

Understanding Pathogenesis Intersects With Effective Treatment for Thyroid Eye Disease

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Abstract

Context: Thyroid eye disease (TED), a vision-threatening and disfiguring autoimmune process, has thwarted our efforts to understand its pathogenesis and develop effective and safe treatments. Recent scientific advances have facilitated improved treatment options.

Objective: Review historically remote and recent advances in understanding TED.

Design/Setting/Participants: PubMed was scanned using search terms including thyroid-associated ophthalmopathy, thyroid eye disease, Graves' orbitopathy, autoimmune thyroid disease, and orbital inflammation.

Main outcome measures: Strength of scientific evidence, size, scope, and controls of clinical trials/observations.

Results: Glucocorticoid steroids are widely prescribed systemic medical therapy. They can lessen inflammation-related manifestations of TED but fail to reliably reduce proptosis and diplopia, 2 major causes of morbidity. Other current therapies include mycophenolate, rituximab (anti-CD20 B cell-depleting monoclonal antibody), tocilizumab (interleukin-6 receptor antagonist), and teprotumumab (IGF-I receptor inhibitor). Several new therapeutic approaches have been proposed including targeting prostaglandin receptors, vascular endothelial growth factor, mTOR, and cholesterol pathways. Of potentially greater long-term importance are attempts to restore immune tolerance.

Conclusion: Despite their current wide use, steroids may no longer enjoy first-tier status for TED as more effective and better tolerated medical options become available. Multiple current and emerging therapies, the rationales for which are rooted in theoretical and experimental science, promise better options. These include teprotumumab, rituximab, and tocilizumab. Restoration of immune tolerance could ultimately become the most effective and safe medical management for TED.

Key Words: Graves' disease, IL-6 receptor, orbit, ophthalmopathy, autoimmunity, IGF-I receptor, TSH receptor, teprotumumab, glucocorticoids

Abbreviations: AE, adverse event; CAS, clinical activity score; CD, cluster of differentiation; EUGOGO, European Group on Graves' Orbitopathy; FcRn, neonatal fragment crystallizable receptor; GD, Graves' disease; IGF-IR, IGF-I receptor; MHC, major histocompatibility complex; MMF, mycophenolate mofetil; OF, orbital fibroblasts; QoL, quality of life; RANTES, Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted; TED, thyroid eye disease; TSHR, thyrotropin receptor; VEGF, vascular endothelial growth factor

Thyroid eye disease (TED, also known as thyroid-associated ophthalmopathy and Graves' orbitopathy) represents a disfiguring and potentially sight-threatening condition most commonly associated with Graves' disease (GD) (1)(Fig. 1). In some instances, TED can accompany Hashimoto thyroiditis. It can inflict substantial physical and emotional morbidity, leading to reduced quality of life (2). Major shortfalls in the management of TED stem from health care provider unfamiliarity with the disease, leading to incorrect diagnosis and therapeutic delays. This is especially true in patients not manifesting obvious thyroid autoimmunity and those in whom subtle signs of periocular inflammation may be misinterpreted. Timely implementation of appropriate medical treatment appears to offer the best clinical outcomes.

TED follows a characteristic disease course, described more than 70 years ago by Rundle and Wilson (3). After typically presenting with subtle signs and symptoms of activity dominated by eyelid retraction, inflammation, and tissue swelling/congestion, some patients with more severe disease develop proptosis and/or diplopia. Mild TED usually does not require

systemic medical therapy and can be managed with local care, such as eyedrops, compresses, tobacco smoke avoidance, and eye protection against strong light and wind. Systemic medical therapy, most frequently glucocorticoids administered either as oral or IV preparations, is typically implemented during the active phase of moderate to severe, symptomatic disease and urgently with development of compressive optic neuropathy. Steroids lessen inflammation-related signs and symptoms of TED (4). Other currently used medical therapies including mycophenolate (MMF), rituximab, selenium, and tocilizumab, may also reduce inflammation and other components of TED activity. Importantly, none of these off-label medications reliably improves diplopia or proptosis and thus none reduces TED severity. Disease activity/progression culminates after 1 to 4 years in chronic, stable disease, when worsening and clinical activity abate. It is during the chronic phase that surgical rehabilitation has been widely viewed as the only therapeutic option remaining for residual disease. These procedures, including orbital decompression, strabismus surgery, eyelid repair, and a variety of cosmetic strategies, are

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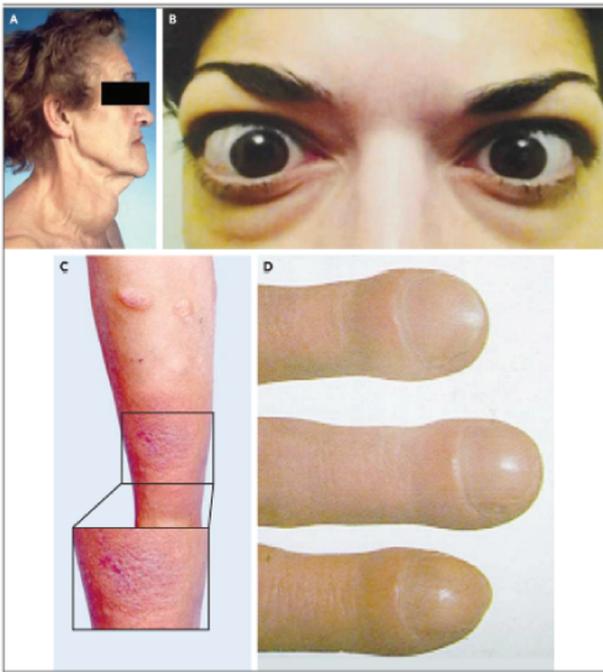


Figure 1. Clinical manifestations of Graves' disease. (A) Diffuse moderately enlarged goiter in a woman with hyperthyroidism from Graves' disease. (B) Moderate to severe thyroid eye disease including bilateral proptosis, periorbital edema, scleral injection, and lid retraction in this patient. (C) Pretibial dermopathy of the plaque form affecting both legs in this patient. (D) Acropachy with fingernail clubbing. From N Engl J Med, Smith T.J. and Hegedus L. Graves' disease. 375; 1552-1565. Copyright © (2016) Massachusetts Medical Society. Reprinted with permission.

typically performed sequentially (5). Any of these surgeries can reactivate TED and lead to imperfect results. Thus, better medical treatment options are very much necessary. But development of targeted and optimally effective drugs requires more complete understanding of disease pathogenesis.

This paper attempts to briefly review basic and clinical research leading directly to development of targeted therapies for TED. It should provide a roadmap followed as scientific insights have emerged from many laboratories. These developments have yielded an underlying rationale for specific TED treatments.

