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PROSTAGLANDIN F2-ALPHA EYE DROPS (BIMATOPROST) IN GRAVES' ORBITOPATHY: A RANDOMISED CONTROLLED DOUBLE MASKED CROSSOVER TRIAL (BIMA TRIAL)

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Abstract

Background: Previous *in vitro* experiments have demonstrated that $PGF_{2\alpha}$ reduced proliferation and adipogenesis in a murine cell line and human orbital fibroblasts derived from subjects with inactive Graves' orbitopathy (GO). The objective of this study was to determine if the $PGF_{2\alpha}$ analogue Bimatoprost is effective at reducing proptosis in this population.

Methods: A randomized controlled double-masked crossover trial was conducted in a single tertiary care academic medical center. Patients with longstanding, inactive GO but persistent proptosis (> 20 mm in at least one eye) were recruited. Allowing for a 15% dropout rate, 31 patients (26 females) were randomized in order to identify a treatment effect of 2.0 mm (p=0.05, two-sided paired t-test, power 0.88). Following informed consent, participants were randomized to receive Bimatoprost or placebo for three months after which they underwent a two-month washout, before switching to the opposite treatment. The primary outcome was the change in exophthalmometry readings over the two 3-month treatment periods.

Results: The mean exophthalmometer at baseline was 23.6 (range 20.0-30.5) mm and the mean age was 55 (range 28-74) years. The median duration of GO was 7.6 (IQR 3.6-12.3) years. The majority were still suffering from diplopia (61.3%) with bilateral involvement (61.3%). Using multilevel modeling adjusted for baseline, period and carryover, Bimatoprost resulted in a -0.17 mm (reduction) exophthalmometry change (95% CI -0.67 to +0.32) p=0.490. Intraocular pressure was reduced -2.7 mmHg (95% CI -4.0 to -1.4) p=0.0070. One patient showed periorbital fat atrophy (PAP) on treatment which resolved on stopping treatment. Independent analysis of proptosis by

photographic images (all subjects) and subgroup analysis on monocular disease (n=12) did not show any apparent benefit.

Conclusion: In inactive GO, Bimatoprost treatment over a 3-month period does not result in an improvement in proptosis.

INTRODUCTION

Graves' orbitopathy (GO) is the commonest extrathyroidal manifestation of Graves' hyperthyroidism. Proptosis may persist after inflammation has subsided in the late "burnt out" phase of GO and the persistent disfigured appearance of the eyes is a source of significant psychological distress and impaired quality of life for sufferers (1). There are no specific medical treatments that target orbital volume reduction in late-stage disease. A UK nationwide survey of patients with GO revealed low satisfaction levels with existing therapies (2).

The main pathological features of GO include expansion of orbital tissue fat, muscle, mononuclear cell infiltration of orbital connective tissue and extraocular muscle, and tissue remodeling, a process that can culminate in fibrosis and diminished eye motility (3). A key mechanism underlying GO is an increase in adipogenesis and muscle associated secretion of glycosaminoglycans (GAG) in the orbit, resulting in an increase in orbital volume and exophthalmos (protrusion of the eye) (4, 5). The opposite effect, enophthalmos (recession of the eye into the orbit), has been described in patients with glaucoma treated with daily Bimatoprost (prostaglandin F2 alpha, $PGF_{2\alpha}$), a prostaglandin analogue used topically in the management of intraocular hypertension (glaucoma). Cases of enophthalmos developing in patients treated with Bimatoprost and other $PGF_{2\alpha}$ analogues have been reported worldwide, albeit in small numbers (6-10). This side effect is more noticeable if only one eye is exposed to treatment as the treated eye is easily comparable with the unexposed eye. However, since most patients receive treatment to both eyes it is possible that the incidence of enophthalmos in Bimatoprost treated patients has been underestimated.

A possible mechanism by which $PGF_{2\alpha}$ agonists might produce enophthalmos is through reduction of orbital fat volume (6). A $PGF_{2\alpha}$ receptor agonist has been shown to be a potent inhibitor of adipose tissue differentiation in new-born rat precursor cells (11). This raises the possibility that $PGF_{2\alpha}$ exerts direct effects on adipose tissue precursors. We have confirmed this finding in *in vitro* studies in our laboratory using 3T3-L1 cell lines and human primary orbital fibroblast cultures (12). This is further supported by Eftekhari et al. who reported that retrobulbar Bimatoprost injections in rats showed histologic evidence of orbital fat atrophy (13). Thus, $PGF_{2\alpha}$ agonists may be effective in reducing orbital fat expansion, ameliorating proptosis, and thus improving quality of life in patients with active and/or inactive disease.

