Targeting CD22 as a strategy for treating systemic autoimmune diseases

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Abstract: B-cells play an important role in the diagnosis and to some extent the pathogenesis of many autoimmune diseases. Specific B-cell directed antibodies are now gaining an increasing role in the management of these diseases. The first antibody target in this regard was CD20, with the development and introduction of rituximab in the management of B-cell malignancies as well as rheumatoid arthritis. A second candidate target is CD22, and the first antagonistic antibody to this B-cell marker is epratuzumab, which appears to function, in contrast to CD20 antibodies, more by modulation of B-cells than by their depletion capacity. Originally developed for the treatment of non-Hodgkin lymphoma, epratuzumab has now been reported to be effective, with a very good safety profile, in two prototype autoimmune diseases, systemic lupus erythematosus and primary Sjögren's syndrome. As such, this new investigational antibody may provide distinct therapeutic effects and may be complementary to the known effects and role of CD20 antibodies.

Keywords: autoimmune diseases, CD22, B-cells, epratuzumab

Autoimmune diseases

Autoimmune diseases comprise more than 80 chronic diseases that affect about 5%–8% of the general population (Jacobson et al 1997), with the prevalence being, in decreasing order, rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS), and systemic lupus erythematosus (SLE). There has been considerable progress made in understanding the immune system during recent decades, resulting in a better appreciation of the role of B-cells in the interaction of innate and adaptive immunity, lymphocyte activation and antigen processing, the principles of immune tolerance, B- and T-cell crosstalk, cytokine signaling, and new approaches of treating autoimmune diseases by depleting or modulating B-cells, including blockade of co-stimulation. This resulted in a plethora of articles and reviews on the importance of B-cells in autoimmunity (Mitchison and Wedderburn 2000; Edwards and Cambridge 2001; Lipsky 2001; De Vita et al 2002; Leandro et al 2002a; Dörner and Burmester 2003; Oligino and Dalrymple 2003; Uchida et al 2004; Park et al 2005; Tedder et al 2005a; Keystone 2005; Viau and Zouali 2005; Dörner 2006; Dörner and Lipsky 2006; Martin and Chan 2006).

These diseases, particularly RA, SLE, and pSS, are complex, usually multi-organ manifestations with a wide heterogeneity in clinical presentations and disease course. Whereas many were traditionally considered to implicate T-cells in their pathogenesis, as referenced above, B-cell disturbances and hyperactivity are now considered to be a hallmark of many of these diseases, as indicated by the development of autoantibodies, and an increased risk of developing B-cell lymphoma, such as in pSS and RA (Voulgarelis et al 1999). Although B-cells were attributed previously only to cause autoantibody production, they have now gained a central role in the pathogenesis of several autoimmune diseases. A breakdown of tolerance mechanisms that normally regulate B-cell development leads to the development of autoimmune diseases (William et al 2006), including

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induction and maintenance of self-reactive B-cell antigen receptor (BCR) complexes (Voulgarelis Dafni et al 1999; Dörner 2006; Dörner and Lipsky 2006; Martin and Chan 2006; Radbruch et al 2006). Because B-cells are considered as being of central importance in the immunopathogenicity, they represent current targets of immunotherapy.

To date, there are a number of therapeutic antibodies targeting B-cell-specific antigens in order to deplete or modulate B-cells, rituximab (anti-CD20 chimeric antibody), ocrelizumab (humanized anti-CD20 antibody), belimumab (anti-BlyS or BAFF human antibody), and epratuzumab (anti-CD22 humanized antibody) that are in advanced clinical trials in several autoimmune diseases (Dörner 2006; Dörner and Lipsky 2006; Edwards and Cambridge 2006; Martin and Chan 2006). A number of other anti-CD20 antibodies (HuMax, hA20 or veltuzumab, ofatumumab) are also in clinical development but no clinical data have been reported so far other than in abstract form.