Deciphering the Pathogenesis of TED

Identifying Pathogenic Autoantigens

Detailed understanding of the molecular and cellular basis for TED has eluded investigators for many decades. The relationship between thyroid autoimmunity in GD and TED was explored in the past. Initial connections between thyroid and orbit were identified once shared thyroid autoantigen expression, including thyroglobulin and TSH receptor (TSHR), were detected in orbital tissues (6, 7). Although the central role for TSHR in GD is now well established, its mechanistic involvement in TED is less clear cut (Fig. 2). There is generally good correlation between TED activity/severity and detectable anti-TSHR antibodies (8), although thyroid-stimulating immunoglobulins (TSIs) are sometimes undetectable in rare patients with severe TED (9). But the importance of TSI actions within the orbit and tissues peripheral to thyroid can only be estimated, based

largely on in vitro studies, in addition to inferences emerging from improved preclinical models (10).

IGF-1 receptor (IGF-1R) has been implicated in TED development, representing a second autoantigen (11). IGF-1R is overexpressed by orbital fibroblasts and B and T cells in GD (12-14). TSHR and IGF-1R form a physical and functional signaling complex (12). Importantly, pathogenic signaling initiated at either receptor is dependent on IGF-1R activity, suggesting a mechanism involving receptor:receptor cross-talk and perhaps TSHR transactivation of IGF-1R (12). In addition, anti-IGF-1R autoantibodies have been detected by some investigators in patients with GD (11, 15-17). In contrast, other laboratory groups have failed to experimentally connect these autoantibodies to GD or TED pathogenesis (18, 19). The central involvement of IGF-1R in mediating GD autoantibody-provoked signaling provided the core rationale for targeting IGF-1R therapeutically. It remains uncertain whether functional, activating autoantibodies directed at epitopes on IGF-1R play an important role in disease development. That issue has generated substantial controversy that remains unresolved. The most likely explanation for disparate findings emerging from various laboratories concerning IGF-1R activating autoantibodies is wide variation in experimental design, culture conditions, and target cell-types used (12, 17, 18, 20-22). Although the precise mechanisms through which TED pathogenesis involves TSHR and IGF-1R remain to be clarified, important evidence clearly implicates both pathways. Potential mechanisms through which TSHR:IGF-1R interactions occur and their impact on TED are detailed in another paper appearing in this issue (23). Importantly, targeting IGF-1R has yielded an effective therapeutic strategy.

Proposed Mechanisms Underlying Disease in the TED Orbit

Importance of circulating and orbit-infiltrating B cells, T cells, and mast cells in the pathogenesis of TED has been investigated for many years by several laboratory groups (24-26). By virtue of their cytokine expression repertoires and interactions with the residential fibroblast population within the orbit, lymphocytes are viewed as critical to the initiation and perpetuation of inflammation and tissue remodeling occurring in TED. Mast cells are also implicated in disease pathogenesis (27). HMC-1 mast cells activate orbital fibroblast production of hyaluronan and PGE₂ (28).

Less well-studied in the context of TED have been monocytes and their putative derivatives, cluster of differentiation 34⁺ (CD34⁺) fibrocytes (29). The fibroblast population inhabiting the TED orbit comprises distinct subsets which can be segregated based on either CD90 (Thy-1) (30, 31) or the myeloid cell marker, CD34 (32). Thy-1⁺ orbital fibroblasts differentiate into myofibroblasts when activated by TGF- β and the activation of the Smad signaling pathway (33), whereas Thy-1⁻ fibroblasts differentiate into mature adipocytes when exposed to peroxisome proliferator-activated receptor γ agonists (34). CD34⁺ orbital fibroblasts (CD34⁺ OF) are putative derivatives of circulating, bone marrow-derived CD34⁺ fibrocytes, and cells involved in wound healing, scar formation, and fibrosis. Fibrocytes cultivated from the blood exhibit a CD34⁺ CXCR4⁺ TSHR^{high} Collagen 1⁺ phenotype. They possess substantial antigen presenting and T-cell costimulatory capacity (35, 36). CD34⁺ OF are uniquely identified in the TED orbit and express multiple thyroid