Rehabilitative surgery is the mainstay of treatment for the late disease phase. However, surgery is not always successful in reducing proptosis and carries the associated risks of anesthesia and local complications (14). Recently Teprotumumab, a human monoclonal antibody inhibitor of IGF-1R has been shown to reduce proptosis in patients with active GO (15), whilst radiotherapy is of questionable benefit in conjunction with steroids (16). However, there remains a major unmet need for medical therapies to reduce residual proptosis in the late-phase (inactive) of GO, a disease stage in which disfigurement and impairment of ocular function persist after resolution of the initial inflammatory process and which affects 5-10 times as many people as the early active phase (17). In our *in vitro* study, the majority of samples studies were from patients with inactive GO, and $PGF_{2\alpha}$ was noted to reduce proliferation and adipogenesis in orbital fibroblasts from both GO and non-GO tissue (12). Even in “burnt out” disease, orbital fibroblasts from GO have a higher proliferation and adipogenesis potential than cells from normal orbits (12). We therefore designed

a randomized double-masked cross-over clinical study to evaluate the impact of Bimatoprost at reducing proptosis in patients with GO.

METHODS

The trial was conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996), the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended in 2006, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The trial has been approved by a local NHS Research Ethics Committee (REC, registration number: 14/WA/0081), the Medicines and Healthcare Products Regulatory Agency (MHRA, registration number: 21323/0043/001-0001) and is registered with ClinicalTrials.gov (registration number: NCT02059655) and the International standard randomized controlled trial network (ISRCTN, registration number: ISRCTN46696624).

This was a single center randomized controlled double-masked crossover trial of Bimatoprost in GO. Allocation of subjects was by remote computerized web-based randomization and minimization over 2 identified factors (degree of proptosis and uni/bilateral eyes involvement) to ensure a balance between the 2 trial arms.

Patients were recruited from the multidisciplinary GO clinic at University Hospital of Wales. All patients had had a previous diagnosis of GO defined by the presence of one or more of the following features: soft tissue changes in the eye, proptosis,

extraocular muscle dysfunction, corneal abnormalities, and optic nerve involvement. The inclusion criteria were stable GO with no reported change in proptosis for at least 6 months, inactive disease with a clinical activity score <3, proptosis (subjective unilateral proptosis confirmed by asymmetry in exophthalmometry of >2 mm or greater than 20 mm on exophthalmometry measurement in one eye), euthyroid (FT3 and FT4 in the reference range) and, if female, using a reliable form of contraception during the trial. The exclusion criteria were age less than 18 years old, dysthyroid optic neuropathy, pregnancy/lactation, on therapy for glaucoma, systemic steroid use, patients with risk factors for cystoid macular edema, iritis or uveitis and allergies to Bimatoprost or preservative. Patients were assessed at screening visit at least 2 weeks prior to a first trial visit to ensure that they had inactive disease. Patients were allocated either Bimatoprost or placebo for 3 months, followed by 2 months washout period before crossing over to the opposite treatment. Bimatoprost 0.03% (Lumigan® Allergan) or placebo (Blumont Healthcare) was administered at a dose of one drop in the affected eye/eyes once daily between 18:00 - midnight starting from the day of allocation. To enhance masking, the placebo contained artificial tears with a similar preservative (Benzalkonium chloride) which will replicate any mild stinging sensation experienced with Bimatoprost. Patients were allowed to use preservative free eye drops for symptomatic dry eyes if needed during the trial which had to be applied at least 30 minutes before/after trial drops application. No other eye drops were allowed during the trial period.

The primary outcome was the change in proptosis with Bimatoprost using the mean improvement of the two eyes where both have been treated. A change of > 2.0 mm in proptosis is considered to be clinically relevant (18, 19). Assuming a standard

deviation of 2.5 mm in proptosis measurements in patients with GO as previously reported [19, 20], we calculated that 26 participants would be needed to be able to identify a treatment effect of 2.0 mm as statistically significant (p=0.05, two-sided paired t-test, power 0.88). Allowing for a 15% dropout rate/incomplete datasets, we recruited 31 participants.

