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# 2021 Update on Thyroid-Associated Ophthalmopathy

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## Abstract

**Purpose:** Our understanding of thyroid-associated ophthalmopathy (TAO, A.K.A Graves' orbitopathy, thyroid eye disease) has advanced substantially since one of us (TJS) wrote the 2010 update on TAO, appearing in this journal.

**Methods:** Pubmed was searched for relevant articles.

**Results:** Recent insights have resulted from important studies conducted by many different laboratory groups around the World. A clearer understanding of autoimmune diseases in general and TAO specifically emerged from the use of improved research methodologies. Several key concepts have matured over the past decade. Among them, those arising from the refinement of mouse models of TAO, early-stage investigation into restoring immune tolerance in Graves' disease, and a hard-won acknowledgement that the insulin-like growth factor I receptor (IGF-IR) might play a critical role in the development of TAO, stand out as important. The therapeutic inhibition of IGF-IR has blossomed into an effective and safe medical treatment. Teprotumumab, a β-arrestin biased agonist monoclonal antibody inhibitor of IGF-IR has been studied in two multicenter, double masked, placebo-controlled clinical trials demonstrated both effectiveness and a promising safety profile in moderate to severe, active TAO. Those studies led to the approval by

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**Authors' contributions** Both authors participated in the writing of the manuscript

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**Conflicts of interest/Competing interests** TJS was issued US patents for the use of IGF-I receptor inhibition in the treatment of autoimmune diseases. These are held by UCLA and the Lundquist Institute. He is a paid consultant for Horizon Therapeutics.

Declarations

**Availability of data and material** N/A

**Code availability** N/A

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the US FDA of teprotumumab, currently marketed as Tepezza for TAO. We have also learned far more about the putative role that $CD34^+$ fibrocytes and their derivatives, $CD34^+$ orbital fibroblasts, play in TAO.

**Conclusion:** The past decade has been filled with substantial scientific advances that should provide the necessary springboard for continually accelerating discovery over the next 10 years and beyond.

### Keywords

Graves' ophthalmopathy; autoimmune; orbit; fibrocyte; insulin-like growth factor receptor

## Introduction

The recently accelerating rate of discovering how thyroid–associated ophthalmopathy (TAO) or Graves' orbitopathy (GO) as it is referred to in Europe, develops and how it might be effectively treated has been propelled by a persistent group of clinical investigators over the past decade.[1] As a consequence of improved research techniques, development of refined preclinical models and more successfully accessed and used patient-derived biological samples, we now better understand the pathogenesis of GO. These insights have resulted directly in the development of fact-based and scientifically plausible medical therapies. In this brief review of advances over the past decade, we attempt to describe which discoveries might directly impact the management of GO. We attempt to cover contributions made by multiple laboratory and clinical investigations including those emanated from our own laboratory at the University of Michigan. We first cover the basic and relevant translational science conducted in the recent past. We then discuss some iof the currently utilized therapeutic approaches. Finally, we briefly describe our view of the future treatments for GO. We acknowledge our selection biases but have attempted to remain balanced in our assessments. We too appreciate the limitations imposed by limited word count. This has resulted unfortunately in many important topics and findings remaining unmentioned. Ultimately, we seek to provide a fact-based review of how several key concepts in this field have matured recently.

### Methods:

Pubmed was searched for relevant articles published in the most recent two decades using search terms such as: thyroid-associated ophthalmopathy, Graves' disease, orbit, autoimmune, Graves' orbitopathy, fibroblast, TSH receptor, IGF-I receptor, thyroid cytokines, biological therapies, thyroid

## Results

### Studies on the Genetic and Epigenetic Basis for TAO

Potential importance of genetic contributions to the development of Graves' disease (GD) and GO has been emphasized for many years, a realization arising in part from recognizing ethnic and family clustering of the disease. Those patients with GD experience a higher prevalence of other autoimmune conditions in themselves and in their family members.

[2–7] Genome-wide association studies (GWAS) have detected polymorphisms associated with GD susceptibility, including thyrotropin receptor (TSHR), CD25, HLA-DRβ-Arg74, PTPN22, ARID5B, CTLA4, CD40, and thyroglobulin (Tg). Some candidates are shared with other autoimmune thyroid diseases, especially Hashimoto's thyroiditis. [8] Single-nucleotide polymorphisms (SNPs) of IL-1β and IL-12 family members, including the IL-23 receptor, have also been identified as candidates for TAO susceptibility. [9–11] Characterization of SNPs suggest that they may not lead to TAO susceptibility specifically, but rather may be linked to GD. [12]

Dysregulated gene expression in GO has been identified with microarray analysis. When compared with healthy orbital tissue, explants and cultured orbital preadipocytes from TAO orbits exhibit overexpression of adipogenesis related genes, the stimulatory Wnt/β-catenin signaling gene, secreted frizzled-related protein-1 (sFRP-1), and intermediate early genes such as CYR61 and EGR1.[13,14] Ezra *et* al. reported that Wnt signaling, IGF-1R, IGF-I, and the downstream transcription factors SGK-1 and c-JUN emerged as prominently expressed.[15] mRNA levels in formalin-fixed adipose tissues from patients with TAO, sarcoidosis, non-specific orbital inflammation, granulomatosis with polyangiitis, and healthy tissues [16] were compared. A greater similarity between inflammatory gene expression profiles was identified in TAO and healthy tissues as compared with those in the other diseases studied. Potentially important limitations of that study, and correctly acknowledged by the authors, included differing disease durations, lack of uniform thyroid function status, and varying steroid and radiotherapy treatment among the tissue donors.

It would appear that genetic variations thus far found in GD and GO account for only ~30% of disease risk. Exploration of epigenetic alterations in GD has been even more limited. Multiple examples of gene hyper-methylation have been reported, including sites within the first TSHR intron and CpG regions of signaling protein-encoding genes expressed by $CD4^+$ and $CD8^+$ T cells.[17] Increased global methylation of orbital fibroblasts from patients with GO (termed GD-OF) have also been observed when compared with control analogues.[18] A potential utility for post-transcriptional repressor microRNAs (miRNAs) and epigenetic modifications as biomarkers in autoimmune disease has been suggested.[19,20] Initial studies of GO orbital fibroblasts have revealed increased expression of pro-lipogenic miR-155 [21] and miR-130a, [22] as well as reduced regulatory microRNA-146a [21,23] and microRNA-27a/b [24] levels. Larger and more comprehensive studies will be necessary before a mechanistic link between variations in epigenetic loci and disease-defining specific protein expression in GO can be made. Thus, although studies have identified potential genetic and epigenetic susceptibility signatures in GD and GO, our understanding of how these might underpin disease pathogenesis remains uncertain.

**Orbital fibroblasts as key players in TAO—**The characteristic pattern of inflammation, volume expansion, and remodeling in GO [25,26] has been widely postulated as resulting from the unique properties of orbital fibroblasts (Fig. 1). Among these unusual attributes are their remarkably robust responses to multiple inflammatory mediators. GD-OF express extremely low levels of secreted IL-1 receptor antagonist (sIL-1RA) when induced by inflammatory cytokines and TSH [27,28] as compared to their counterparts from other anatomic regions. The reduced capacity to generate this antagonist may underlie the

exaggerated induction of genes including those encoding prostaglandin endoperoxide H synthase 2 (PGHS-2), and proinflammatory cytokines such as IL-6 and IL-1β.[29,30] Initially recognized determinants of GD-OF heterogeneity included divergent display of the glycoprotein, Thy-1 (CD90).[31] $Thy1^+$ OFs can differentiate into myofibroblasts when treated with TGF-β. In contrast, Thy $1^-$ GD-OF undergo adipogenesis in response to thiazolidinediones.[31,32] Douglas *et al.* later discovered presence of $CD34^+CXCR4^+Col\ I^+$ GD-OF uniquely in the GO orbit. [33] These $CD34^+$ OFs appear to derive from circulating $CD34^+$ fibrocytes and can be detected *in situ* in GO orbital fat but are uniformly absent in healthy tissues. Fibrocytes express relatively high constitutive levels of MHC class II [34] and can induce antigen-specific T cell proliferation through their display of the co-stimulatory molecules CD80 and CD86, [35,36] and production of adhesion molecules including CD11a, CD54, and CD58.[35] Fibrocytes and $CD34^+$ OF express several proteins historically considered unique to thyroid epithelial cells. These include TSHR, Tg, sodium iodide symporter (NIS), and thyroid peroxidase (TPO).[37–39] The expression of these proteins appears to depend on the actions of thymic autoimmune regulator (known as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy protein, encoded by the AIRE gene). [38] Fibrocytes activated through the TSHR pathway produce high levels of IL-1β, IL-1RA, TNF-α, IL-6, IL-8, and MCP, in response to TSH and M22, an agonist human monoclonal anti-TSHR antibody (TSI). [26,33,40,41] Once in the GO orbit, fibrocytes masquerade as $CD34^+$ OF where they coexist with $CD34^-$ OF. In distinction to circulating fibrocytes, GD-OF express substantially lower MHC class II and the thyroid proteins mentioned above.[37,38,42]

Cytometric sorting into pure $CD34^+$ OF and $CD34^-$ OF has revealed divergent expression of hyaluronan biosynthetic enzymes and cytokines in the two subsets.[43,44] For instance, IL-12p35 is preferentially expressed in $CD34^-$ OF while IL-23p19, TNF-α, and IL-6 levels are substantially higher in $CD34^+$ OF.[43,44] Besides inflammatory changes, orbital congestion and tissue volume expansion are the consequence in part of accumulating hyaluronan. The three mammalian hyaluronan synthase isoenzymes and UDP-glucose dehydrogenase are expressed by GD-OF. Basal and bTSH-induced HAS1 expression localizes to $CD34^+$ OF and fibrocytes, while HAS2 and UDP-glucose dehydrogenase are predominately expressed by $CD34^-$ OF.[44] HAS2 appears primarily responsible for HA synthesis in both cell subsets and the relatively low levels of HAS2 in $CD34^+$ OF appears to represent the basis for substantially higher levels of hyaluronan synthesized in $CD34^-$ OF [44].