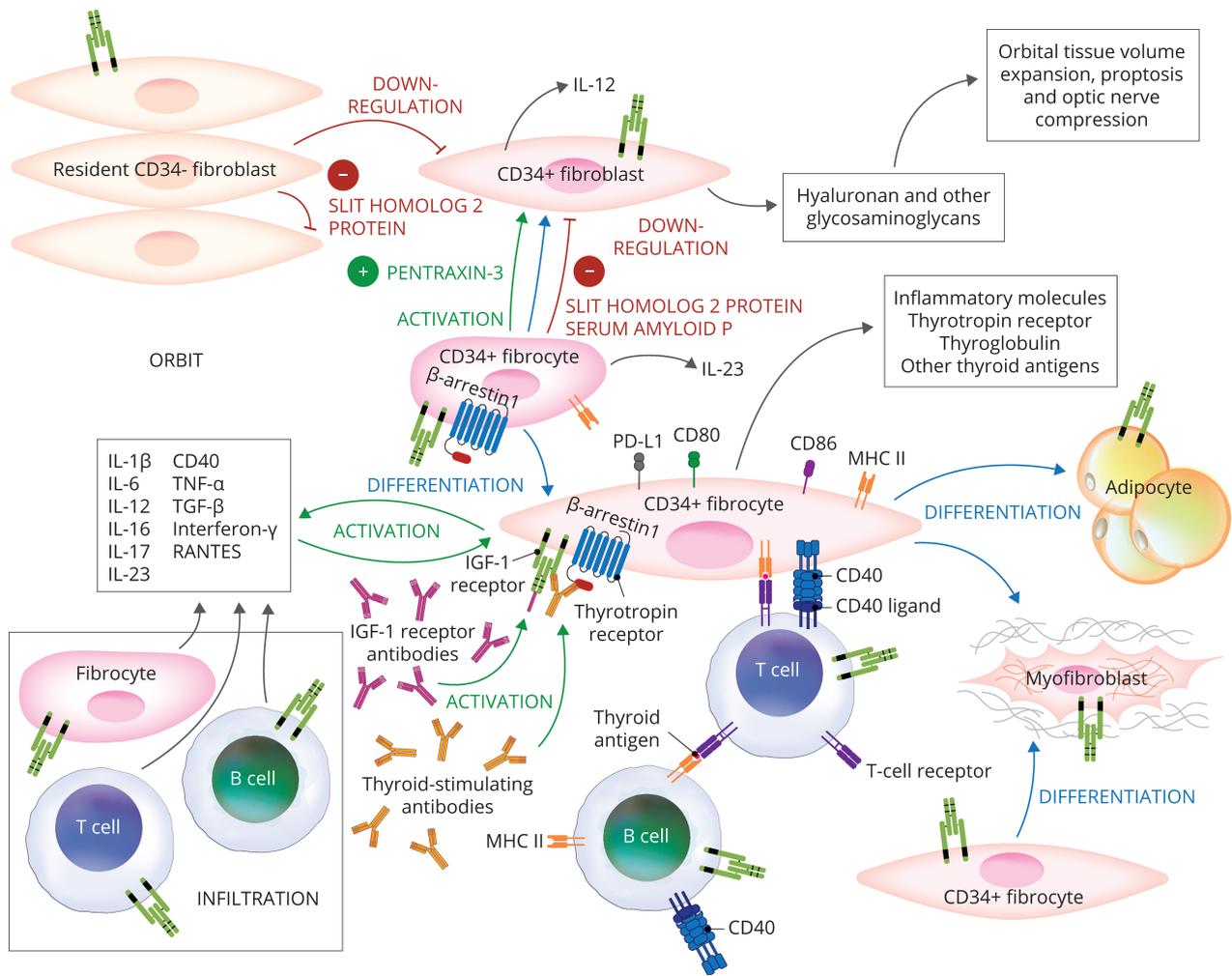


Figure 2. Proposed theoretical model of thyroid eye disease (TED) pathogenesis. Orbital fibroblasts exhibiting robust responses to inflammatory mediators appear to represent the central effector cells. CD34⁺ fibroblasts derived from CD34⁺CXCR4⁺ collagen 1⁺ fibrocytes, monocyte-derived progenitors traffic from bone marrow to the TED orbit. Fibrocytes express several thyroid-specific proteins, including thyrotropin receptor (TSHR), thyroglobulin, thyroperoxidase, and sodium-iodide symporter and express class II major histocompatibility complex (MHC). Fibrocytes and orbital fibroblasts undergo differentiation into myofibroblasts and adipocytes. Slit2 expressed and released by CD34⁺ fibroblasts down regulates expression of many genes expressed by fibrocytes and CD34⁺ fibroblasts. Interleukins 1 β , 6, 8, 10, 12, 16, and 23, tumor necrosis factor α , and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES), CXCL12, and CD40-CD154 are expressed in the TED orbit by various cell types and contribute to the inflammatory milieu. CD34⁺ and CD34⁺ orbital fibroblasts cell-surface display insulin-like growth factor-I receptor (IGF-IR) and express 3 mammalian hyaluronan synthase isoenzymes and UDP glucose dehydrogenase and synthesize hyaluronan. This glycosaminoglycan underlies in part orbital tissue expansion in TED. Hyaluronan synthesis localizes primarily to CD34⁺ orbital fibroblasts.

autoantigens (37, 38). These cells coexist with CD34⁺ OF. The interplay between CD34⁺ OF and CD34⁺ OF subsets results in modulation of the CD34⁺ OF gene expression repertoire, mediated through the Slit2/roundabout 1 pathway (39, 40). Slit2, a TSH-inducible glycoprotein, is expressed and released by CD34⁺ OF and downregulates several immune-related proteins in CD34⁺ OF. These include thyroid autoantigens such as thyroglobulin, TSHR, thyroperoxidase, and sodium iodide symporter. Further, fibrocytes and, to a lesser degree, CD34⁺ OF, display CD80, CD86, PD-L1, and major histocompatibility complex II (MHC II) proteins, the levels of which are also downregulated by Slit2 (36). Fibrocytes and CD34⁺ OF express and release several important cytokines (40). These include IL-1 β , IL-6, IL-8, IL-12, and IL-23. Levels of specific cytokines in CD34⁺ OF are regulated by Slit2 (41).

Debate continues as to whether immune responses occurring in GD and TED are similar or identical in thyroid and orbit, respectively. Recent evidence suggests that

peripheral Th1, Th2, and Th17 biased responses can dominate GD, perhaps at different disease stages. Further, autoimmune processes occurring in rodent models of GD may diverge from those in human beings. Th17 cells have been identified as playing potentially important roles in orbital tissue activation (42). IL-17A can induce Regulated upon Activation, Normal T Cell Expressed and Secreted (RANTES) expression in GD-OF (43) and may promote both inflammation and fibrosis in TED (44). Both autoreactive immunoglobulins and T cells appear to promote inflammation and remodeling of TED orbital tissues (1, 45). CD4⁺ and CD8⁺ T cells infiltrate the orbit as do B cells, CD34⁺ fibrocytes, and mast cells (27, 32, 44, 46, 47). These infiltrating cells generate several cytokines that act on residential fibroblasts to induce TED-relevant cytokines. GD-OF exhibit particularly robust responses to molecular cues within the diseased orbit (48, 49). They generate prostanoids, including PGE₂, when engaged with IL-1 β or CD154. These responses suggest that

prostaglandin endoperoxide H synthase-2, the inflammatory cyclooxygenase, might be therapeutically targeted in TED (50, 51). A number of proinflammatory cytokines and their receptors remain relatively unexplored as clinical targets for TED, including IL-1 β , IL-1 receptor, IL-1 receptor antagonist, IL-8, IL-12, IL-16, IL-17A, and IL-23, RANTES, and interferon γ (40, 43, 52-55). Each appears potentially relevant to TED because all induce target genes in GD-OF at both transcriptional and posttranscriptional levels (56, 57).

Because many of these cytokines comprise regulatory pathways mediating numerous types of immune responses, therapeutically altering their activities could result in off-target consequences. Thus, one could anticipate substantial barriers in bringing novel or repurposed cytokine-targeting medications for TED to the clinic.

Oxidative stress plays important roles in autoimmune disease (58). TED represents a process in which oxidative stress is likely to be involved (59) and therefore might be targeted therapeutically. Caveolin-1 is downregulated in orbital adipocytes, whereas deiodinase 3 and hypoxia-inducible factor-1 α are upregulated in TED-derived orbital adipose tissue (60). Hypoxia-inducible factor-1 α activation has been detected in cigarette smokers developing TED (61). Thiol-disulfide homeostasis may be disrupted in active TED (62). The increased oxidative stress in TED has led some investigators to propose therapeutic antioxidants for the disease (63). It is possible that the benefit of dietary selenium supplementation in deficient patients with TED results from the antioxidant properties of this element (64).

Among the emerging factors determining immune function and underlying autoimmune diseases are the microbiological inhabitants of the human gut, oral mucosa, and other tissue niches (65-68). Attention has been paid to viral constituents of the gut (69). In aggregate, findings to date suggest that probiotic therapies might benefit patients with autoimmune phenotypes (70). The potential relationship between thyroid autoimmunity, TED, and microbiota has prompted several studies yielding interesting relationships (71). In animal models of TED, gut microbiota may in part determine immune responses associated with disease development (72). Changes in gut microbiota have been identified in patients with severe, active TED (73). Propionic acid production by gut bacteria has been linked to alterations in Th17/Treg balance in GD (74). Altering gut flora may alter the resulting disease phenotype in animal models of GD (75). Whether the impact of gut flora modification in animal models of GD will translate into treating patients with TED has yet to be demonstrated.

Vascular endothelial growth factor (VEGF) is elevated in patients with TED (76). Levels of VEGF receptor 2 mRNA and VEGF-A, VEGF-C, and VEGF-D are increased in orbital tissues from patients (77). Tear VEGF levels are elevated in patients with active TED and are reduced in response to glucocorticoids (78).

Current Therapeutic Landscape for TED

Medical treatments historically available for TED have generally failed to meet all needs of many patients, especially those with moderate to severe, debilitating, and vision-threatening disease (Fig. 3). Most cases of mild TED can be effectively managed with conservative, local measures and do not require systemic medications. For moderate to severe active TED, frequently used drugs exhibit broad anti-inflammatory and

immune-suppressing properties, thus limiting both their dosages and duration of exposure. The concerning properties and limitations of these drugs have driven attempts to identify new options for targeted therapy through better understanding of TED pathogenesis. The array of off-label medications currently in use represents in large part several “old standbys.” Many of these lack adequately powered, placebo-controlled studies validating their effectiveness. Newly introduced and repurposed drugs and others “waiting in the wings” promise a dramatically changed treatment paradigm for TED in the immediate future.

