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REVIEW ARTICLE

Targeted biological therapies for Graves' disease and thyroid-associated ophthalmopathy. Focus on B-cell depletion with Rituximab

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Summary

Based on experience from the treatment of other autoimmune diseases and because of the limitations imposed by existing therapeutic options for Graves' disease (GD) and thyroid-associated ophthalmopathy (TAO), rituximab (RTX) was recently proposed as a novel therapy option. Here, we summarize the rationale for using RTX; give an overview of the possible mechanisms of action; and give an account of its effects and side-effects when used in GD and TAO. Scant evidence, originating from only a few methodologically inhomogeneous studies, suggests that RTX may prolong remission for hyperthyroidism over that seen with antithyroid drugs, at least in mild GD. Furthermore, in patients with TAO, who are unresponsive to conventional immunosuppressive therapy, RTX seems efficacious. As we wait for larger-scale randomized studies, RTX, should be considered experimental and reserved for patients who do not respond favourably to conventional therapy. It is the first in what is likely to be a series of new and emerging treatments specifically targeting relevant components of the immune system. Further studies will hopefully lead to improved and better tailored, individualized therapy for GD and especially TAO.

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Graves' disease (GD) afflicts approximately 1–2% in the adult population.[1,2] Its aetiology is incompletely understood and thought to result from a complicated interaction between genetic susceptibility and environmental factors.[3] This interplay might explain its epidemiologic pattern, including geographical variations in prevalence.[3] It is likely that the vast majority of clinicians will at some time during their practice encounter patients with GD, as the disease can be associated with symptoms from a number of organ systems. During the last 50 years, relatively little advance has been made in improving the therapy of this debilitating disease.[4] Therapeutic options for the hyperthyroidism associated with GD – antithyroid drugs, radioiodine therapy and surgical thyroidectomy – allow us to care well for patients with the glandular aspects of the disease. Moreover, adequate data concerning efficacy, side-effect profiles, important prognostic factors (e.g. thyroid size, level of thyroid-stimulating hormone receptor antibodies [TRAbs] and smoking habits) and better patient education have improved our overall ability to individualize therapy and to obtain good clinical outcomes. However, achieving cure of the underlying autoimmune disease in a safe and cost-effective manner remains elusive in the majority of patients. Recurrence rate following antithyroid drugs is usually more than 50%. Eventually, the majority of patients treated with radioiodine become hypothyroid as does many of those undergoing surgical or radioiodine ablation. Therefore, it is not surprising that consensus as to the best therapy for achieving euthyroidy has been difficult to reach.[2,4,5]

Regarding the most incapacitating manifestation of GD, thyroid-associated ophthalmopathy (TAO; also called Graves' orbitopathy), progress in improving therapy has been even more difficult.[6,7] The established methods of treatment, including oral or intravenous glucocorticoids, orbital irradiation, surgical decompression or a combination of these, offer only incremental improvement compared with allowing the disease to take its natural course. Cost-effectiveness is poor and the improvement of quality of life during and following therapy remains modest.[8] Offering patients with GD counselling regarding the pros and cons of radioiodine ablative therapy and cessation of cigarette smoking has positively impacted the prognosis of TAO when considering the cost-effectiveness of intervention.

Since the initial observations dating to 2006, we[9] and others[10] have reported the beneficial effects in GD of B-cell depletion with

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the therapeutic antibody rituximab (RTX). These and subsequent studies have suggested that RTX may benefit patients with GD as well as those manifesting severe TAO. In the following article, we briefly update the potential mechanisms of action of this drug and attempt to dissect the impact of therapy on thyroid function and the clinical behaviour of TAO. Despite favourable initial reports, in the light of the side-effects experienced by several investigators and the substantial cost of RTX therapy, we have tempered our enthusiasm. We now emphasize the great need for additional and more robust studies before we can recommend its use on a wider scale in GD.