Several factors modulate fibrocyte differentiation into fibroblasts, myofibroblasts and adipocytes. Among these, the axonal guidance glycoprotein Slit2, acts through its binding to the second leucine-rich repeat region of roundabout 1 (ROBO1). [45,46] Slit2 inhibits fibrocyte differentiation and attenuates pulmonary fibrosis following bleomycin aspiration in mice.[47] In addition to Slit2, the decameric serum amyloid P (SAP) [48] glycoprotein inhibits fibrocyte differentiation.[49] Long pentraxin-3 (PTX-3) [50] shares a common carboxy-terminal “pentraxin signature” with SAP.[51–53] In contrast to SAP, PTX-3 potentiates fibrocyte differentiation in a FcgR-mediated manner.[54]

Because pure $CD34^+$ OF express higher levels of several genes than do mixed population GD-OF comprising $CD34^+$ OF and $CD34^-$ OF,[37] we postulated that $CD34^-$ OF express and release a soluble modulatory factor(s) that attenuates gene expression. We identified Slit2 as the factor that down-regulates $CD34^+$ OF gene expression.[42] Specifically, rhSlit2 dramatically represses the expression of AIRE, TSHR, Tg, NIS and TPO as well as TNF-α, IL-6, and IL-23 induction by TSH in fibrocytes and $CD34^+$ OF. [42–44] Slit2 knockdown enhances HAS1 expression in $CD34^-$ OF, while reducing both basal and TSH-dependent HAS2 expression.[44] Basal and TSH-dependent HAS2 expression is augmented in fibrocytes by rhSlit2 and this may represent the central mechanism underlying the enhancement in those cells of hyaluronan synthesis.[44] Similar to Slit2, PTX3 was found to be inducible by TSH,[55] actions that can be blunted by IGF-IR inhibition. Teprotumumab, a monoclonal antibody that inhibits IGF-IR activity, reduces IGF-IR and TSHR plasma membrane display on fibrocytes.[56] The complexities of cell-cell interactions present in the heterogeneous GD-OF population highlight the importance of better understanding the characteristics and behavior of each GD-OF subset. Slit2 may represent a heretofore unrecognized endogenous molecular determinant in modulating hyaluronan levels and immune responses within connective tissues. It might be therapeutically targeted in severe TAO. Slit2 is highly TSH-inducible in $CD34^-$ OF [43].

## Characterization of orbit-infiltrating mononuclear cells in GO

The histopathology of GO is characterized by orbital infiltration with professional immune cells, including T and B lymphocytes, macrophages, monocytes, and mast cells.[57] A positive correlation between total orbital tissue lymphocytes and CAS severity has been suggested.[58] An examination of extraocular muscle biopsies suggests that pathogenic $CD4^+$ and $CD8^+$ T cells are among these infiltrating cells and appear to be recruited early in the disease process.[59] Among the cytokines relevant to these T cells are IL-4, IL-10 and IFN-γ. [60] With antigenic stimulation, naive $CD4^+$ T cells develop into Th1 or Th2 cell phenotypes that can be identified within extraocular muscles in a disease-phase-dependent manner.[61,62] $CD3^+CD82ROR\gamma t^+CCR6^+$ Th17 cells appear to play important roles in active GO.[63] Increased expression of receptors for both IL-23 and IL-1 while lower IL-21 receptor levels are found on Th17 cells. $CD34^+$ OF appear to enhance the Th17 phenotype through the prostaglandin $E_2$-EP2/$EP_4$-cAMP signaling pathway.[64] Whether GD is biased towards the Th1, Th2, or Th17 lineage continues to be uncertain.[65,66] It remains possible that a shift in Th bias occurs, at least peripherally, as the duration of GD increases. Peripheral regulatory T cell (Treg) phenotypes have recently been examined *in vitro* in GO.[67] Anti-T lymphocyte globulin stimulated Tregs in TAO display higher CD25, FoxP3, and IL-7 receptor (CD127) levels than do cells from patients with GD but without clinically detectable GO. IGF-I stimulation of PMBCs from these patients enhances Treg abundance.[68]

## Preclinical Models of GO

Effort toward developing rodent models of GO has spanned 20 years and has included a number of near-misses. While several strategies at producing antibody-driven hyperthyroid mice have yielded variably successful models,[69–72] far less convincing has been the development of mice exhibiting periocular manifestations resembling GO. Female BALB/c

mice [69,70] immunized with TSHR A and IGF- 1Rα subunit-encoding plasmids, delivered with muscle electroporation, induced orbital pathology resembling some characteristics of human GO. Anti-TSHR and IGF-1R autoantibodies were detected in animals challenged with hTSHR A, suggesting an association between the immune reactivity toward the two receptors. [70] Zhang *et* al.[71] subsequently modified the injection/electroporation protocol to include intramuscular Ad-TSHR A over 34 weeks compared with injections delivered over 12 weeks.[69,70] These modifications yielded orbital fibrosis and adipogenesis detected by the seventh Ad-TSHR A dosing. Exophthalmia, conjunctival redness, eyelid hyperplasia, and orbital T cell infiltration were also observed in a subset of these mice. Hyperplastic changes could be identified in thyroid glands after four injections. A three-dimensional organoid culture model [73] utilizing a hanging drop plate [74] has shown advantages over monolayer cultures in recapitulating the fibrotic extracellular matrix remodeling observed in GO.[75,76] Studies exploring the pathogenesis of GO in spheroid culture systems may more closely resemble the conditions found *in vivo*.

## The road to specific, fact-based therapies for GO

### Rationale for Glucocorticoid use in GO

Glucocorticoids remain the most widely used pharmacologic agents in the management of active GO. The European Thyroid Association (ETA) recommends intravenous glucocorticoid pulse dosing as first line treatment for active, moderate-to-severe GO. They recommend a cumulative dosage of 4.5g IV methylprednisolone administered over 12 weeks. [77–80] These steroids exert their anti-inflammatory actions largely through gene transcriptional activation and repression. [80] Activation of glucocorticoid-responsive genes occurs through interactions between DNA-bound receptors (GR) and transcriptional co-activator molecules.[81–83]

The effectiveness of glucocorticoids in GO has been assessed in several studies. A single-masked, controlled study of 70 consecutive patients with untreated, active and severe GO involved administration of either oral prednisone (4 g.) or intravenous methylprednisolone (4.5 g.).[84] Intravenous steroid was associated with improved quality of life, milder adverse events, and less-frequent requirement for additional treatment than was oral drug. Bartalena *et* al.[85] examined three different cumulative dosages of intravenous methylprednisolone (2.25, 4.98, 7.47 g). Divided doses were administered weekly for 12 weeks in a multicenter, double-masked randomized control trial. Overall improvement of GO was greatest in those receiving the highest dosage (7.47 g) (52%) when compared to lower dosages (4.98 g group: 35%; $P = 0.03$; 2.25 g group: 28%; $P = 0.01$). Although palpebral aperture changed minimally in all three treatment groups, the clinically inconsequential reduction of proptosis and improvement of subjective diplopia did not differ among the treatment groups. High-dosage glucocorticoids may improve symptoms and benefit dysthyroid optic neuropathy,[86] but are inconsistent in reducing the severity of active GO, including proptosis reduction. Much of the clinical response may be mediated through their attenuation of PGHS-2 expression and $PGE_2$ production. [29,87–89] Among the widely recognized side effects associated with high-dose glucocorticoids is the potential for severe hepatic toxicity. [90,91] Those effects appear to be dose-dependent, with highest risk in patients receiving >8 g

iv methylprednisolone,[92] but severe toxicity has been reported with lower dosages. [93] Given their limited effectiveness, including their inability to meaningfully modify the long-term outcomes of GO, their use should be carefully monitored and weighed against their potential for causing side effects.

### Mycophenolate/Azathioprine

Antimetabolites have been repurposed from their niche in the immunosuppressive regimen of renal transplantation [94] to limited use in GO. Mycophenolate mofetil (MMF) is the pro-drug for mycophenolic acid (MPA).[95] MPA preferentially inhibits type II inosine monophosphate dehydrogenase, the rate limiting-enzyme in *de novo* GTP nucleotide synthesis that is expressed in activated B and T lymphocytes.[96–98] As an anti-epileptic intervention, MMF inhibits the PI3K/AKT/mTOR pathway through pre-translational actions in rodent models as well as attenuating IL-2 and IL-1β.[99] A recent observer-masked randomized trial compared methylprednisolone (cumulative 4.5 g) as a single agent versus steroid combined with mycophenolate sodium (cumulative 120 g) for 24 weeks with a 12 week follow up.[100] The primary outcomes were response rates at 12 and 24 weeks of reduction in ≥ 2 CAS parameters (eyelid swelling, proptosis, lid width, diplopia grade) without simultaneous deterioration in any parameter, and sustained response at week 36. The benefit of adding mycophenolate to steroid was not significant compared to steroid alone in the intention-to-treat population at 12 weeks or in the relapse rate at 24 and 46 weeks. However, *post-hoc* analysis suggested that mycophenolate plus methylprednisolone increased the rate of response at 24 weeks compared to steroid alone. Actions of azathioprine resemble those of MMF with anti-proliferative effects on B and T lymphocytes mediated through *de novo* purine synthesis. [101] As a monotherapy, azathioprine failed to improve proptosis, visual acuity, or palpebral aperture over a 2-year period in moderate to severe GO. [102] Subsequent trials have been inadequately powered statistically to draw firm conclusions. The limited therapeutic value of many agents thus far studied for GO and their potentially serious side effects have driven the search for more targeted therapies.