Steroids Remain the Most Frequently Used Systemic Medications for TED

Because inflammation is frequently a dominant presenting feature of TED, enthusiasm for steroid use, beginning decades ago, made good sense (79). Active, moderate to severe, progressive TED is frequently treated with relatively high-dosage, IV glucocorticoids (80, 81). Steroids exemplify the broadly targeted therapies currently in wide use for TED, given the extensive clinical experience with them, both in formal studies and in real-world patient care (82). In addition, they enjoy relatively low cost and wide availability. Like other nonspecific anti-inflammatory agents, steroids are used off-label for TED. Glucocorticoids act through both nuclear and plasma membrane receptors (Fig. 4). They induce or attenuate expression of several genes, including those encoding proteins involved in proinflammatory pathways in T and B cells, GD-OF, and CD34⁺ fibrocytes. Many of these glucocorticoid steroid effects are mediated through their regulation of transcription factor, nuclear factor- κ B (83-85). The multiple consequential genes lying downstream from glucocorticoid receptor/nuclear factor- κ B interactions are likely to underly, in large part, the common side effects associated with steroid use in TED (86, 87). These off-target consequences severely limit their dosages and treatment duration (1, 88-90).

Pulse IV administration of steroids is preferred over oral dosing based on studies showing greater efficacy and a reduced side effect profile (91, 92). Importantly, steroid popularity, regardless of administration route, rests largely on common interpretations of results from studies/trials not involving placebo controls where clinical activity scores (CAS) improve. Unfortunately, little evidence exists demonstrating the reliable effectiveness of steroids in TED beyond reducing the consequences of inflammation, including pain and ocular discomfort. Many facets of clinical activity typically improve as a consequence of natural disease course, as is pictorialized by the Rundle curve (3). Only a single, placebo-controlled clinical study of steroids has been reported. That report by van Geest et al (93) included 15 patients with moderate-severe TED. The authors concluded steroid superiority over placebo based on responses observed in 6 patients treated with methylprednisolone (6 g total) over 3 months compared with 9 patients receiving placebo. Further, their conclusions were based on outcomes heavily weighted on inflammation and its consequences over a 48-week observation period. Improvement in other parameters, such as proptosis and diplopia, which represent important sources of morbidity and diminished patient quality of life (QoL), were not essential for achieving response in this study. Many more recent studies of steroids have focused on establishing the most effective and safest dosage and route of administration. These have uniformly not included placebo controls (92).

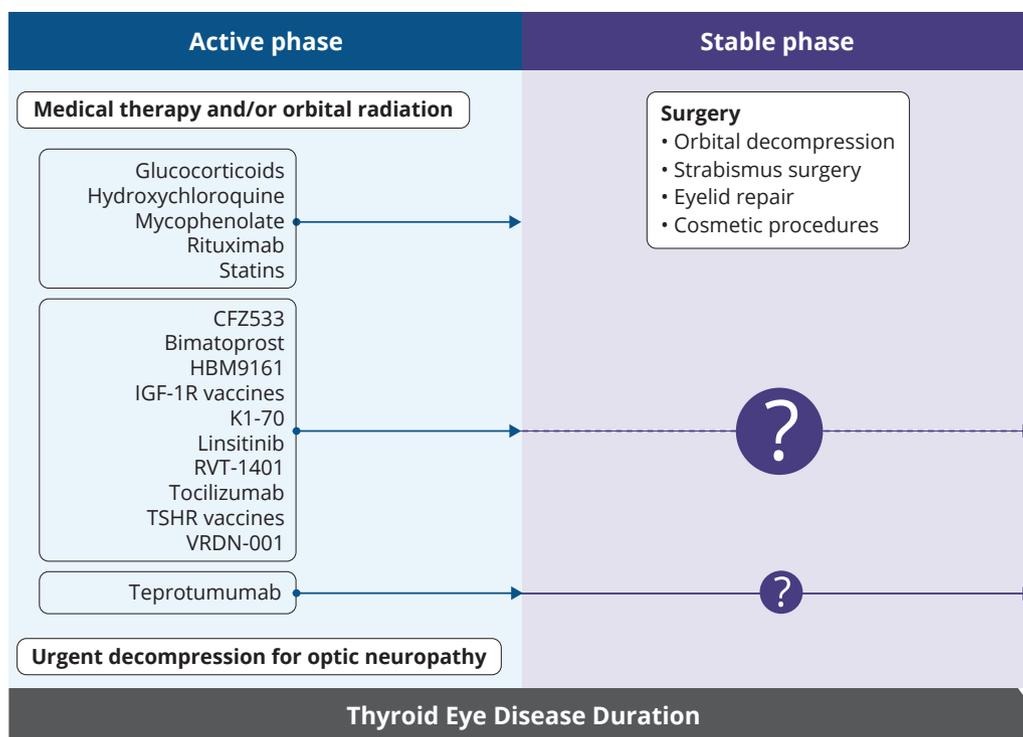


Figure 3. Use of current therapies and those under development in active and stable, moderate to severe TED. Medical treatment for mild active TED is typically limited to local, nonsurgical measures. In contrast, management of moderate to severe, active TED often involves systemic medications. Some of these drugs can reduce disease activity (signs and symptoms related to inflammation) but most appear relatively ineffective in stable TED. Preliminary reports suggest that teprotumumab may also be effective during stable disease, but clinical trials will be necessary to establish its effectiveness in nonprogressive TED. Relative size of question marks reflects the relative uncertainty of specific treatment effectiveness in stable TED. Surgery is typically reserved for TED reaching the stable phase. The exception is the development of vision loss from compressive optic neuropathy or severe anterior eye surface deterioration. Rituximab (anti-CD20 displayed on B cells), tocilizumab (IL-6 receptor inhibitor), teprotumumab, VRDN-001, linsitinib (IGF-IR inhibitors), K1-70 (TSHR inhibitor), RVT-1401 and HBM9161 (FcRn antagonists), CFZ533 (anti-CD40 antagonist), and TSHR and IGF-IR vaccines (for potentially restoring immune tolerance).

Widespread availability of glucocorticoids, prescriber familiarity with their use in TED, and limited access to newly developed biologicals perpetuate calls for their first-line status in Europe. The recently published European Group on Graves' Orbitopathy (EUGOGO) guidelines for medical treatment of TED list high-dosage steroids in combination with MMF as “first-line therapy” (94). Recommendation for this 2-drug approach appears to rest on a single study at 4 different institutions where the medications were combined (95). The relative strength of evidence supporting this combined therapy for TED is discussed in the following section. Another aspect of European preference for steroids stems from the unavailability of 1 newly available therapy. Teprotumumab has not yet been approved for clinical use outside of the United States. Many authors agree that steroids may be very useful in patients acutely experiencing vision threatening compressive optic neuropathy.