Rituximab was the first monoclonal antibody approved by the US Food and Drug Administration for the treatment of B-cell non-Hodgkin's lymphoma (NHL) in 1997, followed by licensing for RA after anti-TNF failure in 2006. The success and the very good safety profile of rituximab therapy in lymphoma, as well as incidental case observations, encouraged many investigators to consider its use in autoimmune diseases. In the last 4-years, clinical trials have shown promising efficacy in various autoimmune diseases (Edwards and Cambridge 2006), such as RA (Edwards et al 2004b; Leandro et al 2002a), Sjögren's syndrome (Pijpe et al 2005), SLE (Leandro et al 2002b), and chronic immune thrombocytopenic purpura (Stasi et al 2001). These studies indicated that circulating B-cells are undetectable after a brief dosing regimen of rituximab. Whether complete depletion of peripheral B-cells and remaining CD20- plasmablasts may be used as a biomarker of clinical response needs further careful analysis in RA. Long-term efficacy and safety was reported in RA (Edwards et al 2004a), which is the first autoimmune disease indication approved recently for rituximab in combination with methotrexate and corticosteroids. Fully human and humanized anti-CD20 monoclonal antibodies are now under further evaluation in autoimmune diseases (Stein et al 2004; Teeling et al 2004; Tahir et al 2005) with ocrelizumab being in advanced trials. Nevertheless, all of these CD20 antibodies appear to markedly deplete circulating B-cells in treated patients. Depleting B-cells is a new and interesting method in the treatment of autoimmune diseases, although there are still too limited long-term safety data, and reduced Ig levels indeed were reported in a 7-year experience in RA (Edwards and Cambridge 2006).

A distinct and novel approach is to modulate B-cell function, perhaps targeting a specific B-cell population or functional target. B-cell survival and selection is determined by BCR specificity, and downstream signaling is affected by the interaction of numerous enzyme cascades, other critical receptors, and target molecules (Dörner and Lipsky 2006). Recently, the CD22 molecule indigenous to B-cells has gained considerable attention as an important co-receptor in BCR function and in autoimmunity, and thus as a potential target for autoimmune therapy (Nitschke 2005; Tedder et al 2005a; Goldenberg 2006).

Current concepts for treating systemic lupus erythematosus

An important consideration in the treatment of SLE is that this heterogeneous disease may have very different organ manifestations, suggesting that the treatment should be determined by the extent of the involvement of major organ systems, so that disease activity determines treatment options. However, with the exception of studies in lupus nephritis, trials evaluating the functional outcome of other organs are limited.

Severe internal organ involvement usually requires immunosuppression. In lupus nephritis, it has been shown that mycophenolate mofetil in combination with corticosteroids (Chan et al 2000; Contreras et al 2004) are sufficient for initial therapy. Rapidly progressive lupus nephritis has been shown to be controlled by iv cyclophosphamide monthly for 6 months (Boumpas et al 1992), and subsequently followed by azathioprine, mycophenolate mofetil or continous iv cyclophosphamide every 3 months. ACE and AT1 inhibitors can reduce proteinuria and retard renal sclerosis in membranous nephritis. Involvement of the central nervous system with encephalopathy and transverse myelitis usually requires high doses of corticosteroids, often combined with cyclophosphamide. Since lupus and also high doses of corticosteroids can induce psychosis, these clinical situations are challenging.

Most of the treatment strategies have been developed or significantly modified within the last decade, especially the use of iv cyclophosphamide and introduction of mycophenolate mofetil for improving the outcome of severe lupus. However, the side effects of non-specifically-acting drugs, as well as the combination with high dose corticosteroids, are serious (ovarian failure, enhanced risk for bladder carcinoma, enhanced infection rate, infusion-related side effects; ie, nausea, vomiting, hair loss). Although cyclophosphamide and corticosteroids have effects on B-cells, the advent of B-cell directed therapies

using specific antibodies against CD20, CD22, and against B-cell survival factors and cytokines (anti-BAFF, anti-BR3, TACI-Ig, anti-IL10, anti-IL6), or agents modulating B-cell receptor strength (LJP394, Epratide) provide different mechanisms of action, potential different efficacies and different adverse events. Currently available data indicate that the side effects of anti-CD20 and anti-CD22 antibody therapies are acceptable, although long-term data are still not available.

Beyond B-cell directed biotherapies, there are numerous new strategies under study, such as peptide vaccinations, neutralizing complement 5, FcγRIIB, PDGF blockade by Gleevec, inducing regulatory T-cells, blocking ligand interactions (ie, ICOS/ICOS-L, CD40/CD40L and CD80/86 blockades), autologous stem cell transplantation, and high-dose cyclophosphamide.