## Repurposing of biological agents to GO

Among the earliest biologics used to treat GO was rituximab (RTX), a chimeric monoclonal antibody consisting of variable light- and heavy-chain murine anti-CD20 regions linked to a human IgG-k region that prolongs the $t_{1/2}$.[103,104] CD20 represents a phosphorylated cell surface protein expressed by both immature and mature B cells yet is undetectable on plasma cells.[105,106] RTX depletes B cells through induction of caspase-dependent apoptosis, antibody-dependent cellular toxicity, and complement-dependent cytotoxicity. [107,108] Developed more than 2 decades ago as therapy for relapse/refractory $CD20^+$ B cell malignancies including non-Hodgkin's lymphoma,[109] it has since found an important place in the therapeutic armamentarium of several autoimmune diseases, achieving U.S. FDA registration for rheumatoid arthritis (RA), Wegener's granulomatosis, microscopic polyangiitis, and granulomatosis with polyangiitis.[110] The available data from three controlled studies of RTX therapy in GD or GO suggested that TRAb levels decline following B-cell depletion, but not to a greater extent than that following treatment with methimazole or glucocorticoids. [111–113] Coetaneous randomized trials published in 2015

were the first to meaningfully test the efficacy of RTX in active GO. [93,114] Both involved a 24-week treatment period. One U.S. study included 13 patients treated with two bimonthly infusions of RTX (1000 mg each) and 12 with two placebo saline infusions. The study was conducted in euthyroid patients with CAS scores ≥ 4 (using a 7-point scale). [114] No differences were observed in the two treatment arms of patients in achieving the primary outcome of CAS reduction ≥2 at 24 weeks. 5/6 cases of moderate-to-severe adverse events were experienced in the RTX arm, including dysthyroid optic neuropathy, infection, vasculitis, and gastrointestinal problems (tongue pain, abdominal pain, and diarrhea), during and following trial completion. The other trial, conducted in Italy, randomized (1:1) euthyroid patients with CAS scores ≥ 3/7 to receive either 7.5 g (cumulative dosage) iv methylprednisolone (n=16) or 1000 mg rituximab twice in a two-week interval (n=15), though this protocol was later amended to a single 500 mg RTX and 11 placebo infusions. [93] A significant reduction in CAS was noted in the RTX arm at week 16 ($p<0.04$); week 20 ($p<0.02$); and week 24 ($p<0.006$). Neither trial yielded evidence that RTX treatment was associated with reductions in proptosis or diplopia. A subsequent post-hoc analysis revealed that the disparities in results from these two trials may have resulted from differences in baseline CAS. Further, the Italian study comprised younger patients with shorter disease duration and lower TRAb levels (P=0.036). [115] Differences in smoking habits and use of radioactive iodine therapy could have also impacted the differing results. Anti-CD19 antibodies may represent a more promising therapy since RTX fails to affect CD20 negative plasmablasts. [116] Inebilizumab (MEDI-551), an affinity-optimized and afucosylated mAb targeting the extracellular loop of human CD19, [117] has shown greater efficacy than RTX in depleting blood and splenic B cells in huCD19/CD20 double transgenic mice at lower mAb dosage, independent of complement-dependent cytotoxicity. [118]

Elevated serum BAFF levels have been described in autoimmune thyroid disease, such as GD, and Hashimoto’s thyroiditis. [119,120] The anti-BAFF monoclonal antibody, belimumab is being compared currently with methylprednisolone for active GO (EudraCT number 2015-002127-26). Interim analysis has failed to identify significant effects of either treatment on proptosis or diplopia. [121]

The MHC class I-related neonatal Fc receptor (FcRn) does not act as a signaling receptor but rather extends the half-life of IgGs in the peripheral circulation. [122] IgG-FcRn interactions are pH-dependent, promoting immunoglobulin recycling. [123] The central role of IgG1 autoantibodies targeting TSHR in GD provides plausible rationale for FcRn inhibitors in GO. Efgartigimod, a monoclonal IgG1 Fc fragment, and rozanolixizumab, a humanized IgG4 anti-FcRn monoclonal antibody, have demonstrated tolerability and accelerated serum IgG and IgG subtype clearance in phase 2 trials in primary immune thrombocytopenia [124] and myasthenia gravis. [125,126] Fully human IMVT-1401 (formerly RVT-1401) underwent a phase 2b trial for safety, tolerability, and pharmacodynamics in GO patients (NCT03938545). Unmasked 12-week data from approximately 40 patients showed dose-dependent increases in total cholesterol and low-density lipoprotein levels in the IMVT-1401 cohort, suspending the study as of this writing. This adverse effect may reflect a previously unrecognized link between GO and the liver. Should they come into clinical use, FcRn-inhibitors will require monitoring for prolonged hypogammaglobulinemia and increased susceptibility to infection.

## Statins as potential therapeutics in GO

The potential of elevated serum cholesterol levels as an independent risk factor for GO has been assessed recently. [127,128] In a cross-sectional study of 250 patients with GD, 133 manifesting GO, a significant correlation was detected between total/LDL-cholesterol and development of GO. [127] A retrospective study found increased serum cholesterol in those with GO, although disease severity and CAS did not correlate with serum cholesterol levels. [128] TSH-β expression in adipose tissue has been linked to total and LDL-cholesterol levels in euthyroid patients with GD, an association that may represent a risk factor for GO. [129] Classically used to manage hypercholesterolemia, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, known commonly as statins, decrease cholesterol production through inhibition of the mevalonate pathway. [130] Statins exhibit anti-inflammatory actions thought to be mediated through cholesterol-independent mechanisms. In this regard, they suppress TLR4/MyD88/NF-κB signaling and TLR2 and TLR4 expression. [131] The protective effect of statins in recently diagnosed GD was examined retrospectively in patients enrolled in a U.S. managed-care network. [132] Multivariable Cox regression revealed that statin use exceeding 60 days over a one-year observation was associated with decreased risk of GO by 40% (HR=0.60 [CI 0.37–0.93]). These effects may be mediated through pleiotropic mechanisms. These findings were supported by Nilsson *et* al. [133] in a registry-based study of more than 30,000 Swedish patients with hyperthyroid GD. In that study, atorvastatin decreased GO risk, with an adjusted HR of 0.78 and 0.91 for men and women, respectively. These effects were marginally superior to other lipid-lowering agents (HR=0.87 [CI 0.75–1.01]) [133] This therapeutic approach with statins carries potentially severe side effects, including liver dysfunction. [134] With the development of agents targeting pathways specific to GO, it seems unlikely that lipid-lowering agents will find widely-embraced traction in treating this disease.

## Repurposed anti-cytokine therapy in GO

A number of studies examining the potential roles of cytokines in the pathogenesis of GO have been conducted over the last decade. In general, these have concentrated on molecules implicated previously in other autoimmune diseases. The CD40/CD40 ligand (aka CD154)/receptor cognate is among these targets. Increased CD40 levels were found in GD-OF. [30] When treated with interferon γ (IFN-γ) or TSH, CD40 expression in GD-OFs is further enhanced. [30,135,136] CD40 belongs to the tumor necrosis α (TNFα) receptor superfamily. It undergoes trimerization after binding to CD154, recruits TNFα receptor associated factors (TRAFS) to the cytoplasmic domain and interfaces with signal transduction pathways. [137] Constitutively expressed NF-κB1 p65 and the up-regulation of NF-κB2 p52 in GD thyrocyte cultures following CD40 activation implicates both canonical and non-canonical NF-κB activities in GD. [138] CD40-CD154 ligation results in transcriptional activation of the PGHS-2 gene, hyaluronan synthesis and production of $PGE_2$and proinflammatory cytokines.. [30,87,139,140] These include ICAM-1, IL-6, IL-8, and macrophage chemoattractant protein (MCP-1). Confocal microscopy recently revealed TSHR-CD40 co-localization on the surface of fibrocytes. [136] In autoimmune diseases such as systemic lupus erythematosus and RA, aberrant CD40-CD154 interactions may result in abnormal humoral- and cell-mediated immunity. [141] The CD40 pathway

has consequently become an attractive therapeutic target. Unfortunately, early-stage clinical trials of a monoclonal antibody targeting CD40L have revealed platelet-targeted thromboembolic adverse events. [142,143] The human anti-CD40 monoclonal IgG1 blocking antibody, iscalimab (CFZ533), can suppress T cell-dependent Ab responses and abrogated germinal center formation in cynomolgus monkeys. [144] CFZ533 contains a N297A silencing mutation that disables the mAb from binding Fcγ receptors, impeding antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). [145] An open-label phase II trial of CFZ533 in which a 12-week treatment period was conducted in 15 hyperthyroid patients receiving the drug. [146] This was followed by a 24 week follow up period. The antibody bound CD40 displayed on peripheral blood B cells for up to 20 weeks. Its administration was associated with euthyroid status in 7/15 patients (47%). TSHR-Ab levels normalized in 4 (27%) patients at week 20. 4/7 responders relapsed over the follow-up period, the majority of whom required rescue antithyroid drugs. Placebo-controlled studies will be necessary to more fully evaluate whether targeting the CD40–CD40L pathway in GD and GO might be effective and safe. Anti TNF-α biologics have shown effectiveness in RA. [147,148] Pilot studies in GO have been inadequately powered. [149–151]