Steroid therapy in TED is frequently associated with development or worsening carbohydrate intolerance/diabetes mellitus, hypertension, Cushing syndrome, increased infections, thrush, psychiatric events, and accelerated bone loss (88, 96). These side effects are similar to those widely recognized in patients undergoing systemic, supraphysiological steroid therapy for other medical indications. In addition, high-dose IV pulse steroids can result in severe liver toxicity, although this is rare (89, 90). From aggregate evidence, one can conclude that high dosages of steroids, administered either orally

or IV, are associated with increased incidence of side effects (4, 80).

In summary, many steroid-treated patients with TED experience reduced CAS by diminishing inflammatory-related signs and symptoms of active, progressive TED. Benefit of steroids is limited by the lack of consistent response regarding proptosis and diplopia, both of which greatly impact the morbidity of TED. Importantly, steroid therapy in TED does not appear to improve disease outcomes or alter its clinical course. Thus, many steroid-treated patients with relatively severe TED must undergo subsequent surgical procedures, typically performed once the active phase has run its course. Further, exposure to therapeutic dosages of glucocorticoids can be associated with potentially serious side effects. Recent progress in identifying more effective and safer therapeutic options has stirred controversy as to the likely future role of systemic steroids in TED (80).

Mycophenolate

Antimetabolites such as MMF have been used for some time to immunosuppress renal transplant patients and thus reduce organ rejection (97). MMF is the pro-drug of mycophenolic acid that inhibits type II inosine monophosphate dehydrogenase, the rate limiting-enzyme in de novo GTP nucleotide synthesis. This enzyme is expressed widely in activated B and T lymphocytes (98-100). MMF inhibits PI3K/AKT/mTOR and thus attenuates the actions of IL-2 and IL-1 β (101). Thus,

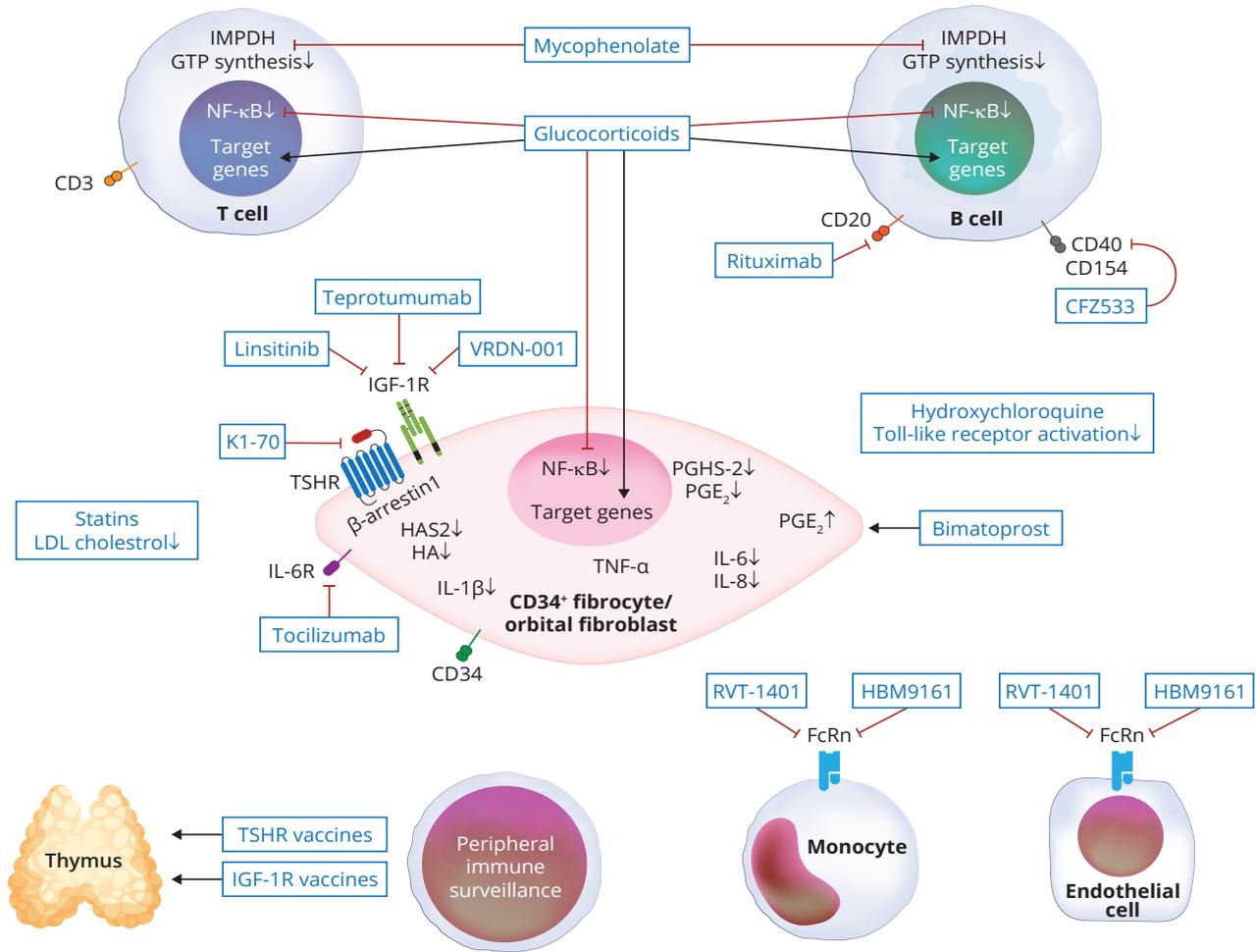


Figure 4. Putative molecular targets for current medical therapies and those under development. Glucocorticoid steroids target many cell types, where they induce several target genes while inhibiting the expression of others. Many of their anti-inflammatory actions are mediated through nuclear factor-κB. Other agents are more target-specific, such as mycophenolate (targets inosine monophosphate dehydrogenase [IMPDH] and GTP synthesis in lymphocytes), rituximab (anti-CD20 displayed on B cells), tocilizumab (IL-6 receptor inhibitor), teprotumumab, VRDN-001, linsitinib (IGF-1R inhibitors), K1-70 (TSHR inhibitor), RVT-1401, HBM9161 (FcRn antagonists), CFZ533 (anti-CD40 antagonist), and TSHR and IGF-1R vaccines. It is possible that additional, as-yet unidentified targets may play important roles in mediating clinical responses.

its cellular targets, by and large, are indiscriminate, leading to a wide scope of immunologic consequences.