Rationale for the use of rituximab in GD (Fig. 1)

RTX is a human/murine chimeric monoclonal antibody, which targets CD20, a transmembrane protein, present on immature and mature B cells, but absent on most pro-B cells and plasma cells.[11] It was originally utilized in the treatment of B-cell lymphomas, an indication first identified in 1997 (reviewed in reference[12]). Subsequently, RTX proved useful in the treatment of a variety of autoimmune diseases.[13] Among the first were IgM antibody-related polyneuropathies in 1999[14] and rheumatoid arthritis (RA) in 2001.[15] The rationale behind the use of RTX in these and allied diseases remains the putative role of autoantibodies (i.e. rheumatoid factors and, as subsequently shown, anticyclic citrullinated peptid antibodies in RA) in their pathogenesis. It was believed that disruption of pathogenic autoantibody generation through B-cell depletion would yield long-term remission.[16] As GD is in part an autoantibody-mediated disease where TRAbs (or more specifically the stimulating fraction of TRAbs, known as TSAbs) are pathognomonic for GD, Hasselbalch[17] and Wang and Baker,[18] as the first, suggested the potential benefit of RTX therapy in GD. In addition to the thyroid-stimulating hormone and the antibodies directed against it, the insulin-like growth factor receptor (IGF-1R) has been demonstrated by one group to be over-expressed on orbital fibroblasts[19] in TAO and its ligation by IGF-1 or IgG's from patients with GD results in the production of powerful T-cell chemoattractants.[20] Moreover, both T and B lymphocytes are skewed towards the IGF-1R$^+$ phenotype, resulting in enhanced proliferation and survival.[21,22]

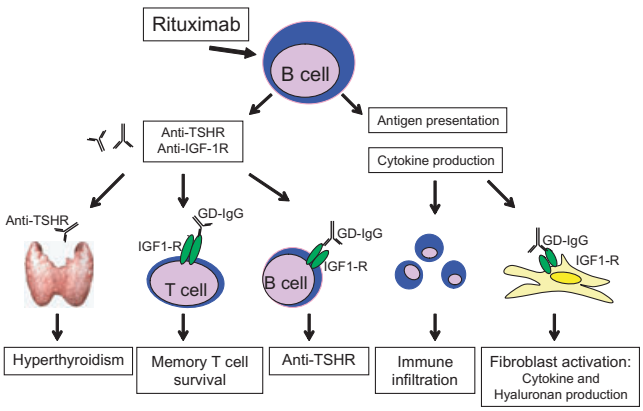

Fig. 1 The multifaceted role of B cells in Graves' disease (GD). Rituximab specifically eliminates peripheral CD20$^+$ B cells. B cells produce autoantibodies to the TSHR and IGF-1R, which leads to hyperthyroidism and potentially fibroblast activation. B and T cells from Graves' patients display increased expression of the IGF-1R and activation of this receptor via GD-IgG enhances B-cell autoantibody production and T-cell survival. Furthermore, B cells are excellent antigen-presenting cells and produce cytokines which may be central to immune infiltration and the exuberant inflammatory response demonstrated in patients with GD.

In addition to their role as precursors for antibody-secreting plasma cells, B cells are highly efficient antigen-presenting cells (APCs).[23] Studies using animal models of RA[24] and type 1 diabetes mellitus[25,26] have pointed towards a critical role for B cells as APCs in disease pathogenesis. Accordingly, a beneficial effect of RTX has been demonstrated in classical T-cell-mediated autoimmune diseases, such as multiple sclerosis[27] and type 1 diabetes.[28] Because they can take up minute amounts of antigen via their high-affinity antigen receptor, antigen-specific B cells are efficient APCs, even at very low antigen concentrations.[23] It should be noted that an (self) antigen associated with IgG-containing immune complexes may engage B cells expressing rheumatoid factor as antigen receptor in antigen presentation.[29] Moreover, as we have previously shown regarding human thyroglobulin, B cells may take up self-antigen in the context of complement-activating immune complexes, through the complement receptor 2 (CR2, CD35), and in so doing, initiate a T-cell response.[30,31]

In addition to their roles in antibody production and as APCs, B cells are capable of producing a variety of cytokines. These include pro-inflammatory cytokines such as IL-6, tumor necrosis factor-α (TNF-α) and lymphotoxin,[32–34] and anti-inflammatory cytokines including IL-10 and transforming growth factor β (TGF-β).[33–35] Recently, an IL-10-producing subset of B cells, known as regulatory B cells (Bregs) or B10 cells, has been associated with protection against experimental autoimmune encephalitis[35] and arthritis.[36] Moreover, B cells can induce T-cell anergy through their TGF-β production.[37] Somewhat paradoxically, B cells seem to play detrimental roles under certain conditions while exerting protective effects in others. This may be explained by the protective actions of B cells during the autoimmune disease induction, prior to their pathogenic roles following disease initiation.[38]