At each visit, patients underwent ophthalmological assessment including assessment of proptosis (using an Oculus© exophthalmometer), intraocular pressure in primary position and up gaze, logMAR visual acuity, clinical activity score (CAS), palpebral aperture, Gorman's diplopia score, corneal integrity, quality of life questionnaires (GO quality of life questionnaire (GO-QOL) and EQ-5D-5L) and health economic assessment using modified client service receipt inventory (CSRI) for GO (see supplementary material - BIMA protocol). Color photographs of the eye in the lateral and anterior views were taken according to a standard operating procedure (see supplementary material - SOP). Photograph exophthalmometry measurements were made following 200% magnification from standard view either from lateral canthus or nasal bridge to the corneal apex by a masked assessor. Any adverse events were recorded in the patient's diary. Thyroid function tests (TSH, FT3, FT4) were performed at the beginning, middle and end of trial visits to ensure patients remained euthyroid. Secondary outcomes were change in GO-QOL, change in intraocular pressures (IOP) in primary and chin forward position, side effect profiles of Bimatoprost, and health economic evaluation. The ophthalmology assessment was carried out by either one of two assessing ophthalmologists. We conducted an initial exophthalmometer alignment phase whereby the assessors were calibrated by multiple exophthalmometer readings on the same non-trial subjects in the clinic and

adjustments were made to ensure their readings were comparable. Subjects were not necessarily assessed by the same assessor at each time point. In order to ensure maintenance of masking, during each trial visit the assessors did not have access to baseline values or any prior measurements and clinical notes.

The mean change in proptosis measurement in the placebo phase and Bimatoprost phase was compared with a paired t-test. This was carried out using the mean improvement of the two eyes where both have been treated or the change in one eye where only one was treated. Multilevel model in STATA version 12.1 (STATACORP, College Station, TX) using demographic and clinical variables (including baseline, the order of treatment and carryover effects) was also used to adjust for unexplained variance and in order to obtain better estimates of effect size with tighter confidence intervals. The results are expressed as an effect in millimeters from the treatment arm controlling for the placebo effect with 95% confidence intervals (95% CI) and p-values. Secondary and other outcomes were summarized with descriptive statistics.

There were 3 patients who were deemed to be protocol non-compliant with inclusion criteria who had FT4 levels above the reference range with normal FT3 during the screening period. This was due to a misinterpretation of the inclusion criteria whereby the definition of euthyroid was FT3 and FT4 in the reference range. Instead, the result of either FT3 or FT4 was used to define the euthyroid state. These three patients were clinically euthyroid during randomization. A sensitivity analysis was done after the exclusion of these three subjects to determine any effect on the study conclusions.

RESULTS

Recruitment and retention

Seventy-two patients were invited initially of which 33 agreed for the trial enrolment. One patient was ineligible on screening and one patient chose not to take part due to fear that Bimatoprost might change her iris color. Thirty-one patients were subsequently randomized and underwent the first phase of the trial successfully. Unfortunately, one patient from the Bimatoprost starting group died at the end of first washout period due to pulmonary embolism which was not considered to be related to the investigational product. Therefore, 30 patients were entered into the second phase of the trial. One patient from the placebo starting group did not return for visit 4 (end of second phase assessment) due to the withdrawal of consent. Twenty-nine patients entered the second washout phase and completed the trial (Figure 1).

Demographic and baseline characteristics

Out of 31 patients, there was a female preponderance with a 5:1 ratio and a mean age of 55.2 (range 28-74) years. The median duration of GO was 7.6 (IQR 3.6-12.4) years. The majority were smokers at diagnosis (74.2%), but this reduced to 38.7% after the diagnosis. 61% were still suffering from diplopia (19/31) and 19/31 (61%) had bilateral involvement. There was a good balance between the 2 treatment allocations with some differences in smoking history but not at trial entry and more patients with constant diplopia in the Bimatoprost first starting group (Table 1). Thyroid function tests were unchanged throughout the study period.