MMF was repurposed for use in TED, a practice with thinly documented benefit as the rationale derives from limited studies. A randomized clinical trial comparing MMF and steroids in 174 patients with moderate to severe TED was conducted in China (96). The primary outcome was “overall response,” consisting of at least 3 of the following elements: improvement in CAS ≥ 2 points/disease inactivation (CAS ≤ 3), soft-tissue improvement by 1 grade of eyelid swelling, eyelid erythema, conjunctival redness/edema, proptosis improvement ≥ 2 mm, eye movement improvement, diplopia improvement, and increased visual acuity ≥ 2/10, adjudicated at weeks 12 and 24. Analysis of week 24 data indicates a higher overall response rate for MMF compared with steroids. CAS, diplopia, and proptosis (2-3 mm reduction) responses were greater in those receiving MMF. The same study found more modest but sizable improvement in these parameters following glucocorticoid therapy. Another, randomized, single masked study compared methylprednisolone

(cumulative 4.5 g) alone over 6 weeks versus in combination with MMF (cumulative 120 g) for 24 weeks (EUDRACT number 2008-002123-93) in 164 patients at 4 European centers (95). The primary outcome was response rate at 12 and 24 weeks, including CAS reduction of > 2 parameters (eyelid swelling, proptosis, lid width, diplopia grade) in the absence of worsening of any parameter, and sustained response at week 36. Addition of MMF to steroid offered no additional benefit to glucocorticoids alone in the intention-to-treat population at weeks 12 and 24 and week 46 relapse rates. Post hoc analysis of week 24 data suggested that MMF combined with methylprednisolone increased response rate compared with steroids alone. Safety evaluation of MMF in TED, whether as a single agent or combined with steroids, was also limited in terms of observational duration and numbers of patients followed. Importantly, primary responses in the European study did not require meeting disease severity endpoints. Because neither study was placebo controlled, the potential contributions of either medication, administered as a single agent or in combination, to naturally occurring clinical improvement

cannot be determined unambiguously. The Chinese study revealed improved proptosis and diplopia, responses not seen in reports from other clinical studies. Smith (102) commented on what he considered to be important weaknesses in the existing evidence supporting the 2-drug therapy proposed by EUGOGO (94). Those comments stressed the importance of evenhandedness in evaluating newly introduced medications for TED, a goal he felt was not achieved by the guideline authors. Those authors have in turn responded, noting that teprotumumab availability is currently limited to North America. Importantly, they left unaddressed the limited evidence for efficacy and lack of adequate long-term follow-up specifically regarding combined steroid and MMF therapy (103). An analysis of safety data from the published trials with MMF suggests that the drug, alone or in combination with steroids, may be well-tolerated (104).

Untargeted Therapies

Hypercholesterolemia has been associated with a seemingly vast array of human diseases, including those of established or putative autoimmune nature (105, 106). An approach to potentially enhance the effectiveness of steroids in TED was examined in a study in which statins were administered as therapy in 88 patients with TED and low-density lipoprotein cholesterol levels between 2.97 and 4.88 mmol/L in an open label, phase 2 trial conducted at a single institution (STAGO) (107). Patients were randomized to receive either methylprednisolone 500 mg/wk for 6 weeks followed by 250 mg/wk for an additional 6 weeks alone or in combination with oral atorvastatin (20 mg/d) for 24 weeks. Primary response was achieving 2 of the following: (1) exophthalmos reduction ≥ 2 mm or more; (2) clinical activity score reduction ≥ 2 points; (3) eyelid aperture reduction ≥ 2 mm; (4) disappearance/improvement of diplopia; and (5) improved visual acuity ≥ 0.2 decimals. The investigators interpreted their study results as demonstrating that the addition of atorvastatin to high-dose steroids improves the clinical outcomes of patients with TED and hypercholesterolemia. They further state an intention to conduct a phase 3 trial where patients with TED are included, regardless of whether they exhibited elevated serum cholesterol levels. Concern arises for administering 2 medications with potentially severe side effects, especially to patients without medically indicated treatments for lipid abnormalities. Such an approach could limit enthusiasm for participation in such a study. The potential for statins to cause autoimmune diseases (108, 109) makes them a particularly dubious choice as therapy for TED. Several experimental studies suggest that lowering serum cholesterol levels, in and of themselves, may be ineffective in altering the development or activity/severity of autoimmune diseases such as TED (110). In any event, calls for caution in the use of statins in individuals with autoimmune diseases have been voiced (111).

Hydroxychloroquine has been used to treat cultured GD-OF and OFs from healthy donors. The drug inhibited cell proliferation, adipogenesis, hyaluronan accumulation, and autophagy while enhancing apoptosis in GD-OF (112). Whether these observations suggest therapeutic utility in TED is uncertain.

Steroid-sparing drugs such as methotrexate were examined for their potential to reduce needed dosage of IV methylprednisolone in 24 moderate-to-severe TED patients

with vision, inflammation, strabismus and appearance (VISA) 5.5/10 in a retrospective study (113). All patients received IV methylprednisolone combined with a steroid-sparing drug. The authors reported reduction in the methylprednisolone dosage (mean, 2.72 g) compared to the EUGOGO recommended 4.5 g in 22 patients.

Birth of Biological Therapies for TED

Rituximab

The relatively poor effectiveness of steroids and other nonspecific medications for TED in reliably reducing proptosis and diplopia has resulted in a search for better, more specific therapies with fewer side effects. Among these, rituximab, an anti-CD20 monoclonal antibody targeting a subset of B cells, was among the first examined, initially in case reports/series (114, 115), and subsequently in 2 small, single institution trials (116, 117). The study from Milan examined 32 patients randomized to receive either methylprednisolone (7.5 g) or rituximab (2 g or 500 mg) (116). The primary endpoint was improvement in CAS, whereas the secondary responses included changes in proptosis, diplopia, eye muscle motility, lid fissure, and QoL score at week 24 following treatment initiation. Rituximab was found more effective than steroids in reducing CAS (116). The other trial, conducted at the Mayo Clinic, Rochester, MN, compared rituximab (2 g) with placebo in 25 patients and failed to detect differences in inflammation responses between the 2 treatment arms at 24 weeks (117). Neither study revealed a proptosis response. Differences in patient age, disease duration, and TSH receptor antibody test levels were postulated as potential explanations for the divergent results from the 2 trials.

In aggregate, the existing evidence fails to unambiguously support rituximab as superior to placebo or glucocorticoids in the treatment of active TED. Further, anti-CD20 B-cell depletion does not appear to reliably improve either proptosis or diplopia, 2 major manifestations negatively affecting QoL. Given the absence of a placebo comparator in the Italian study, it is not possible to determine whether either steroids or rituximab offers clinical benefit beyond that attributable to the natural course of TED (3). Thus, further consideration of rituximab as therapy for TED will require additional clinical studies involving larger patient cohorts and appropriate controls.

Teprotumumab

Rationale for therapeutically targeting IGF-IR in TED was born out of empirical observations suggesting that this tyrosine kinase receptor was involved in the pathogenesis of GD and its connective tissue manifestations (118). The therapeutic landscape for TED changed following 2 clinical trials for teprotumumab (119, 120) and its subsequent approval by the US Food and Drug Administration in January 2020 (121). For the first time, a registered medical therapy for TED has become available for wide clinical use. Teprotumumab, a fully human IGF-IR inhibitor, was developed and evaluated initially for treatment of several types of cancer but clinical responses to it and other similar molecules proved inadequate to sustain those programs (122). Thus, the drug became available for repurposing.