In autoimmune thyroid disease (AITD), the gland often exhibits a lymph node-like architecture, where germinal centres harbour somatic hypermutation and affinity antibody maturation.[39] These germinal centres support the development of specific memory B cells and plasma cells, and thyroid autoantibody generation.[39,40] We have recently shown that B cells are completely absent from thyroid and colon within 1 week and at 69 days, respectively, following RTX therapy.[41,42] Moreover, others have shown the absence of B cells from additional examples of inflamed tissue.[43,44] Taken together, these data indicate that RTX mediates complete B-cell depletion in inflamed tissues manifesting autoimmune diseases by disrupting germinal centres.

With regard to TAO, Salvi *et al.*[10] reported an almost complete absence of lymphocytes in an orbital biopsy taken 10 months after RTX therapy. In contrast, we[45] found that the B-cell content in the orbital tissue following RTX in a patient with TAO was comparable to that in another patient not receiving the therapy. It is unclear whether the presence of B cells in that case reflects insufficient

depletion or repopulation of lymphocytes. Clearly, additional sampling, perhaps undertaken at earlier time points, will prove necessary to establish the completeness and durability of RTX-dependent B-cell depletion in orbital tissue.

The effect of rituximab on thyroid function

Three studies have been conducted examining the effect of RTX on thyroid function in GD.[46–48] We must acknowledge limited data from the few patients thus far reported (Table 1). These preliminary observations currently preclude drawing any conclusions. Thus, enthusiasm generated by the novelty and potential benefit of RTX therapy in GD must be tempered by the shortcomings of each of these studies. Many factors varied among individual patients and between the three studies. Among them, duration of disease, interval since diagnosis, primary disease *vs* recurrence, familial *vs* sporadic AITD, duration and type of treatment for hyperthyroidism, presence of TAO, initial thyroid size, TRAb levels, smoking habits, RTX dose, follow-up intervals and inclusion of control subjects all varied between the three studies.[46–48]

El Fassi *et al.*[46] treated prospectively a group of 20 patients with newly diagnosed but untreated hyperthyroidism with methimazole (MMI) for approximately 4 months until they became euthyroid. Ten of these were then treated with RTX, two of whom manifested TAO. All were followed off MMI until hyperthyroidism recurred. Within approximately 1 year, all the patients not receiving RTX had relapsed, while 4 of 10 patients treated with RTX were euthyroid and have subsequently remained so for up to 30 months. TRAb levels appear predictive of this sustained remission, as all four had values below 5 IU/l. Further, in support of an effect of RTX, all patients with similar low TRAb levels who did not receive RTX relapsed within a year. Salvi *et al.*[47] studied nine patients (Table 1), of whom seven manifested TAO. At the time of RTX therapy, four were hyperthyroid and had yet to be treated and five were euthyroid. Among these five, some were in remission, others were undergoing treatment with MMI or were receiving levothyroxine following surgical thyroidectomy. Eight patients were re-assessed 12 months after receiving RTX, while one was re-examined 5 months later. The authors concluded that RTX failed to affect thyroid function, as those in remission remained euthyroid, while those who were hyperthyroid and remained untreated showed no improvement in their thyroid function. These were thus started on MMI. Finally, Heemstra *et al.*[48] treated 13 patients with relapsing GD of whom three manifested mild TAO. Ten patients were transiently treated with MMI. There was no control group and follow-up examination occurred 26 weeks following administration of 1 g of RTX i.v. twice with an interval of 2 weeks. Four patients failed RTX treatment and received radioiodine therapy. Serum free-T4 concentrations declined while serum TSH levels increased in the remaining nine patients with normalization occurring in five. These nine patients have remained euthyroid for a median of 18 (range 14–20) months since RTX administration. Pretreatment levels of TRAb were relatively low (median 4 IU/l, range 0·2–6·3), congruent with those reported in the study of El Fassi *et al.*[46] Despite the small number of patients included and the lack of control subjects, the study of Heemstra *et al.*[48] taken together with ours[46] suggests that RTX may influence remission rates in GD, especially in patients with low TRAb levels.