CD22 molecule

CD22 is a 135-kD type I transmembrane sialoglycoprotein of the immunoglobulin (Ig) superfamily that is the focus of two recent comprehensive reviews (Nitschke 2005; Tedder et al 2005b). The expression of CD22 is specific to B-cells and is developmentally regulated so that it is expressed at low levels in the cytoplasm of pro-B and pre-B-cells, and its localization shifts to the cell surface and higher expression on mature IgM+, IgD+ B-cells (Dörken et al 1986). CD22 is strongly expressed on follicular, mantle, and marginal-zone B-cells, but is weakly present in germinal B-cells (reviewed in (Nitschke 2005; Tedder et al 2005b)).

CD22 consists of 7 extracellular Ig domains and a full-length 141 amino acid cytoplasmic tail (Wilson et al 1991; Engel et al 1995). Human CD22 genes have 15 or more exons, where the single Ig domains are encoded by exons 4–10 and the transmembrane and cytoplasmic domains encoded by exons 11–15. Immunoprecipitation studies have shown that different monoclonal antibodies to CD22 react with different Ig domains. Epratuzumab has been found to compete with the RFB4 antibody, which binds to the third Ig domain (Stein et al 1993).

Functionally, CD22 is involved in regulating B-cell function by CD19 and B-cell antigen receptor (BCR) signal transduction, BCR-induced cell death, and the survival of B-cells in peripheral organs. Because CD22 is considered to be a regulator of B-cell functions and survival, it appears to be an important link for modulating humoral immunity and also the proliferation of B-cell lymphomas. Therefore, CD22 is a candidate target for therapeutic antibodies (Tedder et al 2005b; Goldenberg 2006; Steinfeld and Youinou 2006).

In vitro assays indicate that CD22 plays a role in intercellular adhesion, involving α2-6-linked sialic acid residues (Stamenkovic and Seed 1990; Engel et al 1993). CD22 is thus considered to be a member of the sialoadhesin or SigLec subclass of the Ig superfamily, characterized by functioning as lectins (Engel et al 1993; Kelm et al 1994), whose members include other sialic acid-binding receptors, such as sialoadhesin and CD33. CD22 adhesion has been implicated in a variety of functions, such as interacting with neighboring leukocytes, regulator of signaling, such as generating either costimulatory, resulting in cell proliferation, or apoptotic signals on B-cell function after being treated with CD22 antibodies (Chaouchi et al 1995; Doody et al 1995; Tuscano et al 1999). In an in vitro assay system, immobilized epratuzumab crosslinked with anti-IgG had different effects, depending on the cell type studied. In the situation of B-cell lymphomas expressing CD22, epratuzumab, when immobilized, inhibited cell proliferation, but did not do so when not immobilized, even when crosslinked (Carnahan et al 2003, 2007). CD22 has been reported to be a negative regulator of signaling (Otipoby et al 1996; Sato et al 1996; Nitschke et al 1997; O'Keefe et al 1999). However, our own studies with human B-lymphoma cells suggest an involvement of CD22 with the BCR, where it can inhibit proliferative functions (Carnahan et al 2007) that may be operative in epratuzumab's activity against NHL and certain autoimmune diseases. Blocking CD22 in vivo in different settings and involving different B-cell populations needs to be studied further in order to further elucidate the complex role of CD22 and various antagonistic antibodies in B-cell development and in B-cell diseases.

Epratuzumab

Epratuzumab was derived from the murine IgG2a monoclonal antibody, LL2 (originally named EPB-2) generated against Raji Burkitt lymphoma cells, and found to be highly selective for normal B-cells and B-cell tumors, but not reactive with Hodgkin's disease, solid tumors, or non-lymphoid tissues (Pawlak-Byczkowska et al 1989). It was shown to bind to the third Ig-like domain of CD22 (Stein, Belisle, Hansen, and Goldenberg 1993). The antibody was found to rapidly internalize (10^7 molecules/min) into CD22-positive lymphoma cells) (Shih et al 1994), and induces CD22 phosphorylation (Carnahan et al 2003).

Epratuzumab (hLL2), the humanized $IgG_{1(\kappa)}$ form of the murine LL2, was further developed for clinical use (Leung et al 1994). Its known mechanisms of action differ from the anti-CD20 monoclonal antibody which depletes

very efficiently CD20+ B-cells, rituximab, by CD22 phosphorylation (Carnahan et al 2003) and by affecting BCR (via immobilized immunoglobulin crosslinking), as well as inducing moderate, but significant, ADCC, without showing direct apoptotic or complement-mediated killing (Carnahan et al 2003). Epratuzumab has also been shown depletion of circulating B-cells when given to patients with NHL (Leonard et al 2002; Leonard et al 2003), SLE (Dörner et al 2006), or pSS (Steinfeld et al 2006), but markedly less than rituximab (Reff et al 1994), yet has been active therapeutically in all three diseases. Epratuzumab at the dose of 360 mg/m^2 in 250 mL 0.9% NaCl is administered i.v. as a slow infusion (less than 1 hour). In order to minimize hypersensitivity, patients were premedicated with acetaminophen and antihistamine.