Eighty-eight patients with active, moderate to severe TED were enrolled in an initial phase 2 trial, whereas a phase 3

trial enrolled 83 patients in North America and Europe. Both studies were double masked and multicentered. Patients in both trials were within 9 months of initially developing TED, were clinically active (clinical activity scores ≥ 4 on a 7-point scale), and were clinically euthyroid at baseline. None had undergone orbital radiotherapy or remedial surgery for TED. Enrolled patients must have received less than 1 g of prednisolone or equivalent previously and must have undergone a 6-week steroid washout period. Patients were randomized to either placebo or teprotumumab treatment (1:1). All participants were administered a total of 8 infusions, each delivered at 3 weekly intervals over the 24-week treatment phase. If the initial infusion of 10 mg/kg per body weight teprotumumab was uneventful, subsequent doses of 20 mg/kg per body weight were administered for the remaining infusions. Responses to therapy were adjudicated at week 24. For the phase 2 study, the primary endpoint was an aggregate of ≥ 2 -point improvement in CAS using a 7-point scale AND ≥ 2 mm proptosis reduction in the more severely affected (study) eye. The phase 3 trial had an endpoint of reduction from baseline in proptosis ≥ 2 mm in the study eye.

A total of 29/42 patients in the intention-to-treat cohort receiving teprotumumab met the primary response at 24 weeks in phase 2. In contrast, 9/45 patients treated with placebo responded ($P < 0.001$). The primary outcome in phase 3 was achieved by more patients receiving active drug (teprotumumab, 83% vs placebo controls, 10%, $P < 0.001$). When intention-to-treat data from the 2 trials were pooled (including 84 patients receiving teprotumumab and 87 treated with placebo), more teprotumumab-treated patients (65/84, 77%) achieved a reduction of ≥ 2 mm in proptosis at week 24 versus placebo (13/87, 15%); stratified treatment difference 63%; 95% CI, 51-75 ($P < 0.0001$) (123). Responses tended to be durable (67% and 69% of proptosis and diplopia responders, respectively maintained 51 weeks after the final dose of teprotumumab).

The safety profile of teprotumumab was encouraging (119, 120, 123). A total of 63/67 (94%) of teprotumumab and 59/60 (98%) of placebo-treated patients experienced mild to moderate (grade 1 or 2) adverse events (AEs), whereas 3 (4%) receiving teprotumumab had serious AEs related or possibly related to the drug. These included diarrhea, infusion reaction, and confusion (potentially Hashimoto encephalopathy), the last of these leading to study discontinuation. Muscle cramping (18%; 95% CI, 7.3-28.7), hearing loss or abnormalities (10%), and hyperglycemia (8%, 1.7-15.0, grade 2-3) were the most commonly reported AEs. Glycemic excursions were most commonly detected in patients with baseline diabetes mellitus or carbohydrate intolerance. In general, glycemic control was restored with medication adjustment. Those dosage requirements reverted to baseline requirements following the treatment phase. Hearing abnormalities have also been identified in clinical use, likely reflecting the importance of the IGF-I pathway and ear development and function (124-126). Sensorineural hearing loss, tinnitus, patulous Eustachian tube, hypoacusis, hyperacusis, and autophony have been reported (126-129). Although some of the cases of hearing abnormalities have proven transient or improved, others have continued to the present time. Issues concerning impaired cognition have also arisen in a single elderly patient (130). The important role of IGF-I and its pathway in central nervous system development and function provide plausibility for an association between IGF-IR inhibition and cognitive

changes/decline (131, 132). Clearly the AEs associated with teprotumumab require continued pharmacovigilance and potentially additional studies.

Details concerning the mechanisms of action for teprotumumab remain incomplete. The drug may act on tissues peripheral to the orbit (36). Circulating fibrocytes from teprotumumab-treated patients exhibited a dramatic reduction of CD80, CD86, PD-L1, and MHC class II surface display. Further, teprotumumab treatment was associated with marked reduction of IL-17A- and interferon γ -expressing T cells, actions mediated through gene transcription and mRNA stability. Those observations suggest that teprotumumab clinical responses involve its effects on T-cell activation, mediated through antigen-presenting cells such as fibrocytes. Teprotumumab actions within the orbital tissues also remain plausible. Impact of IGF-IR disruption has been shown in cultured GD-OF. A very recent report has implicated cell-extrinsic pathways in GD-OF mediating apoptosis because of IGF-IR inhibition (133). Whether either/both intraorbital and/or peripheral mechanisms are involved in clinical responses to teprotumumab will require further studies. Additional anti-IGF-IR inhibitory molecules are being evaluated for their efficacy in TED, including VRDN 001, which is undergoing evaluation in a phase 1 trial (NCT05176639). A phase 2b trial for linsitinib, a tyrosine kinase inhibitor of IGF-IR and the insulin receptor, is being investigated for its benefit in TED (NCT05276063).

Emerging Medical Therapies for TED

Tocilizumab

Among the more plausible candidates within the therapeutic pipeline for TED is tocilizumab, an IL-6 receptor (IL-6R) antagonist (134). The plasma membrane IL-6R comprises 2 transmembrane protein subunits, IL-6R α (gp80) and IL-6R β (gp130). The receptor interfaces with Janus kinase and STAT3, which are integral to its downstream signaling and target gene activation. IL-6 induces several important genes, including immediate-early genes and those encoding critically important transcription factors (135, 136). IL-6 has been implicated in several autoimmune diseases, including GD and TED (137, 138). Underlying this involvement are its diverse actions in determining the characteristics of immune responses and T-cell polarization. Tocilizumab has been used clinically for treating rheumatoid arthritis and systemic sclerosis (139, 140). The drug was first examined in TED when Pérez-Moreiras et al (141) performed an uncontrolled, single institution trial of 18 patients with active disease, the majority of whom (15/18) experienced substantial reduction in proptosis (mean, 3.92 mm), whereas 13/18 patients exhibited diplopia responses. This was followed by a placebo-controlled, randomized study including 32 patients with moderate to severe, steroid-resistant TED (142). Proportion of patients with ≥ 2 -point change in CAS from baseline at week 16 was the primary response. A total of 93.3% of patients receiving active drug responded, compared with 58.8% on placebo. Mean proptosis reduction was 1.5 mm in tocilizumab-treated patients compared with 0 mm in placebo. An open-label, unmasked, and uncontrolled observational study of 48 steroid or selenium treatment-resistant patients was conducted at several centers in Spain (143).

Improvement in disease activity, intraocular pressure, and best-corrected visual acuity was reported. In another study, 21 patients were treated with either tocilizumab (7/21) or rituximab (14/21) (144). The primary endpoint, CAS reduction ≥ 2 points, was achieved in 7/7 patients receiving tocilizumab and in 9/14 rituximab-treated patients. As with many clinical studies in TED patients, small numbers, lack of standardization and placebo controls, and heavy reliance on CAS-related response parameters reduce the strength of argument for tocilizumab as effective in this disease. Future randomized, double masked trials of tocilizumab seem warranted, given the preliminary, encouraging results. These should include placebo controls and adequate statistical power.