The effect of rituximab on autoantibodies

El Fassi *et al.*[46] demonstrated that TRAb levels decrease at similar rates in patients regardless of whether they are receiving RTX as a single agent (median 29%), RTX combined with MMI (36%) or only MMI (64%) at 13 weeks. Subsequent levels were found to decline in patients treated with RTX, while the levels remained relatively constant in those not receiving the drug. Salvi *et al.*[47] found a mean decrease of 40% in TRAb levels in RTX-treated patients after 30 weeks, similar to that observed in patients treated with i.v. glucocorticoids. In the study by Heemstra *et al.*,[48] the median TRAb levels of the nine responders decreased by 52% within 26 weeks following RTX administration. Thus, a moderate decrease in TRAb levels occurred following RTX therapy in all three studies, but the decline was similar to that in patients treated with MMI or prednisolone.

The report by El Fassi *et al.*[49] of a decrease (median 84%) in cAMP production in TSHR-transfected CHO cells elicited by sera collected from patients treated with RTX, strongly suggests that TSAb activity had declined within 20 weeks of therapy. Those patients not treated with RTX failed to exhibit a decrease despite similar decreases in total TRAbs in the two groups.[46] It remains obscure how TSAb production can be specifically affected by RTX.

Table 1. Pertinent clinical data in patients with Graves' disease (GD), with or without thyroid-associated ophthalmopathy (TAO), treated with rituximab. Influence on thyroid function and TAO

Study and reference number	Number and sex	Rituximab dose	Thyroid state	Initial CAS	Final CAS	Side effects	Remission-rate
El Fassi *et al.* 2007[46]	10 F. 2 with TAO	375 mg/m^2 weekly for 4 weeks	Euthyroid	5 and 6	1 and 2	5 of 10	4 of 10*
Salvi *et al.* 2007[47]	7 F; 2 M 7 with TAO	1 g twice with 2 week interval	4 hyperthyroid 5 euthyroid	Mean 4·7	Mean 1·8	3 of 9	NA
Heemstra *et al.* 2008[48]	9 F; 4 M 3 with mild TAO	1 g twice with 2 week interval	Hyperthyroid	NA	NA	2 of 13	9 of 13†
Khanna *et al.* 2010[66]	4 F; 2 M All with TAO	1 g twice with 2 week interval	Euthyroid	Mean 5·3	Mean 1·8	3 of 6	NA

F, females; M, males; TAO, thyroid-associated ophthalmopathy; CAS, clinical activity score; NA, not applicable.
*Follow-up 14–30 months post rituximab therapy.
†Follow-up 14–30 months post rituximab.

Certain specificities of autoreactive plasma cells are more short-lived than plasma cells producing antimicrobial antibodies and disappear more rapidly following B-cell depletion,[50] and this may also apply to TSAb-producing plasma cells. As expression of the TSH-receptor can be up-regulated upon stimulation[51] and the A subunit of the receptor can be shed from thyroid cells,[52] intrathyroidal TSAb-producing B cells may stimulate the release of their cognate antigen, and disruption of thyroid germinal centres by RTX may interrupt this positive feedback loop. However, because of the small number of sera with TRAb levels sufficiently high to allow this kind of analysis, the findings of a differential effect of RTX on TSAb level compared with that on TRAbs need to be confirmed in a larger study.[49]

The data presented by Salvi *et al.*[47] indicate an approximately 65% decrease in TPOAb levels by 50 weeks following RTX administration, findings congruent with our results.[49] While the effect of RTX on anti-thyroglobulin antibodies was not examined by El Fassi *et al.*,[46] Salvi *et al.*[47] indicated a non-significant 50% decline in those antibodies within 8 weeks in the patients treated with RTX. To date, no reports have appeared concerning potential effects of RTX on other autoantibodies in TAO, including those against collagen XII[53] or insulin-like growth factor receptor.[54]

Treatment with RTX, usually in combination with cyclophosphamide and prednisolone, generally has modest effects on levels of circulating IgG and IgA. This includes antimicrobial and self-directed antibodies.[49,55–61] In contrast, RTX often lowers levels of IgM, including rheumatoid factor.[61,62] We know now that plasma cells can be especially long lived,[63] which explains why detectable levels of IgGs persist, even during B-cell depletion. On the other hand, levels of some autoantibodies, including anti-dsDNA, anti-C1q and ANCA, can fall markedly after initiation of RTX treatment.[60,61,64]