Chemistry

Epratuzumab is a humanized MAb of $IgG_{1(\kappa)}$ class, which specifically binds to the CD22 antigen in B-cells. The construct encoding epratuzumab was created by grafting the complementarity-determining regions (CDR) of the murine parental origin antibody in a human IgG_1 genetic backbone (Leung et al 1994, 1995). The resulting epratuzumab contains the original murine sequence only at the antigen-binding sites, comprising about 10% of the molecule, the remainder being the human framework sequences. The antibody is formulated in 0.04 M sodium phosphate–0.15 M sodium chloride, pH 7.4, buffer with 0.075 % polysorbate 80.

Pharmacokinetics and pharmacodynamics

To date, pharmacokinetic (PK) results are available for unlabelled epratuzumab from patients enrolled in Phase I/II chemotherapy-refractory NHL, SLE, and pSS studies. Pharmacokinetic analyses showed that mean maximum antibody levels generally increased with increasing epratuzumab dose. The mean serum half-life ($t_{1/2}$) increased from 6.9-days to 26.5-days between the first and the fourth infusion (Leonard et al 2002, 2003), while the highest serum values (C_{max}) increases with subsequent doses, findings likely due to the saturation of CD22-binding sites. Epratuzumab serum levels were still detectable at week 12 post-infusion. Non-compartmental pharmacokinetic analysis indicated a serum half-life ($t_{1/2}$) after the fourth infusion of 15 ± 8 days in pSS patients (Steinfeld et al 2006) and 23-days in NHL patients (Leonard et al 2003).

Drug administration and interactions

No drug interaction has yet been reported with epratuzumab. In addition, neither an overdose effect of epratuzumab nor an antidote to overdose is known. However, due to the potential for hypotension, it is recommended for subjects receiving daily anti-hypertensive medication that the medication be held until after completion of their epratuzumab infusion. It is noteworthy that, in contrast to many other antibodies in human use, epratuzumab has been infused within 1 hour and has shown good tolerance, with usually only grade 1 or 2 reactions (according to NCI CTC grading).

Epratuzumab in SLE and primary Sjögren's syndrome

Two Phase II open label studies were conducted in patients with autoimmune diseases.

The first study of anti-CD22 in the treatment of 14 patients with moderately active SLE was reported using the BILAG (British Isles Lupus Assessment Group) scoring method to categorize SLE activity levels (Dörner et al 2006). Patients received 360 mg/m^2 epratuzumab intravenously every 2 weeks for 4 doses, with analgesic/antihistamine premedication (but no steroids) prior to each dose. Evaluations at 6, 10, 18, and 32 weeks (6 months post-treatment) follow-up included safety, SLE activity (BILAG score), blood levels of epratuzumab, B- and T-cells, immunoglobulins, and human anti-epratuzumab antibody (HAHA) titers. Total BILAG scores decreased by ≥50% in all 14 patients at some point during the study (including 77% with ≥50% decreases at 6 weeks), with 92% having various levels of decreases continuing to at least 18 weeks (where 38% showed ≥50% decreases). Almost all patients (93%) experienced improvements in at least one B- or C-level disease activity at 6 weeks, and all patients achieved improvement in at least one BILAG body system at 10 weeks. Complete resolution of all BILAG B-level disease activities was seen in three patients at 18 weeks. Epratuzumab was tolerated well, with a median infusion time of 32 min. Drug serum levels were measurable for at least 4 weeks post-treatment and detectable in most samples at 18 weeks. B-cell levels decreased by an average of 35% at 18 weeks and remained depressed at 6 months post-treatment. Changes in routine safety laboratories were infrequent and without any consistent pattern, and there was no evidence of immunogenicity or significant changes in T-cells, immunoglobulins, or autoantibody levels. Of note, the anti-CD22 antibody preferentially reduced naïve CD27- B-cells and has been shown by in vitro studies to inhibit proliferation of activated B-cells, especially blocking TLR-dependent activation by CpG and to a lesser degree by CD40/CD40L (Jacobi et al 2007). Further randomized trials in SLE are underway to evaluate efficacy and safety of epratuzumab in SLE.