K1-70, A Monoclonal TSHR Blocking Antibody

Plausibility for therapeutically targeting TSHR derives in large part from finding the receptor expressed in orbital tissues (145). Therapeutic interruption of TSHR activity has long been viewed as the most logical and obvious approach to medically managing GD and TED. In fact, many investigators have advocated that TSHR and the stimulatory autoantibodies directed against it represent the only therapeutic targets against which TED severity and activity might be mitigated. But the biology of human disease rarely proves that simple, given the elaborate interplay occurring between genes, cells, and signaling pathways. To determine the therapeutic potential of TSHR inhibition in GD, both monoclonal antibodies and small molecules targeting that receptor protein have been under development for many years (146, 147). One such blocking antibody, K1-70, was found to inhibit cAMP generation *in vitro* (147) and reduce circulating total and free T4 levels *in vivo* in rats (148). Those studies were followed with pharmacokinetic investigation in rats and cynomolgus monkeys (149). Eighteen patients with GD were enrolled in a phase 1 interventional trial of K1-70 in GD (NCT02904330), which was very recently reported (150). Findings from that study included improvements in thyroid function tests and manifestations of TED. As many as 5 patients experienced clinically meaningful reductions. Earlier, Ryder and colleagues (151) reported metastatic follicular thyroid cancer and TED presenting in a single patient treated with lenvatinib, radioiodine ablation, and K1-70 (made available through US Food and Drug Administration expanded access). Within 22 days of her initial dose of K1-70 (administered intramuscularly), the patient experienced decreased proptosis (2-mm bilateral reduction from baseline) and improved clinical activity score from 6/7 to 0-1/7 while continuing to smoke cigarettes. At this point, the patient underwent ophthalmic surgery for the correction of diplopia. The patient continued receiving K1-70 until, after 16 doses, each at 3-week intervals, the drug was withheld for radionuclide scanning. Her TED then worsened but responded to glucocorticoids. Over the course of her K1-70 treatment, serum TSAb levels decreased but increased after the therapy pause. This single case, although complicated by comorbidities, and the positive results of the phase 1 study, could reflect potential benefit of TSHR inhibition in TED. These results will require later stage, controlled trials before any conclusions can be drawn as to the role of TSHR inhibition in TED therapy.

Targeting CD40/CD154 Bridge

Another potential therapeutic approach for TED involves interrupting the CD40/CD154 pathway. This cascade represents an important conduit through which molecular information is conveyed between T and B cells. Moreover, it has been therapeutically targeted in other autoimmune diseases. Thyroid epithelial cells in GD express elevated CD40 levels (152), and this receptor mediates the induction in orbital fibroblasts by CD154 of IL-6, IL-8, prostaglandin endoperoxide H synthase 2, and hyaluronan (51). A recent clinical study demonstrated the response to CFZ533, an anti-CD40 monoclonal antibody, in hyperthyroid patients with GD (153). Seven of 15 patients achieved a biochemical euthyroid state with the drug. Follow-up studies will be necessary.

Neonatal Fragment Crystallizable Receptor

The neonatal fragment crystallizable receptor (FcRn) plays a determinative role in regulating IgG and albumin clearance from the circulation and degradation through lysosomal pathways (154). Targeting FcRn with monoclonal antibodies can disrupt FcRn-IgG interactions, thereby shortening the circulating half-life of pathogenic IgG autoantibodies (155). This approach has been applied with apparent success to myasthenia gravis (156). Two such therapeutic antibodies are being developed to target FcRn in patients with GD and TED, including RVT-1401 (Immunovant) and HBM9161 (Batoclimab, Harbour Biomed). The trial of RVT-1401 has been suspended over concerns of drug-induced hypercholesterolemia.

Bimatoprost

EP2-specific agonists activate cAMP-mediated responses in GD-OF (157). More recent studies have demonstrated that bimatoprost inhibits adipogenesis in these cells (158). PGF_{2 α} and EP₂ agonists are active in 3-dimensional OF organoids, where they downregulate fibronectin, collagen 1, and collagen 6 gene expression while upregulating IL-6 (159). A small phase 1 clinical trial is being conducted for TED with proptosis reduction being the primary response (NCT03708627).

Immune Tolerization

Restoring immune tolerance has been attempted in a number of autoimmune diseases, including type 1 diabetes mellitus (160), systemic lupus erythematosus (161), and neuromyelitis optica (162). This strategy offers the potentially enormous advantage of leaving host defense completely intact while, in some instances, curing the underlying disease. One retolerizing approach currently under study uses noninflammatory mRNA vaccines (163). Another involves intradermal injection of antigen-processing independent epitopes (termed apitopes) (164). ATX-GD-59, which contains a combination of 2 TSHR peptides, was evaluated in a phase 1 study of 12 hyperthyroid patients (165). Seven of 10 patients receiving all 10 doses at week 18 of the study exhibited improvement or normalization of thyroid function, whereas 3 subjects developed worsening thyrotoxicosis. Antigen-specific immune retolerization as a treatment in TED seems possible: at least 2 autoantigens with likely important involvement in disease pathogenesis, TSHR and IGF-IR, have been identified, and their roles in disease development partially characterized. These advancements should “set the table” for successfully reestablishing immune tolerance.

Concluding Remarks Concerning Therapies for TED

Traditional medical management of TED, including the use of anti-inflammatory drugs such as glucocorticoid steroids, has served an important role in reducing some aspects of disease burden. However, these agents have failed to consistently improve several important disease manifestations, including proptosis and diplopia. These disease-related signs and the resulting symptoms can substantially reduce QoL, including the inability to engage in normal daily activities. Historical limitations in treatment have frequently resulted in loss of personal and financial productivity and diminished self-image. Steroids can offer temporary relief of signs and symptoms related to inflammation and congestion in TED but are not disease modifying. Further, they are frequently associated with serious side effects. Absence of placebo-controlled trials of steroids, in my view, substantially weakens the case promoting their continued use in TED, either as a single agent or in combination with MMF. That drug combination is currently advocated as “first-line” therapy in the recently published 2021 EUGOGO guidelines (94, 95). Lack of availability and the unfamiliarity of newly developed or repurposed therapies for any disease can slow drug acceptance and uptake. Attitudes toward previously unavailable treatments vary among patients and their health care providers, as has been the case with drugs being introduced for TED.

Despite the reticence expressed by some, novel therapies, including those repurposed from other diseases or developed de novo, have ushered in a new era of TED treatment. Among these are medications specifically targeting pathogenic mechanisms identified from studies conducted in the research laboratory. Teprotumumab, a monoclonal anti-IGF-IR inhibitor, was recently US Food and Drug Administration-approved for TED. It is effective in reducing inflammation, diplopia, and proptosis, thereby improving QoL. Continued vigilance remains critical when assessing the effectiveness, durability, and safety of all newly introduced drugs. Restoration of immune tolerance to TSHR, IGF-IR, and potentially other important autoantigens involved in TED pathogenesis remains the ultimate goal of many researchers. Its attainment could offer disease-free life without ongoing treatment.

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T.J.S. was issued patents covering treatment of TED and other autoimmune diseases with inhibitors of the insulin-like growth factor I receptor. These are held by UCLA/Lundquist Institute. He is a paid consultant for Horizon Therapeutics and Veridian

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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