Inter-operator comparison

Fifteen non-trial patients were assessed by the 2 assessors by exophthalmometry after a period of calibration between assessors involving 5 patients. Compared to assessor 1, the regression coefficient of assessor 2 was 0.93 (95% CI 0.83 to 1.03) mm. There was a positive Pearson correlation with r=0.9652 (p<0.0001) between the 2 assessors (Supplement Figure 1 and 2).

Primary outcome analysis

The mean baseline exophthalmometer readings of treated eyes in the Bimatoprost starting group was 24.1 (SD 2.9) mm and 23.1 (1.9) mm in the placebo starting group (Table 1). The mean change across all affected eyes in the Bimatoprost phase was +0.17 mm (95% CI -0.35 to +0.69) versus +0.26 mm (95% CI -0.51 to +1.03) in the placebo phase. This was not statistically different with a p value = 0.845 (Figure 2). A sensitivity analysis was done after exclusion of the three protocol non-compliant subjects. There was no difference between the 2 groups p=0.727. Using pkcross function on the STATA, there were no period (p=0.38) or carryover (p=0.46) effects observed.

Multilevel modelling

Data were also analyzed using a multilevel model in STATA which will also enables to use one data point for those patients who were unwilling or unable to proceed to the second phase of the protocol, thus using all available data as efficiently as possible. In this process, each patient's eye outcome measured was nested within each individual patient.

Crude analysis adjusted for baseline (model 1) did not show any treatment effect on the exophthalmometer readings with a coefficient of -0.27 mm (95% CI -1.43 to +0.89, p=0.648). Adding multilevel modelling correcting for baseline and phase of treatment (model 2) resulted in a treatment coefficient of -0.17 mm (95% CI -0.67 to +0.32), again not statistically significant p=0.490. Carryover adjustment was omitted because of collinearity with the phase of treatment. Adding the assessors to the model did not improve the model with a treatment effect of -0.16 mm (95% CI -0.65 to 0.33, p=0.531) and an assessor coefficient of -0.34 mm (95% CI -0.96 to 0.27, p= 0.274). Removing 3 patients with protocol deviation resulted in a model 2 treatment coefficient of -0.06 mm (95% CI -0.56 to +0.45, p=0.827) and a model 3 treatment coefficient of -0.04 mm (95% CI -0.55 to +0.46, p= 0.861). Using response to 10% drop in IOP as a surrogate marker for compliance showed no statistically significant treatment effect on proptosis as measured on the exophthalmometer (Table 2).

Exophthalmometer change in patients with unilateral proptosis

There were 12 patients with unilateral proptosis. In these patients, only one eye with proptosis was treated whilst the other eye was not treated and served as a control. Analysis of the exophthalmometer reading revealed predicted baseline exophthalmometer differences with a higher exophthalmometer mean in the treated eye of 22.17 mm (95% CI 21.16 to 23.17) versus 20.33 mm (95% CI 19.14 to 21.52) in the untreated eye (p=0.0032). Treatment with Bimatoprost did not result in a statistically significant reduction in exophthalmometer results with a mean change of +0.08 mm (95% CI -0.66 to +0.82) in the treated eye compared to 0.67 mm (95% CI -0.58 to +1.92) in the untreated eye (p=0.1516).

Exophthalmometry and photographic assessment correlations

Proptosis measurements were also made by photographic assessment of the patient photos taken during the trial. The measurements were taken either from the lateral canthus or nasal bridge to the corneal apex by a masked assessor (Figure 3). All data from 5 visits were used for this analysis. Results of the Spearman correlation indicated that there was a significant positive association between exophthalmometer and lateral canthus measurements (Spearman rho 0.609, $p<0.0001$). There was a significant negative correlation between exophthalmometer and nasal bridge measurements (Spearman rho -0.396, $p<0.0001$) (see Figure 4 and Figure 5). The later finding was expected as the measurement was taken from nasal bridge to corneal apex, i.e. the more proptosis, the lesser the distance between the corneal apex to the nasal bridge.