Another open-label trial was conducted (Steinfeld et al 2006) in 15 patients with pSS. In this two-center study, the patients were on symptomatic treatment for at least 6 months prior to entry and were not on steroids or immunosuppressive drugs (hydroxychloroquine, methotrexate, cyclosporin, sulfasalazine, or corticosteroids were not allowed during the study and were discontinued at least 4 weeks before study entry). They received the same infusions of epratuzumab every other week for 4 doses, as in the SLE trial. The patients had active pSS based on increased erythrocyte sedimentation rates (ESR) and/or serum IgG levels, as well as at least one systemic manifestation. A composite endpoint involving the objective measures of eyes and mouth dryness, fatigue, and two laboratory parameters (ESR and IgG), was used to provide a clinical measure of response. Accordingly, a patient responded if there was a ≥20% improvement in at least two of these parameters. This study found that 53% of the patients achieved a clinically meaningful response 24 hours following the fourth infusion, or at 6 weeks. A higher response rate (67%) was observed at 32 weeks, or about 6 months after completion of therapy, suggesting possible recovery or regeneration of the glandular tissues. Circulating CD19+ B-cell levels decreased (mean change from baseline ±50%) for at least 12 weeks after the last infusion. However, no improvement in the antinuclear antibody titers was observed from baseline values.

Further trials with epratuzumab in patients with SLE are currently being conducted by UCB SA of Brussels, which licensed the product from Immunomedics, Inc., for autoimmune disease indications.

Safety and tolerability

Epratuzumab has been generally well tolerated in the studies cited above. Most reactions were transient, mild-to-moderate adverse events that resolved without sequelae, including nausea, fatigue, and general pain. The most treatment-related events were transient mild-to-moderate (Grade 1–2) infusion reactions, occurring mainly during the initial infusion. In order to minimize hypersensitivity, patients were premedicated with acetaminophen (0.5–1 g) and antihistamine (25–50 mg p.o. or IV benedryl, polaramin, or an equivalent). However, patients with pSS appeared to have a higher rate of adverse events. One patient experienced a moderate-severe acute infusional reaction (flushing, dyspnea, nausea, vomiting, nasal mucosa swelling, and glottis pressure) during the first infusion. 93% of the pSS patients completed all infusions. Another patient prematurely terminated the third infusion after experiencing a grade-3 acute infusion reaction. Adverse events were reported in 10/16 patients, with 4 having severe events. Interestingly, pSS patients treated with rituximab (Pijpe et al 2004) were reported also to have a higher rate of side effects, including the development of serum sickness. Standard safety laboratories showed no consistent pattern of change, and infrequent post-treatment increases in NCI-CTC v 3.0 toxicity grades for these laboratories were all limited to changes of at most one grade level. No change in serum IgG, IgA, or IgM levels was reported in both NHL and autoimmune disease studies.

Human anti-human antibody (HAHA) titers were determined in collected blood samples by Immunomedics, Inc., to determine the agent's immunogenicity. In NHL and Sjögren's syndrome studies, only 2.4% of the patients had positive ELISA assay results, all borderline positives or low-level values, and there were no associated clinical events (Steinfeld et al 2006). No evidence of immunogenicity was reported in the SLE study (Dörner et al 2006).

Conclusion

More than 300 patients with B-cell NHL received epratuzumab either in single-agent or in combination studies with rituximab. A small number of approximately 30 patients with autoimmune diseases (SLE and Sjögren's syndrome) have received epratuzumab in 2 single-agent Phase II clinical trials. Epratuzumab was infused, ususally within an hour, with minimal toxicity at the dose of 360 mg/m^2 for 4 doses. Most treatment-related events were transient mild/moderate infusion reactions and preferentially seen in pSS patients. There was no evidence of changes in hematology, serum chemistry, or immunoglobulins with epratuzumab, except for a circulating B-cell drop of up to about 35%–50%, which was maintained for several months. Evidence of clinical efficacy in indolent and aggressive NHL and in both SLE and pSS suggests that epratuzumab is a promising new therapeutic agent for the treatment of B-cell malignancies and B-cell-implicated autoimmune diseases.

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