IL-6 is pleiotropic cytokine that promotes Th17 development in naïve T cells in conjunction with TGF-β and IL-23 [152]. It can inhibit TGF-β-induced Treg differentiation. [153] IL-6 classically activates early acute response genes through the STAT-3 pathway. [152] Serum IL-6 levels are elevated in GD [154,155] and particularly high in GO. [156] Both TSH and the TSIs can induce IL-6 expression in GD-OFs and fibrocytes, effects mediated through gene promoter activation and enhanced mRNA stability.[40] IL-6 production in fibrocytes is up-regulated in a cAMP independent mechanism in fibrocytes. Instead, it is mediated through TSH activation of AKT, PKC, and PDK1 pathways.[40] PKCβII, a PKC isozyme associated with lymphoproliferative disorders, [157,158] becomes phosphorylated in fibrocytes following TSH stimulation. Dominant PKC isoenzyme expression appears to switch from PKCβII to PKCμ isoenzyme as fibrocytes differentiate into GD-OFs. $CD34^{-}$ fibroblasts may impose this shift in expression which reverts to that resembling fibrocytes following $CD34^{+}$ OF isolation. [40,157,158] Tocilizumab (TCZ) is an inhibitory recombinant, humanized IL-6 receptor monoclonal antibody that binds both soluble and membrane-bound receptors. The IL-6 receptor has been implicated in multiple autoimmune diseases including RA. [159,160] In an uncontrolled study of 18 steroid-resistant patients with active GO, CAS improved in all subjects, proptosis decreased in 72%, and ocular motility improved in 83.3%. [161] No relapses were observed at the end of a 9-month follow-up period. A subsequent, randomized, placebo-controlled trial conducted by these same investigators in Spain included 32 patients with glucocorticoid-resistant GO (defined as CAS ≥ 4 on a 10 point scale) for a mean duration of one year. [162] TCZ was administered intravenously over 12 weeks, followed by a 28 week follow up period. 93.3% patients receiving TCZ achieved the primary outcome of improved CAS ≥ 2 points at week 16 compared to 58.8% of those receiving placebo ($P = 0.04$). The median baseline CAS in both treatment arms was 5, with patients receiving TCZ achieving CAS < 3 at week 16 ($P=0.005$). Those receiving TCZ experienced a greater reduction in proptosis (median of −1.5 mm) compared with placebo (0.0 mm, $P = 0.01$) but proptosis reduction

was minimal and transient. The authors correctly considered their study limited by the relatively small cohort studied, prolonged duration of GO prior to trial enrollment, and a potentially insufficient treatment period. [162] A retrospective study of 54 steroid-resistant patients with GO duration <24 months [163] revealed significant differences from baseline at weeks 4, 8, 12, 16 and follow-up (mean= 22 month) in CAS and TRAb levels ($p<0.001$ at all- time points). 37/50 (74%) patients achieved an absolute CAS of 0 or 1, 42/54 (78%) experienced proptosis reduction ≥ 2 mm, 33/44 (75%) had eyelid retraction reduction ≥ 2 mm, and diplopia improved in 19/28 (68%, $p<0.001$ for all variables). 7.4% of patients experienced disease relapses. Twenty-six patients experienced mild to moderate adverse events. Future prospective, placebo-controlled studies might help clarify the potential benefit of IL-6 receptor inhibition in glucocorticoid resistant TAO.

## TSHR as a therapeutic target

Loss of immune tolerance to TSHR and generation of TSI are central events underlying development of hyperthyroidism in GD. [76] Among the pieces of circumstantial evidence that these anti-TSHR antibodies also play roles in GO are the closely associated onset of both thyroid dysfunction and ocular manifestations. [1,164] TSHR is a member of the rhodopsin-like G protein coupled receptor family, all members of which possess seven plasma membrane spanning regions. [165] TSHR undergoes intramolecular proteolytic cleavage at the hinge region, where ~50 amino acids are removed, leaving two components; an A-subunit containing a leucine-rich domain and a B-subunit consisting of the transmembrane domain. These subunits are linked by two disulfide bonds. [166,167] Cleavage occurs through actions of a metalloprotease and multimerization of the shed A-subunit, resulting in enhancing induction and affinity maturation of pathogenic anti-TSHR antibodies. [166,168] Unique from other members of this glycoprotein hormone receptor family (including follicle-stimulating receptor and luteinizing receptor), TSHR is characterized by its relatively high constitutive (basal) activity. [169] TSH and GD-IgG actions in GD-OF and fibrocytes are mediated through surface TSHR and result in the induction of several cytokines, including IL-1 receptor antagonist, IL-6, IL-8, IL-12 and IL-23. [27,40,56,170] In recent years, interest in treatments for GD and GO have focused on interrupting TSHR signaling, either with monoclonal antibodies or allosteric small-molecule inhibitory ligands. Anti-TSHR monoclonal antibodies with antagonist (K1–70), agonist (M22 and K1–18), and dual antagonist and inverse agonist (5C9) activities have been developed and are at various stages of testing. [171–175] K1–70 was derived from the serum of a hypothyroid patient demonstrating both TSHR-cAMP stimulating and blocking activity in TSHR-transfected CHO cells. [171,172,176] It binds the concave surface of the leucine-rich repeat domain of TSHR. [177] K1–70 reduces total and free serum thyroxine ($T_4$) in rats [176] and is currently being evaluated in a phase I clinical trial for GD hyperthyroidism (NCT02904330). 5C9 inhibits both constitutive and agonist-driven cAMP production in TSHR-transfected CHO cells. [175] Further, it blocks activities of gain-of-function TSHR mutants, including Ser281Ile, Ile568Thr, and Ala623Ile. [175] In contrast to activating anti-TSHR antibodies that bind the extracellular region of the receptor, small molecule antagonists bind the cleft between helices and extracellular loops of the transmembrane domain. In so doing, they can modulate ectodomain-mutated receptor activity at micromolar concentrations, both *in vivo* and in cultured cells. [178–181]

ANTAG3 decreases endogenous TSH-driven serum $FT_4$, as well as the induction by thyroid releasing hormone of NIS and TPO expression in an *in vivo* murine model. [181] This inhibition lacks specificity for TSHR as cross-reactivity with the luteinizing and follicular stimulating hormone receptors was detected. Another TSHR antagonist, s37a, was identified using high-throughput screening. [178] This molecule exhibits TSHR-selectivity and awaits further study.

## Targeting IGF-IR pathway may shift the treatment paradigm for GO

The absence of detectable TSIs in a small subset of patients with GO [182,183] has suggested the possible involvement of another autoantigen target in the development of GO. Thus, we began our examination of other cell-surface receptors that might play roles in the disease and therefore might represent therapeutic targets for GD and GO. [184,185] Besides its behavior as a prototypical tyrosine kinase receptor, IGF-IR can signal through non-canonical mechanisms as a partner of the G-protein-coupled receptor (GCPR) family. [186–188] Although β-arrestins are recognized as adaptors of GCPR endocytosis and transducers of signaling from seven-transmembrane receptors, [189] they can also bind to agonist-occupied IGF-IR and facilitate clathrin-mediated receptor internalization. [190] Girnita *et* al. [191,192] advanced the important concept that β-arrestin 1 represents a cofactor through its association with the oncoprotein E3 ubiquitin ligase $Mdm2_{161–400}$. It can facilitate IGF-IR ubiquitination and degradation. In addition to direct receptor desensitization, β-arrestin 1 initiates MAPK/ERK activation, which can occur in the absence of detectable IGF-IR tyrosine kinase activity. [192] These effects mirrored those of an IGF-IR inhibiting therapeutic antibody that incompletely inhibited the IGF-IR complex, instead forming a partially active receptor which induced β-arrestin-dependent phosphorylated ERK signaling. [193] Deletion of the β-arrestin 1 binding site on the IGF-IR C-terminal tail (Δ1245) in fibroblasts rendered those cells resistant to either anti-IGF-IR antibody- or IGF-I induced degradation, thus demonstrating biased signaling.[193]

Anti-IGFIR autoantibodies were insinuated in the pathogenesis of GO in 1993 by Weightman *et* al, [194] when these authors demonstrated that GD-IgG could displace radiolabeled IGF-I from its high affinity surface binding sites on fibroblasts. The identity of those binding sites was subsequently established by Pritchard *et* al. [184,185] as IGF-IR and not IGF binding proteins. Further, GD-IgG could mimic rhIGF-I in inducing expression of IL-16, a CD4-specific chemoattractant, and RANTES, a C-C chemokine, in GD-OF. Those effects are mediated through activation of the Akt/mTOR/FRAP/$p70^{s6K}$ pathway. The effects of GD-IgG on IL-16 expression were attenuated with rapamycin, a macrolide specifically targeting mTOR protein kinase. [184] Further, the murine monoclonal anti-IGF-IR inhibitory antibody (1H7) [185] and transfection of GD-OF with a dominant-negative mutant IGF-IR (486/STOP) [195] could both attenuate induction of IL-16 and RANTES expression. Subsequent studies revealed that induction by GD-IgG of hyaluronan accumulation in GD-OF could be attenuated by 1H7. [196] IGF-I could mimic the effects of GD-IgG, but rhTSH failed to do so. Using multi-parameter flow cytometry, IGF-1R was found to be overexpressed in GD-OF, [185] and by peripheral lymphocytes [197,198] when compared to cells from healthy donors. Later studies from Tsui *et* al. [199] demonstrated that GD-IgG can activate Erk p42/p44 in GD-OF, as can IGF-I, effects that are attenuated

by 1H7. In contrast, rhTSH failed to activate Erk *in vitro*. Orbital fibroblasts from healthy donors failed to exhibit an Erk response to either GD-IgG or IGF-I. Thus, it appears that the phenotype of GD-OF differs from that of orbital fibroblasts from healthy donors.