There was no difference between placebo and Bimatoprost with regard to photo measurement results of the lateral canthus to corneal apex distance with placebo (mean change of +1.30 mm; 95% CI -0.74 to +3.35) compared to Bimatoprost +0.98 mm (95% CI -1.25 to +3.20) (p=0.8160). Similarly, there was no difference between placebo and Bimatoprost nasal bridge to corneal apex measurement results with placebo treatment resulting in a mean change of -0.50 mm (95% CI -4.18 to +4.08) compared to Bimatoprost with a mean change of +1.30 mm (95% CI -5.65 to +8.25) (p=0.6870). There was no significant change observed in the subset of patients with unilateral proptosis (n=12) with Bimatoprost treatment resulting in a lateral cantus measurement change of -0.32 mm (95% CI -4.41 to +3.76) versus untreated of +1.09 mm (95% CI -4.07 to +6.26) (p=0.5252). Likewise, Bimatoprost treatment resulting in

a nasal bridge measurement change of +3.10 mm (95% CI -13.53 to +19.73) compared to untreated eye of +6.43 mm (96% CI -3.99 to +16.86) (p=0.6318).

Secondary outcome analysis

In general, patients scored highly on the total visual score using the GO-QOL questionnaire throughout trial visits with a range of mean total visual scores of 79 to 85. With regard to treatment, there was no change in the total visual scores. The change was calculated by subtracting post-treatment score against baseline score. A positive value would indicate an improvement in the quality of life and a change of at least 6 points was considered a minimal clinically important difference. The mean changes for Bimatoprost was 0.8 (95% -7.1 to 8.7) versus placebo -0.6 (95% CI -6.5 to 5.2) (p=0.7930). There was a good negative correlation between the Gorman diplopia score and the total visual score (Spearman's rho -0.5118, p<0.0001). This negative correlation persisted even after removing patients treated with prisms (Spearman rho -0.5111, p<0.0001).

Patients scored lower throughout trial visits with regard to total appearance score with the mean ranging from 52 to 58. No change in total appearance score was seen at 3 months after Bimatoprost treatment with a mean of 0.4 (95% CI -3.6 to 4.5) versus placebo 2.2 (95% CI -5.2 to 9.5) (p=0. 0.6897). There was no correlation between the Gorman diplopia score and the total appearance score (Spearman's rho –0.0785, p=0.3396). This correlation became significant after removing patients treated with prisms, albeit remaining a rather weak association (Spearman rho -0.2282, p<0.0115).

During trial visits, the mean IOP measured in the primary position was within the normal reference range between 16 to 18 mmHg. As expected, Bimatoprost caused a reduction in IOP with a mean change of -2.7 mmHg (95% CI -4.0 to -1.4) compared to placebo with a mean change of 0.3 mmHg (95% CI -1.4 to 2.1) (p=0.007), consistent with compliance with the medication. We found chin forward position did not alter intraocular pressure significantly. There was no difference in NHS health economics consumption between Bimatoprost and placebo period.

Bimatoprost was associated with patient-reported conjunctival hyperemia and headache (Supplementary Table 2). Apart from patient-reported side effects, objective assessments of photographs were also made by an independent masked assessor. Patients treated with Bimatoprost had a higher than placebo detectable skin discoloration, eyelashes elongation, and eyelid redness (Supplementary Table 3). Only 1 patient (3.2%) developed observable periorbital fat atrophy which was the desired effect in this trial (Figure 6). This was a 57-year-old female patient who was a current smoker with 5 years history of GO. She was previously treated with IV steroids, radiotherapy, cyclosporin and Rituximab. The fat atrophy lasted for 2 months following the washout period. In this patient, at baseline the right eye exophthalmometer measurement was 23 mm and the left eye was 24 mm. Following 3 months on Bimatoprost, there was a reduction of 2 mm of the right eye and 1 mm of the left eye. These then returned to baseline following a washout period of 2 months.

DISCUSSION

This is the first clinical trial assessing the effects of $PGF_{2\alpha}$ in stable inactive GO. This trial did not show any clinical benefit of Bimatoprost on reducing proptosis. This finding was confirmed on photographic measurements and despite the effect on IOP and appearance changes (lashes elongation, conjunctival hyperemia and skin changes) suggesting good compliance. The standard deviation was consistent with power calculations suggesting that we were not underpowered and unlikely that the effect was missed. This is in contrast with the *in vitro* findings (20, 21), anecdotal case reports in people without GO (6-8) suggesting adipocyte differentiation inhibition with Bimatoprost. The findings also contrast with the results obtained with Teprotumumab, a human monoclonal antibody inhibitor of IGF-IR shown to reduce proptosis. The success of Teprotumumab might be attributed to the fact it was used in active GO and it targets a different pathophysiological mechanism.