The effect of rituximab on TAO

When considering evidence for a positive impact of RTX on the clinical course of TAO, concerns similar to those articulated with regard to the glandular component of GD appear to apply. In addition, the well-recognized limitations in evaluating and classifying patients with TAO confound any assessment of the studies performed. These barriers merit attention.[65] In the three studies, only 17 patients with TAO were included[9,47,66] (Table 1). While these studies report remarkable improvement in the activity and severity of TAO following RTX therapy, the substantial limitations of all three preclude drawing any firm conclusions.

The study of El Fassi *et al.*[9] included two female ex-smokers who had previously received both glucocorticoids and retrobulbar irradiation. Both were euthyroid and had completed their course of steroids at time of RTX therapy. Eight months after RTX administration, the clinical activity score (CAS) had decreased from 6 to 2 in one patient and from 5 to 1 in the other. Disease severity (soft tissue changes and eye motility) was significantly improved in both patients, as was proptosis. Effects were evident as early as 1 month post-therapy and have lasted for more than 1 year without additional therapy.

Seven of nine patients followed prospectively by Salvi *et al.*[47] had active TAO, while the others manifested lid signs. Evaluated 7 months after RTX therapy, the CAS decreased from a mean of 4·7–1·8. Disease severity, including soft tissue inflammation, was reduced significantly and ocular motility improved. The effect was evident at 1 month after therapy and has lasted for at least 1 year.

Most recently, we reported that in six patients unresponsive to glucocorticoid therapy and presenting with severe and active TAO, RTX had a rapid and sustained therapeutic effect on both activity and severity of TAO.[66] CAS decreased from a mean of 5·3–1·8 after 8 weeks had elapsed following therapy and continued for an average of 6 months. Four of the six patients had presented with optic neuropathy. Visual acuity improved in all within 4 weeks of RTX therapy and returned to premorbid levels within 2 months. Tapering of glucocorticoids, given concomitantly in all patients, was uniformly well-tolerated. Not surprisingly none of the patients experienced improvement in extra-ocular motility or proptosis. In contrast to the other articles, failure of RTX in improving TAO in a single patient was recently reported.[67]

Taken together, these limited studies on a heterogeneous cohort of patients suggest that RTX may prove efficacious in those patients with TAO who are most needy. But controlled studies must now be conducted. In view of the high cost of RTX and the reportedly serious side effects, such studies must focus on both efficacy and on carefully defining the subgroup of patients most appropriate for inclusion. Awaiting such studies, it is our opinion that RTX be used when evidence-based therapy has failed or is contraindicated.

Possible mechanisms for RTX action

A series of animal studies[24–26,68,69] and clinical trials of RTX in T-cell-mediated autoimmunity[27,28] has taught us that RTX targets the APC functions of B cells. It remains likely that abolished antigen presentation by B cells also plays a significant role in the clinical improvement observed in GD and TAO following RTX therapy. In TAO, attenuation of antigen-presentation by B cells seems a plausible explanation for the clinical effect of RTX. This is entirely consistent with the view that TAO is T-cell mediated, and because a role for auto-antibodies in the pathogenesis of this component of GD has yet to be established convincingly.

RTX therapy alters the behaviour of other immune cells besides B cells. For instance, administration of the drug to patients with RA up-regulated expression of B-cell-activating factor, IL-10 and CD86 in monocyte-derived macrophages.[70] Another important potential aspect of RTX actions on immune function concerns its impact on B-cell cytokine production. In patients with RA, B-cell depletion results in a dramatic elevation of serum IL-8 levels.[71] The markedly elevated levels of IL-5, IL-6 and IL-10 found in untreated individuals with HIV infections were reduced following RTX therapy.[72] Serum TNF-α levels were also reduced within 2 days of RTX administration to patients with systemic lupus erythematosus (SLE).[73]