Subsequent studies performed in other laboratories have examined the ability of GD-IgG to provoke tyrosine phosphorylation in IGF-IR, an issue left unexplored by Pritchard *et* al. [184,185,199] Those later studies by in large have been unsuccessful in demonstrating that GD-IgG can activate IGF-IR tyrosine kinase. These findings have sparked debate as to whether increased levels of anti-IGF-IR antibodies are relevant to GO. The reports of Varewijck *et* al. [200] and Minich *et* al. [201] described studies using transfected HEK 293 cells to detect anti-IGF-IR antibodies but arrived at disparate results. Using an immunoprecipitation-based assay, Minich *et* al. [201] found similar anti-IGF-IR autoantibody levels in sera from healthy and GO patients. The authors treated HepG2 and MCF-7 cells with GD-IgG alone or with IGF-I. The Igs failed to stimulate IGF-IR autophosphorylation in Hep G2 and attenuated IGF-I-dependent IGF-IR activation. GD-IgGs also inhibited MCF7 breast cancer cell proliferation, suggesting that GD-IgG contains an IGF-IR antagonist/inhibitor. [201] That study used purified GD-IgGs, potentially removing endogenous serum factors that could affect cellular responses to Igs. It should be noted that both HepG2 and MCF-7 cell lines synthesize IGF-II. [202] Thus, endogenous IGF-II could alter IGF-1R receptor activation, potentially masking any effects of GD-IgG. In other studies, [203] GD-IgG stimulated HA secretion in GD-OFs in the absence of detectable IGF-IR autophosphorylation. No evidence of IGF-IR-stimulating activity was found in GD-IgG. [203] 1H7 and another IGF-IR inhibiting monoclonal antibody inhibited IGF-I-induced HA secretion in primary GD-OF cultures, leading the authors to conclude that GD-IgG does not bind to or activate IGF-IR. Further, they speculate that TSHR mediated signaling comprises both IGF-IR dependent and independent components. The use of non-standardized experimental conditions and study designs, uncertain culture medium content, and different cellular targets and assays for detecting IGF-IR activation remain possible explanations for widely dissimilar results. The potential impact of endogenous IGF and IGFBP production in these cell models may also confound interpretation of the experimental results thus far reported.

Growing understanding of how GCPRs and TKRs interact [204,205] has coincided with the evolving concept of IGF-IR playing a meaningful role in GO. Using confocal microscopy, Tsui *et* al. [199] reported that IGF-IRβ forms a signaling complex with TSHR on the plasma membranes and within the cytoplasm and perinuclear compartments of GD-OF, thyroid epithelial cells, and orbital fat *in situ*. Immunoprecipitation with either anti-IGF-IRβ or anti-TSHR monoclonal antibodies brought both proteins out of solution. [199] Of note, IH7 blocked the actions of IGF-I, rhTSH and GD-IgG in provoking ERK1/2 phosphorylation in primary human thyrocytes, indicating that signaling initiated through either receptor is dependent on IGF-IR activity. [199] In aggregate, these observations provided a rationale for interrupting the IGF-IR pathway as therapy in GD and GO. More recently, Krieger *et* al. [206] suggested that β-arrestin 1 could provide scaffolding for the TSHR-IGF-IR protein complex as the basis for receptor crosstalk.

## Therapeutic Targeting of IGF-IR in GO

Teprotumumab (R1507) is a 150 kDa fully human monoclonal anti-IGF-IR antibody without detectable agonistic activity toward canonical IGF-IR signaling or binding affinity for the insulin receptor. [207] It binds with selectivity and high affinity to the extracellular α-chain of IGF-IR, [208] blocking its binding pocket and thus precluding IGF-I and IGF-II ligation. Once bound, teprotumumab promotes internalization of the antibody-receptor complex, promoting its degradation. [207] Its pharmacokinetic disposal exhibits a dose-dependent dual elimination pathway; non-linear (saturable) kinetics at lower concentrations and linear (nonsaturable) clearance at higher concentrations, with a uniform steady-state volume of distribution across all doses. [209] The aberrant signaling of IGF-IR and its binding proteins in tumors provided rationale for targeting the IGF-pathway in cancer, [210,211] yet clinical trials involving several anti-IGF-IR monoclonal antibodies have failed to improve disease outcomes. [212]

In collaboration with the study sponsor, River Vision Development, a randomized, multicenter, double-masked, placebo-controlled therapeutic trial (NCT01868997) for teprotumumab was organized for active, progressive and moderate to severe GO, based almost entirely on *in vitro* studies (Fig. 2). [213] Eighty-eight patients with active (defined by clinical activity score (CAS) ≥4 on a seven-point scale), moderate-severe GO were randomly assigned to receive either placebo or active drug. Inclusion criteria included ages 18–75 years with onset of periocular manifestations within 9 months of enrollment, chemical euthyroidy (serum $FT_4$ and $FT_3$ within 50% above or below the normative upper and lower assay limits) and prior cumulative glucocorticoid dosage ≤ 1 g with a 6-week washout period. Exclusion criteria included pregnancy, prior surgical intervention for GO and previous immunotherapy with RTX. Also excluded from the trial were patients with optic neuropathy. Patients were stratified at each institution with regard to smoking status. The intention-to-treat cohort consisted of 87 patients, 42 received teprotumumab and 45 were assigned to the placebo arm. Patients were assessed clinically at baseline and at 6-week intervals over a 24-week span during which they received 8 infusions of either placebo or teprotumumab. The initial drug dosage was 10 mg/kg of body weight, which was increased to 20 mg/kg for the remaining infusions if the initial infusion was uncomplicated. The primary response was the aggregate at 24 weeks of a reduction in CAS ≥ 2 points and proptosis ≥ 2 mm in the study eye (the more severely affected eye) without contralateral (fellow) eye worsening. Secondary responses included reduction from baseline in CAS ≥ 2 points and reduction in proptosis ≥ 2 mm measured as continuous variables, as well as improvement in quality of life score (GO-Qol questionnaire). [214] Thirty-seven (88%) patients receiving teprotumumab and 39 (87%) receiving placebo completed the intervention phase of the study. Those patients who did not complete the week 24 clinical assessment for any reason were judged as treatment failures in the intention-to-treat analysis. Twenty-nine out of 42 (69%) patients in the teprotumumab cohort met the primary response compared to 9/45 (20%) of those receiving placebo at week 24 ($p < 0.001$) (Fig. 3). The interval from baseline to achieving primary response was significantly shorter in the teprotumumab group; patients in this treatment arm had greater responses at weeks 6, 12, and 18 ($p < 0.001$). A “high response” of ≥3 mm proptosis reduction and ≥3 CAS reduction occurred in 21/42 (50%) patients in the teprotumumab group as compared to those receiving placebo (4/45,

9%) ($p<0.001$). The visual-functioning GO-QOL score also improved significantly at 24 weeks ($p < 0.001$) with teprotumumab. In contrast, the appearance subscale failed to achieve statistical significance despite marked improvement with treatment duration. Seventeen of 42 patients (40%) receiving teprotumumab experienced proptosis reduction ≥ 4 mm at week 24. This magnitude of proptosis reduction is unprecedented among previously developed medical therapies and is equivalent to the best reported surgical outcomes.

Subsequently, a pivotal multicenter phase 3 OPTIC trial (NCT03298867) was conducted with a similar design as that of phase 2 with minor modifications. Age eligibility was extended to 18–80 years and excluded individuals with a history of inflammatory bowel syndrome. [215] 83 patients underwent randomization in the intention-to-treat population, with 41 assigned to teprotumumab and 42 to placebo. The primary endpoint was proptosis responder rate of ≥ 2 mm reduction in the study eye at week 24. Following the 24 week treatment period, an open-label extension trial, OPTIC-X (NCT03461211,) was offered to proptosis non-responders and to relapsing responders, irrespective of the treatment the received during OPTIC. This 24 week extension included 8 additional infusions of teprotumumab every 3 weeks. The fraction of patients with proptosis response of ≥ 2 mm in those receiving teprotumumab at week 24 (83%) in OPTIC was significantly greater than in the placebo arm (10%; $p < 0.001$). The number needed to treat was 1.36 (Fig. 4). Thus the vast majority of patients in the teprotumumab group achieved the primary outcome. The magnitude of proptosis reduction with teprotumumab was −2.82 mm vs. −0.54 mm in placebo. Six patients treated with the active drug who underwent orbital MRI at baseline and again at week 24 experienced a mean reduction in extraocular muscle and orbital fat volumes of 1.56 $cm^3$ (35%) and 2.67 $cm^3$ (17%), respectively (Fig. 5). Follow-up of integrated responses at 7 weeks and 51 weeks after final dose for proptosis in 62/71 (87%) and 38/57 (67%) of patients respectively; 38/58 (66%) and 33//48 (69%) for diplopia, respectively [215]. An ophthalmic composite outcome included improvement in ≥1 eye from baseline without deterioration in either eye in ≥2 of the following: absence of eyelid swelling; CAS ≥2; proptosis ≥2 mm; lid aperture ≥2 mm; diplopia disappearance or grade change; or 8 degrees of globe motility improvement. The composite response was achieved in 66/72 (92%) at 7 weeks and 48/58 (83%) at 51 weeks.

Adverse events (AEs) during treatment in the intention-to-treat populations from both phase 2 and 3 studies were pooled for analysis. [216] Sixty-three (94%) of 67 patients receiving teprotumumab and 59 (98%) of 60 patients in the placebo group experienced mild to moderate (grade 1 or 2) AEs, with 3 patients (4%) experiencing serious AEs related or possibly related to teprotumumab. These included diarrhea, infusion reactions, and Hashimoto's encephalopathy (co-incident) which prompted study discontinuation. The prominent AEs with risk difference within the teprotumumab cohort were muscle spasm (18%), hearing loss (10%), and hyperglycemia (8%). As IGF-I and IGF-IR signaling are intimately involved in glucose homeostasis, [217] these were associated with pre-existing diabetes mellitus. Glycemic control was restored with diabetes medication adjustment. Increased drug requirements returned to those at baseline following the end of exposure to teprotumumab.