The lack of the effects in the primary analysis might be due to several explanations. We are fully aware that the 2 main mechanisms of GO are adipogenesis and hyaluronan accumulation (22). In the burnt out stage, fibrosis will predominate. The topical eye drops might be absorbed less freely due to the inflammatory/fibrosis process. In the search for stable disease in order to show the effect of $PGF_{2\alpha}$, we might have chosen the wrong stage of the disease which is predominantly caused by hyaluronan deposition or fibrosis rather than adipogenesis. Adipogenesis starts early in the disease and it has been shown that it may continue even in the inactive disease stage (23). $PGF_{2\alpha}$ inhibits adipogenesis per se but does not affect lipolysis and hence has no impact on an already fully mature adipocyte (20). Not all glaucoma patients treated with $PGF_{2\alpha}$ develop periorbital fat atrophy with an estimated incidence of

24.1% (24). Some patients with GO have predominantly fat excess whilst the others have muscle predominant disease (25). This suggests the possibility of a subgroup of subjects that are more susceptible to the effect of Bimatoprost who could be identified by screening using orbital imaging. Perhaps a treatment of 3 months' duration is not long enough to see the intended reduction in proptosis. However, this seems unlikely as there was sufficient time to see fat atrophy. Compliance also might be an issue, although the changes in IOP on treatment suggest this is unlikely, and we did not find a statistically significant treatment effect after adjustment made for compliance using a reduction in IOP as a surrogate marker. Periorbital fat atrophy was observed in 1 subject (3%) of our patient population suggesting that periorbital fat atrophy is different from general fat reduction. Perhaps the periorbital effect seen is mediated via a different mechanism such as activation on matrix metalloproteinases (26).

The assessment of exophthalmos was robust with exophthalmometer and supported with photographic assessments conducted by an assessor who was masked to the treatment phase. In the current study, we had 2 trained assessors. Assessors were assigned to the trial patients at each trial visit according to assessors' availability. To reduce inter-rater variation, our assessors were calibrated by multiple exophthalmometer readings on the same non-trial subjects in the clinic and adjustments were made to ensure their readings were comparable. Photographic measurements also provided further independent confirmation of the exophthalmometer results.

The strengths of this trial include its cross-over design with no period or carry-over effects. There was good patient retention and good compliance as evidenced by the fall in IOP in the treatment phase. The success of the masking process was analyzed

by asking patients and assessors directly and by the independent masked assessor on photographic assessment. Assessors guessed treatment allocation incorrectly in 56.7 % of the patients. Approximately 27% of the patients on placebo thought that the prominence of their eyes improved compared to 43% treated with Bimatoprost. Just above 40% of the patients in both phases preferred the treatment. 43% of subjects in the placebo phase were unsure of treatment allocation and a further 10% guessed incorrectly; 29% in the Bimatoprost phase were unsure and 32% guessed incorrectly when asked about their treatment allocation suggesting that masking was successful.

In summary, Bimatoprost treatment over 3 months in inactive GO does not result in improvements in proptosis and this information should prevent clinicians trialing this approach further and causing side-effects unnecessarily. Future trials should be done on early stage GO and active disease. Periorbital fat atrophy appears to be an idiosyncratic reaction to Bimatoprost rather than a routine event in inactive GO patients. The BIMA study has demonstrated that crossover studies can be performed reliably in patients with persistent proptosis due to thyroid eye disease and that this study design is acceptable to patients. The BIMA study also has shown that over 60% of patients with residual proptosis in thyroid eye disease also have double vision (diplopia). IGF-1R antagonists have shown promise in active disease but still only surgical treatments are available in burnt out disease. Hence, there are still large unmet needs in this patient group.