Of particular relevance for GD, it is conceivable that B cells support inflammatory plasma cell niches where the production of auto-antibodies occurs and that RTX therapy destroys such inflammatory niches.[74] This mechanism might enhance the turnover of

autoantibody-producing plasma cells, and in so doing reduce autoantibody production. The available data from three studies of RTX in GD or TAO suggest that TRAb levels decrease following B-cell depletion to a similar extent to that seen following treatment with MMI or prednisolone.[46–48] However, the prolonged remission observed in patients with low baseline TRAb levels indicates that pathogenic antibody specificities may have been temporarily removed from the repertoire of the repopulating B cells. A similar phenomenon was observed in SLE where RTX treatment causes a temporary decrease in the proportion of specific $CD27^+$ memory B cells producing disease-associated auto-antibodies encoded by the V_H4-34-gene.[75]

Side effects of rituximab

In deciding on disease management, the potential side effects of RTX therapy need to be balanced with disease severity and suitability of therapeutic alternatives. In uncomplicated GD, inexpensive and time-proven therapies make the use of RTX relatively unattractive.[4,76,77] The threshold for its use differs substantially in severe TAO, where our options are considerably more limited. All studies examining RTX therapy in GD and TAO have reported the occurrence of side effects, but with varying prevalence and severity.[42,46–48,66] Pretreating with 1 g of acetaminophen and 2 mg of i.v. clemastine, El Fassi *et al.*[46] encountered side effects in 5 of 10 patients following the initial RTX infusion. Four patients developed hypotension, two became nauseated, one became febrile, another complained of chills and one developed sinus tachycardia. Two of these patients received antihistamine and one was given glucocorticoids. Four days after the second infusion, two patients developed serum sickness (joint pain and fever), one of whom subsequently developed diarrhoea and iridocyclitis a year later. Another had recurrent fever, symmetric polyarthritis and ulcerative colitis diagnosed 1–2 months after the second infusion.[42] The latter process is remarkable as administration of RTX to patients with ulcerative colitis has lead to its exacerbation.[78] This finding suggests that B cells might play a protective role mediated by IL-10, which may then override any detrimental aspects of B-cell function. Therefore, RTX should be administered with caution in patients with concomitant inflammatory bowel disease.

In the study of Salvi *et al.*[47], 1 g of paracetamol and 10 mg chlorphenamine were given as pretreatment. Only three of nine patients had mild side effects during the first RTX infusion, such as a mild fever, which was treated by 100 mg hydrocortisone i.v. Heemstra *et al.*[48] gave 10 mg dexamethasone and 2 mg clemastine i.v. and reported no other side effects than temporary joint pain in two patients who had no clinical signs. Khanna *et al.*[66] administered 100 mg i.v. methylprednisolone, 1 g acetaminophen and 50 mg diphenhydramine as premedication. One patient developed a urinary tract infection, one had worsening hypertension and one died from sudden cardiac arrest 3 months after the second infusion.

The relatively small numbers of cases thus far reported make drawing any valid conclusions impossible with regard to efficacy or side effects. Reconciling the types and severities of the complications thus far encountered in patients with GD with those experienced in other diseases where RTX is administered may provide valuable insights. Until results from a randomized study with standardized recruitment become available, any assessment of side effects remains difficult. These studies are apparently underway,[79,80] although their scope and size may prove inadequate. Unfortunately, several obstacles remain concerning the evaluation of clinical efficacy in TAO.[65] Until these are resolved, interpretation of any clinical trial will be confounded with the same problems that have plagued this field for decades.

Conclusions and areas for future research

Treatment of Graves' disease (GD) complicated by thyroid-associated ophthalmopathy (TAO) remains difficult and imprecise, largely because we have not yet determined many aspects of disease pathogenesis. On the horizon is the potential for utilizing several highly specific agents that target components of the immune system.[79,80] The advantage they appear to offer results from their targeted mechanisms of action. Among them, rituximab (RTX) may prove effective and seems to interrupt many aspects of the immune response that we predict would underlie this disease. Clearly, the clinical assessment is in its very early stages and will require additional and more robust trials. But if we are to approach treatment of this disease in a more systematic manner, we must learn much more about its cause, identify the aberrant molecular and cellular events that lead to its manifestations and to develop animal models in which we might screen drug candidates.[79] In addition, we can almost certainly gain from the experience in developing therapies for related autoimmune diseases, where greater progress is being made.

Competing interests/financial disclosure

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