a single treatment course with rituximab does not increase the incidence of infectious complications, which suggests that during B-cell depletion the innate immune system as a “first-line defense”, including natural killer cells and T-cells, as well as circulating antibodies and long-lived plasma cells provide adequate protection to exogenous antigens (McLaughlin 2001).

Lastly, B-lymphocyte depletion is an anticipated side effect of rituximab and the time to reconstitution differs for each patient and for each underlying rheumatic disease. The mean time to reconstitution is around 6–9 months (Edwards et al 2005). A decrease of serum concentration of immunoglobulins (IgG, IgM, IgA) below normal values seldom occurs. Also, long-term humoral memory derived from long-lived plasma cells, as measured by serum antibodies against the recall antigen tetanus toxoid, does not seem to be affected by a single treatment course of rituximab.

## Patient-focused perspectives

Few studies so far have addressed patients’ satisfaction and acceptability of rituximab treatment, although new data will appear. One spin-off study from the REFLEX-trial investigated quality of life as measured by the SF-36. This study showed that rituximab resulted in a significantly greater improvement in quality of life scores on all 8 domains of the SF-36 (Kielhorn et al 2006). In addition, two cost-analysis studies performed by the Health Economics and Strategic Pricing of F.Hoffmann-La Roche (Alvarez et al 2006; Lewis et al 2006), the pharmaceutical company producing rituximab, showed by a Markov Model that rituximab combined with methotrexate could achieve a gain of 0.48–0.63 Quality Adjusted Life Years (QALYs) as compared to current practice for refractory RA patients, which included TNF-blocking treatment. Importantly, these analyses compared treatments over an average time of approximately 18.5 years. Clearly, these studies need to be reproduced with adequate control groups as well as by independent groups, to enable relevant socioeconomic evaluations.

## Conclusions

Anti-CD20-mediated B-cell depletion by rituximab has proven to be a valuable expansion of the therapeutic armamentarium in the rheumatologic practice. Several studies have now established its efficacy and safety for treating refractory RA patients. Based on the evidence from three large randomized trials, rituximab treatment is a treatment

option for RA patients failing TNF-blocking therapy. Future studies will have to show whether B-cell depletion is superior to TNF-blocking therapy in earlier stages of disease. In addition, the exact mechanism through which rituximab treatment results into clinical improvement still needs to be clarified. Recent studies have made it clear that depletion of CD20+ B-cells has led to significant decreases of autoantibody titers, but the biologic relevance of these observations is still unclear. Undoubtedly, as rituximab will be more and more prescribed by rheumatologists, further insight into the effects of B-cell depletion will be revealed. Lastly, studies analyzing cost-effectiveness will determine for a large part the availability of rituximab for RA patients in the near future and studies defining predictive factors of responsiveness to rituximab may therefore be of high importance. Recently, one study identified predictive determinants in serum and synovium of RA patients who have a high a-priori change to achieve a good response upon rituximab treatment (Teng, Levarht et al 2007). Because of increasing expenses to provide biologic treatments, such as rituximab, to RA patients, these studies obviously need to be further substantiated.

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