In January 2020, teprotumumab became the first medical therapy approved by the US FDA for the treatment of GO.[218] Following its approval, case reports have been published suggesting that teprotumumab may effectively treat compressive optic neuropathy secondary to GO. [219–221] The effectiveness of the drug must now be studied in formal trials and compared with the orthodox regimen of high-dosage steroids and urgent surgical decompression. Teprotumumab might also be effective in chronic, non-progressive GO. [222,223]

### Concluding Remarks

The past decade has witnessed substantial progress in understanding and treating GO. For the first time, a specific therapy, based on recognition of the immunologic pathogenesis of GO, has been developed and was recently approved by the U.S. FDA. Teprotumumab, market as Tepezza, is now available in North America but has not yet been introduced elsewhere. Importantly, that drug, an IGF-IR inhibitory monoclonal antibody, was developed as a therapeutic based entirely on insights into the pathogenesis of GO. It therefore serves as example of the power of basic and translational science that can result in effective treatment. We envision future insights providing a springboard for development of even better therapeutic options over the coming decade. Further, we predict that the ultimate treatment for GD and GO will involve the restoration of immune tolerance to the relevant autoantigen(s), sparing patients chronic exposure to immunomodulatory agents. Among the new approaches in early-stage development are those using synthetic peptides resembling T cell epitopes, some of which have shown promise in protecting against autoimmune induction. [224,225] The potential for capturing CAAR-T cell technology [226,227] to generate memory cells against pathogenic B cells and thus promoting long term remission in GO, is an exciting possibility based on our growing understanding of how the disease develops.

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## Glossary

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|---|---|
| **Ad-TSHR A** | Adenovirus expressing the extracellular A-subunit of the human TSH receptor (TSHR) |
| **AE** | Adverse event |
| **AIRE** | thymic autoimmune regulator |
| **AKT** | Protein kinase B |
| **AP-1** | Activator protein-1 |
| **ARID5B** | AT-Rich Interaction Domain 5B |
| **BAFF** | B-cell activating factor |
| **CAAR-T cells** | Chimeric autoantibody receptor expressing T cells |

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| **cAMP** | Cyclic adenosine monophosphate |
| **CAS** | Clinical activity score |
| **CFZ533** | Iscalimab |
| **CHO** | Chinese hamster ovary cell |
| **CTLA4** | Cytotoxic T-lymphocyte-associated protein 4 |
| **COX-2** | Cyclooxygenase-2 |
| **CYR61** | Cysteine-rich angiogenic inducer 61 |
| **Dsg3** | Desmoglein-3 |
| **EGR-1** | Early growth response protein 1 |
| **ERK** | Extracellular signal-regulated kinases |
| **ETA** | European Thyroid Association |
| **GcgR** | Fc gamma receptor |
| **FcgRI** | High affinity immunoglobulin gamma Fc receptor I |
| **FcgRIIa** | Low affinity immunoglobulin gamma Fc region receptor II-a |
| **FcRn** | Neonatal Fc receptor |
| **FRAP** | FK506-binding protein 12-rapamycin-associated protein 1 |
| **FT3** | Free triiodothyronine |
| **FT4** | Free thyroxine |
| **GD-IgG** | Graves’ disease immunoglobulins |
| **GD-OF** | Graves’ disease orbital fibroblasts |
| **GO** | Graves’ orbitopathy |
| **GO-QOL** | Graves’ ophthalmopathy quality-of-life scale |
| **GR** | Glucocorticoid receptor |
| **HAS1** | Hyaluronan synthase 1 |
| **HAS2** | Hyaluronan synthase 2 |
| **HLA-DRβ-Arg74** | Human Leukocyte Antigen – DR isotype -- variant containing Arginine at position 74 |
| **HMG-CoA** | 3-hydroxy-3-methylglutaryl coenzyme |
| **IFN-γ** | Interferon gamma |

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|---|---|
| **IGF-I** | Insulin-like growth factor 1 |
| **IGF-II** | Insulin-like growth factor 2 |
| **IGF-1R** | Insulin-Like Growth Factor 1 Receptor |
| **IgG-k** | Immunoglobulin G kappa light chain |
| **LRR** | Leucine-rich repeat |
| **MHC class I** | Major histocompatibility complex I |
| **MHC class II** | Major histocompatibility complex II |
| **MMF** | Mycophenolate motefil |
| **MPA** | Mycophenolic acid |
| **NF-κB** | Nuclear factor-κB |
| **NIS** | Sodium iodide symporter |
| **OD** | Oculus dexter |
| **OF** | Orbital fibroblast |
| **OS** | Oculus sinister |
| **p70s6K** | Ribosomal protein S6 kinase beta-1 |
| **PDK1** | Pyruvate dehydrogenase kinase 1 |
| **PGE2** | Prostaglandin E2 |
| **PGHS-2** | Prostaglandin endoperoxide H synthase 2 |
| **PI3K/AKT/mTOR** | Phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) |
| **PKC** | Protein kinase C |
| **PKCβII** | Protein kinase C beta type 2 |
| **PMBC** | Peripheral blood mononuclear cell |
| **PPARγ** | Peroxisome proliferator-activated receptor gamma |
| **PTPN22** | Protein tyrosine phosphatase, non-receptor type 22 |
| **PTX-3** | Pentraxin-3 |
| **qRT-PCR** | Real-Time Quantitative Reverse Transcription PCR |
| **RA** | Rheumatoid Arthritis |
| **RANTES** | Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted |

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|---|---|
| **RTX** | Rituximab |
| **ROBO1** | Roundabout 1 |
| **RhSlit2** | Recombinant human Slit homolog 2 protein |
| **SAP** | Serum amyloid P |
| **sFRP-1** | Secreted frizzled-related protein-1 |
| **SGK-1** | Serine/threonine-protein kinase Sgk1 |
| **sIL-1RA** | Secreted IL-1 receptor antagonist |
| **SLE** | systemic lupus erythematosus |
| **Slit2** | Slit homolog 2 protein |
| **SNP** | Single-nucleotide polymorphisms |
| **STAT-3** | Signal transducer and activator of transcription 3 |
| **TAO** | Thyroid associated Ophthalmopathy |
| **TCZ** | Tocilizumab |
| **Tg** | Thyroglobulin |
| **TNFα** | Tumor necrosis factor |
| **TPO** | thyroid peroxidase |
| **Trab** | Thyrotropin receptor antibody |
| **TRAFS** | TNFα receptor associated factors |
| **Treg** | T regulatory cells |
| **TSH-β** | Thyroid stimulating hormone beta protein |
| **TSHR** | Thyrotropin Receptor |
| **TSI** | Thyroid stimulating immunoglobulin |
| **UGDH** | UDP-glucose dehydrogenase |
| **Wnt** | Wingless/Integrated |
| **1H7** | Murine monoclonal anti-IGF-IR inhibitory antibody |
| **486/STOP** | Dominant-negative mutant IGF-IR |

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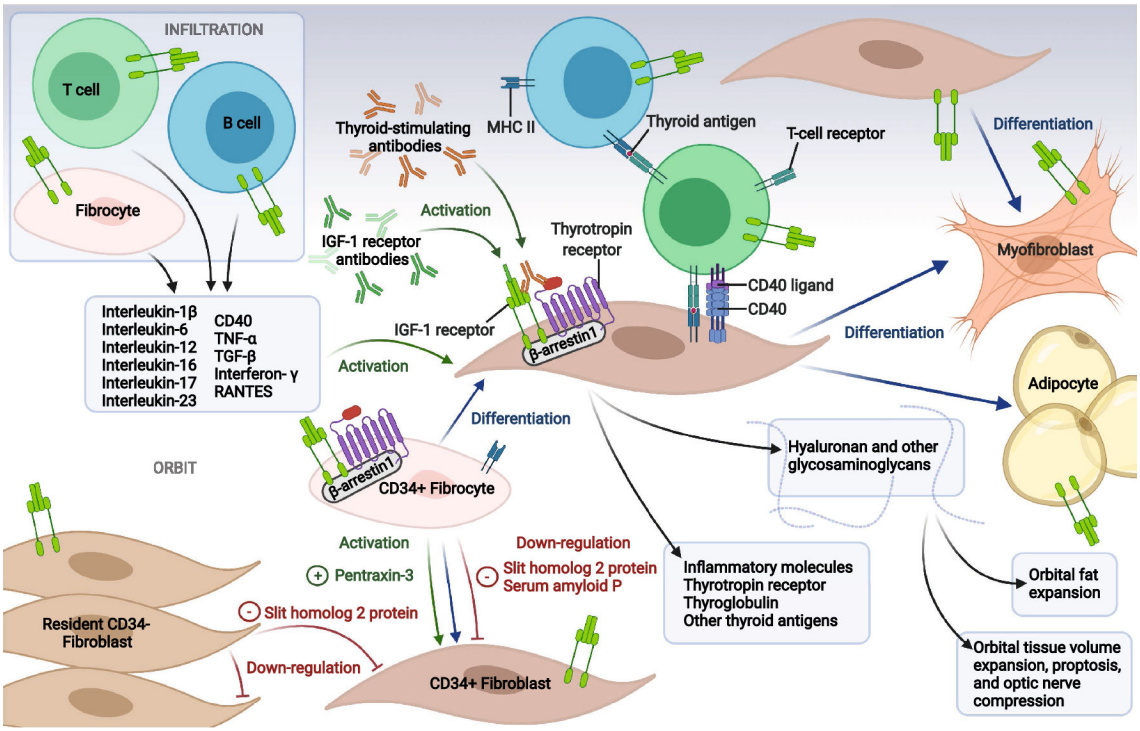


**Figure 1.**

Theoretical concept of TAO pathogenesis. $CD34^+$ fibrocytes of the monocyte lineage infiltrate the orbit where they can differentiate into $CD34^+$ fibroblasts, adipocytes, and myofibroblasts. $CD34^+$ fibroblasts co-populate the orbit with $CD34^-$ fibroblasts in an admixture (GD-OF). $CD34^+$ fibrocytes express thyroid autoantigens, including thyrotropin receptor (TSHR), thyroglobulin (Tg), thyroperoxidase (TPO), and sodium-iodide symporter (NIS), the expression of which depends on the autoimmune regulator (AIRE) protein. Fibrocytes can present antigens to T cells which in turn support IgG1 production in B cells. When activated, fibrocytes and orbital fibroblasts produce many cytokines, including interleukins 1β, 6, 8, 10, 12, 16, 23, TGF-β, tumor necrosis factor α, regulated on activation, normal T expressed and secreted (RANTES,) CXCL-12 and CD40 ligand (CD154). Many genes expressed by fibrocytes are also detected in $CD34^+$ fibroblasts but at considerably lower levels. These lower levels result from actions of Slit2 which is expressed and released by $CD34^-$ orbital fibroblasts. Orbital fibroblasts display the tyrosine kinase, insulin-like growth factor-I receptor (IGF-IR), a therapeutic target for TAO. Cytokine-activated orbital fibroblasts express all three mammalian hyaluronan synthase isozymes and UDP glucose dehydrogenase (UGDH), with the majority of hyaluronan synthesis attributable to activities of HAS2 expressed by $CD34^-$ fibroblasts. Created with BioRender.com

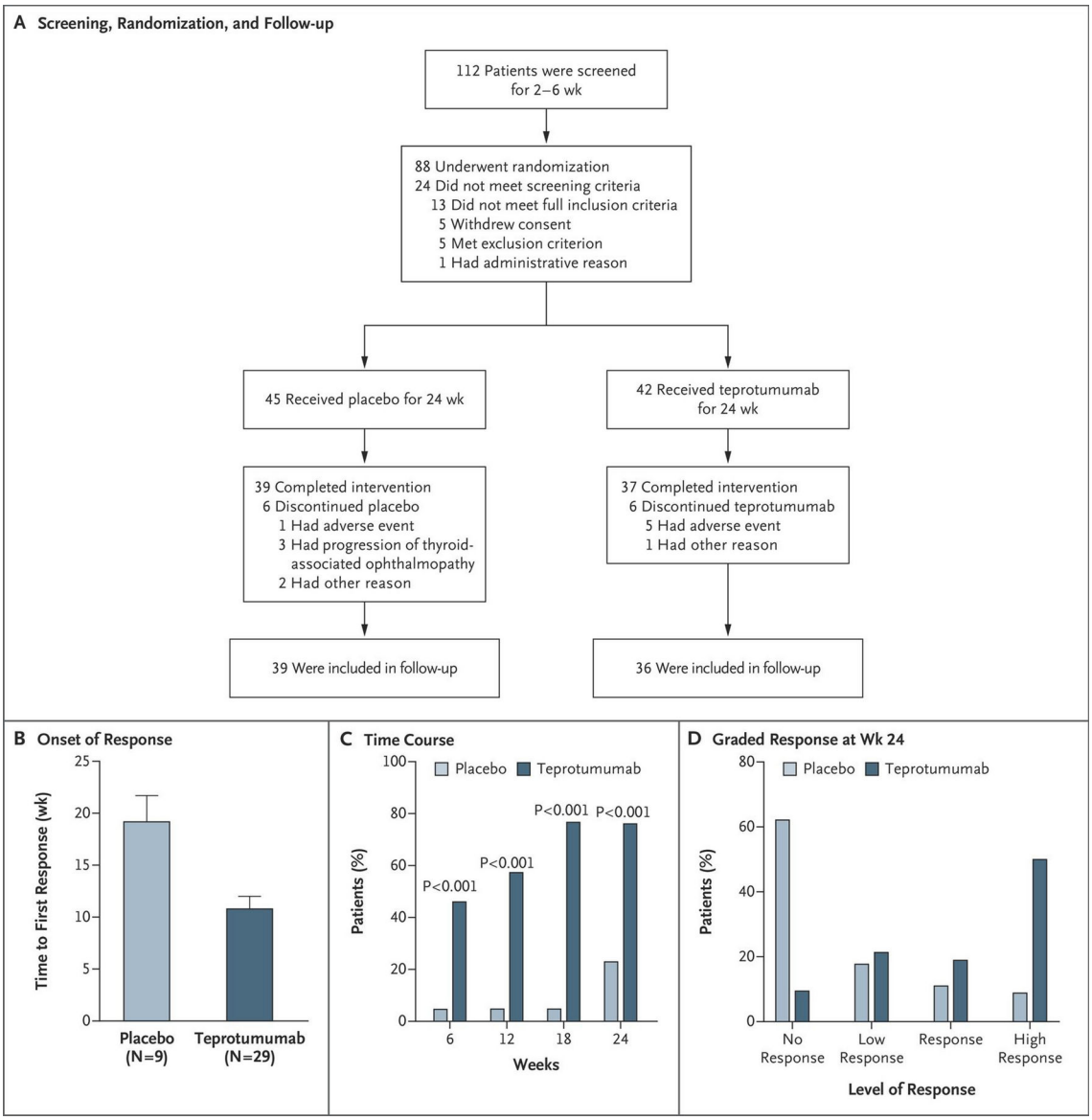


**Figure 2.**

Phase II trial design and therapeutic response. (a) Strategy for screening patients, their randomization into the two treatment arms for a 24-week intervention phase, and follow-up. One patient after screening failed to meet inclusion criteria, and withdrew prior to treatment initiation. (b) Analysis of the primary response (a reduction of ≥ 2 mm in proptosis and an improvement of ≥ 2 points on a 7-point scale in clinical activity score) in the study eye. (c) Time course in patients meeting response criteria at 6,12,18 and 24 weeks during treatment. (d) Grading the response at week 24. A high response denoted decrease of proptosis ≤ 3 mm and clinical activity score reduction ≤ 3 points. A response indicates a reduction ≥ 2 mm but < 3 mm in proptosis, and ≥ 2 points but < 3 points in Clinical Activity Score. A low response indicates reductions ≥ 1 mm but < 2 mm in proptosis and ≥ 1 point but < 2 points in Clinical Activity Score. No response indicates that the patient did not meet any response criteria or

missed evaluation at week 24. Reprinted with permission from Smith TJ et al N Engl J Med. 2017; copyright 2017 Massachusetts Medical Society

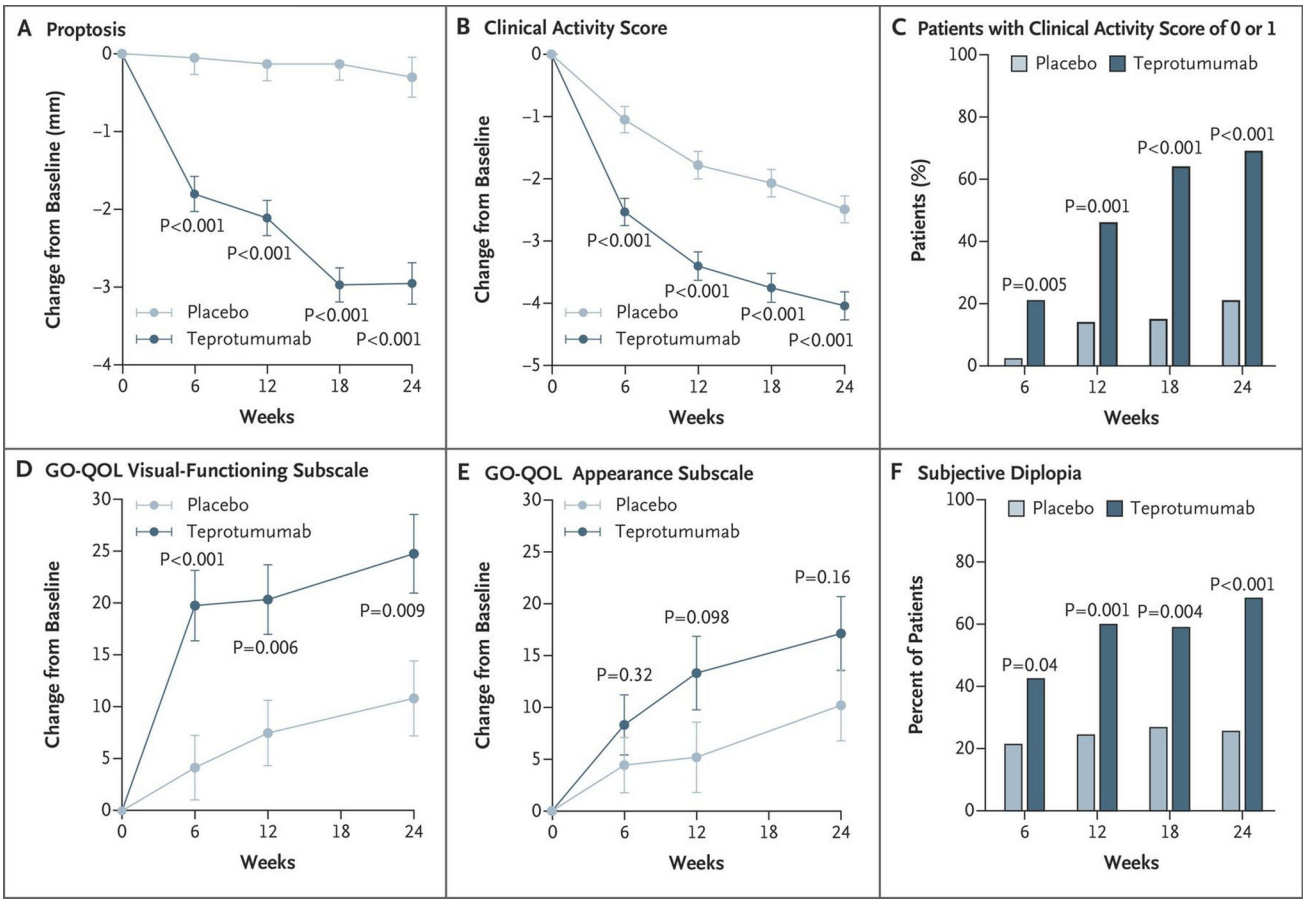


**Figure 3.**

Secondary efficacy points of Phase II trial. Vertical bars in panels a,b,d, and e represent standard error (SE). (a) Reduction in proptosis from baseline over 24 weeks. (b) Changes in clinical activity score from baseline. (c) Percentage of patients with clinical activity score of 0 or 1 at week 24. (d) Change from baseline in visual-functioning subscale of the Graves' ophthalmopathy–specific quality-of-life scale (GO-QOL) from baseline. Scores on the visual-functioning subscale range from 0 to 100, with a change of 8 points considered clinically relevant. e) Change from baseline in GO-QOL appearance subscale. (f) Response in subjective diplopia. Reprinted with permission from Smith TJ et al N Engl J Med. 2017; copyright 2017 Massachusetts Medical Society.

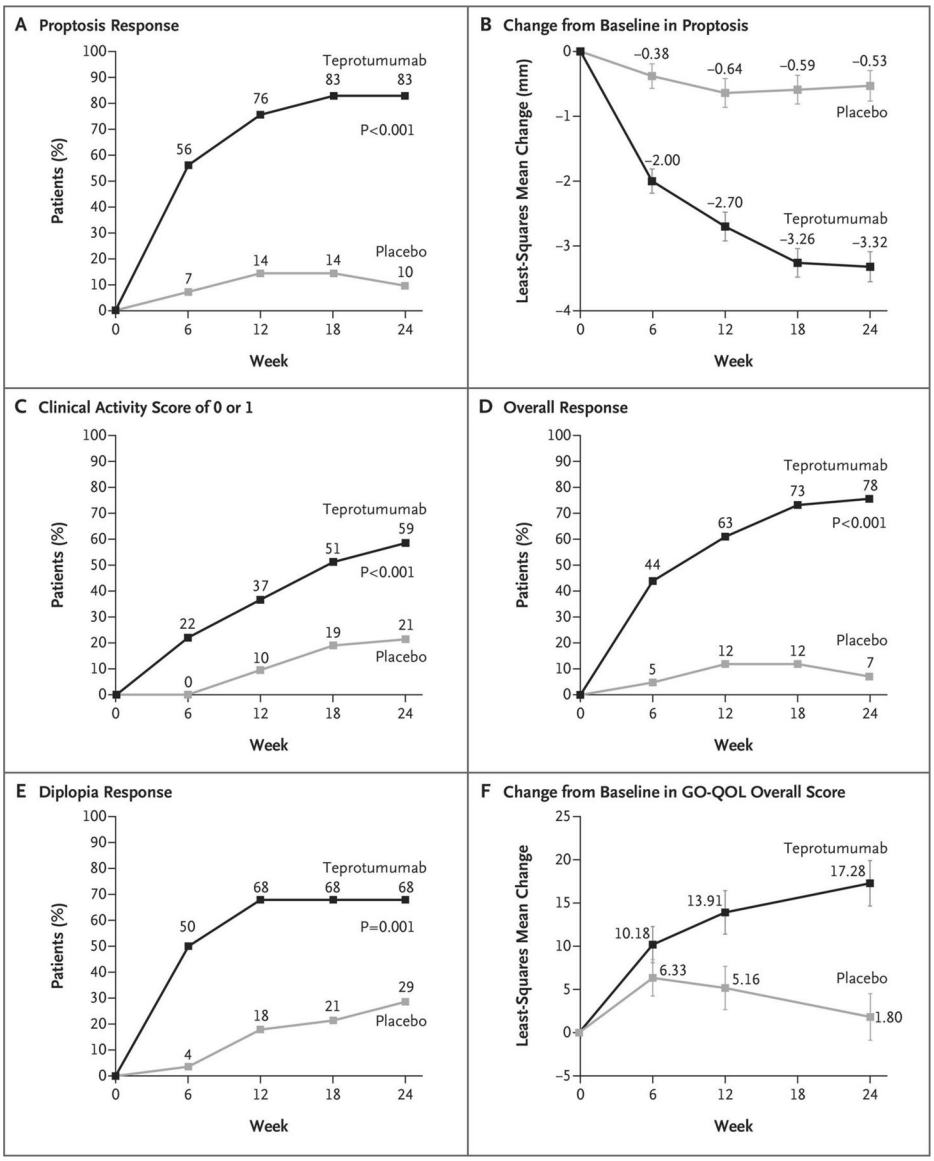


**Figure 4.**

Efficacy outcomes for phase III trial. The p values for the between-group differences at week 24 (panels a, c, d, and e) were adjusted for multiplicity. I bars in panels c and f represent SE. (a) The primary response of proptosis response (b) the change from baseline in proptosis (c) The percentage of patients with a Clinical Activity Score of 0 or 1 at week 24 (d) The percentage of patients with an overall response (e) The percentage of patients with a diplopia response (f) The change from baseline in the overall score on the GO-QOL questionnaire. Reprinted with permission from Douglas RS et al N Engl J Med. 2020; copyright 2020 Massachusetts Medical Society

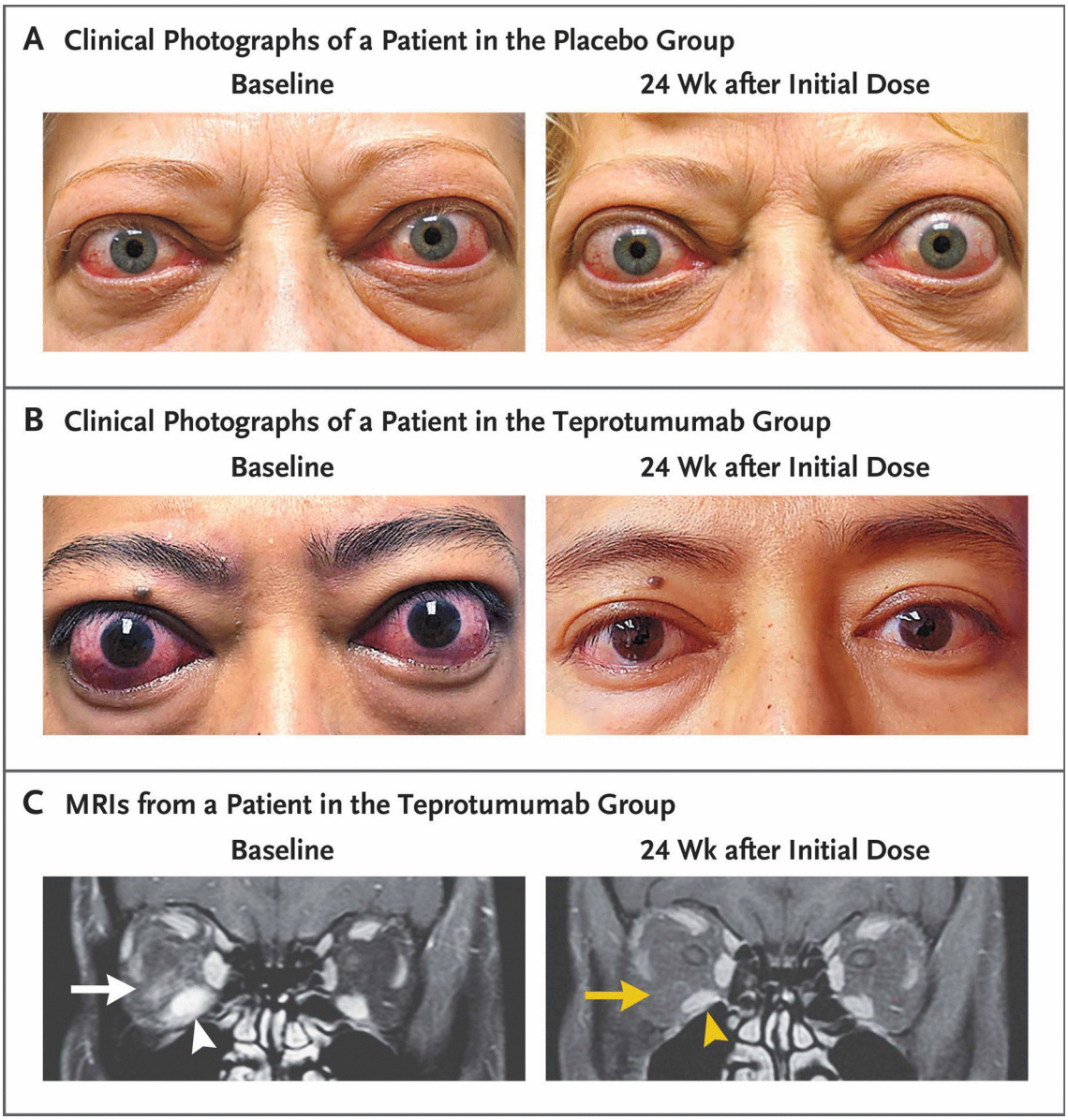


**Figure 5.**

Phase III trial facial appearance and orbital MRIs at baseline and 24 weeks after initial saline or teprotumumab infusion. (a) Frontal view of a patient receiving placebo. At baseline, considerable bilateral proptosis (OD, 29 mm and OS, 27 mm) and inflammatory signs (Clinical Activity Score 7 OD and 5 OS). Proptosis and inflammation persist at week 24. (b) Frontal view of a patient receiving teprotumumab. At baseline, proptosis (24 mm OU), edema, upper and lower eyelid retraction, and multiple inflammatory signs (Clinical activity score 5 OU) are present. Reductions of proptosis of 5 mm OU and reduction of CAS 4 points OU observed at 24 weeks (c) T1-weighted coronal and contrast-enhanced, magnetic resonance images at baseline and week 24 in a patient treated with teprotumumab. Enhancement of inferior rectus muscle (OD, white arrowhead) and adipose tissue (white arrow) reflect findings of inflammation and edema. The respective inferior rectus muscle

(yellow arrowhead) and orbital fat are shown at week 24 (yellow arrow). The inferior rectus volume was reduced by 49% by Materialise software (yellow arrowhead). Proptosis reduction decreased 5 mm from baseline at week 24. Reprinted with permission from Douglas RS et al N Engl J Med. 2020; copyright 2020 Massachusetts Medical Society