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Table 1: Baseline demographics of the study population. Data presented as means (standard deviation or range) unless stated otherwise or % (patient number/total).
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Treatment allocations	**All patients**	**Bimatoprost first n=16**	**Placebo first n=15**
Female (%)	83.9 (26/31)	87.5 (14/16)	80.0 (12/15)
Caucasian (%)	93.5 (29/31)	87.5 (14/16)	100 (15/15)
Age (years), mean (range)	55.2 (28-74)	55.2 (31-70)	55.2 (28-74)
BMI (kg/m2)	29.0 (6.5)	28.8 (6.3)	29.2 (7.0)
Symptom duration before diagnosis (months), median (IQR)	4.0 (1-6)	3.5 (2-6.5)	4.0 (1-6)
GO Duration (years), median (IQR)	7.6 (3.6-12.3)	8.8 (3.5-14.4)	7.1 (4.3-12.3)
Smokers at diagnosis (%)	74.2 (23/31)	81.2 (13/16)	66.7 (10/15)
Current smoking (%)	38.7 (12/31)	37.5 (6/16)	40.0 (6/15)
No. cigarettes/week, median (IQR)	70 (10-105)	70 (2-70)	70 (14-140)
FT4 (pmol/L), median (IQR)	15.9 (13.5-17.4)	16.7 (15.9-18.4)	13.9 (12.5-15.2)
TSH (mU/L), median (IQR)	0.87 (0.12-2.6)	0.76 (0.15-1.22)	1.45 (0.12-5.33)
Total Diplopia (%) *Intermittent* *Inconstant (gaze-evoked)* *Constant*	61.3 (19/31) 25.8 (8/31) 16.1 (5/31) 19.4 (6/31)	62.5 (10/16) 18.8 (3/16) 12.5 (2/16) 31.3 (5/16)	60.0 (9/15) 33.3 (5/15) 20.0 (3/15) 6.7 (1/15)
Eyes treated (%) *Both*	61.3 (19/31)	62.5 (10/16)	60.0 (9/15)
Clinical activity score (%) 0 1 2	67.7 (21/31) 19.4 (6/31) 12.9 (4/31)	75.0 (12/16) 18.8 (3/16) 6.3 (1/16)	60.0 (9/15) 20.0 (3/15) 20.0 (3/15)
GO severity *Mild*	31/31	16/16	15/15
Exophthalmometer (mm)	23.6 (2.5)	24.1 (2.9)	23.1 (1.9)
Palpebral aperture (mm)	11.1 (2.0)	11.8 (2.0)	10.4 (1.7)
Previous treatments of GO (%) Selenium Steroid Other immunosuppressant Radiotherapy Decompression Blepharoplasty	 29.0 (9/31) 51.6 (16/31) 22.6 (7/31) 35.5 (11/31) 19.4 (6/31) 35.5 (11/31)	 25.0 (4/16) 37.5 (6/16) 31.3 (5/16) 37.5 (6/16) 18.8 (3/16) 25.0 (4/16)	 33.3 (5/15) 66.7 (10/15) 13.3 (2/15) 33.3 (5/15) 20.0 (3/15) 46.7 (7/15)

The diplopia severity was assessed by Gorman score and GO severity according to EUGOGO criteria. BMI, body mass index; IQR, interquartile range; FT4 (9.0-19.1 pmol/l); TSH (0.30-4.4 mU/l).

Table 2: Beta coefficient of Bimatoprost effect on exophthalmometer readings using multilevel modeling with each treated patient's eye within the patient. Minus protocol deviation indicated 3 patients removed from the analysis due to the stated reason. Minus IOP non-responder indicated removal of eyes from analysis with at least a 10% reduction in intraocular pressure (surrogate marker to compliance).

Model	N	Eye (numbers)	Outcome data points	β coefficient	95% CI	p value
All patients						
Model 1	31	50	96	-0.22	-0.75, 0.32	0.424
Model 2	31	50	96	-0.17	-0.67, 0.32	0.490
Model 3	31	50	96	-0.16	-0.64, 0.33	0.531
Minus protocol deviation						
Model 1	28	46	88	-0.06	-0.60, 0.47	0.814
Model 2	28	46	88	-0.06	-0.56, 0.45	0.827
Model 3	28	46	88	-0.04	-0.55, 0.46	0.861
Minus IOP non-responder (10% IOP drop)						
Model 1	27	46	88	-0.37	-0.94, 0.19	0.192
Model 2	27	46	88	-0.29	-0.81, 0.24	0.283
Model 3	27	46	88	-0.27	-0.78, 0.25	0.313

Model 1 Adjusted for baseline
Model 2 Adjusted for baseline, phase and carryover.
Model 3 Adjusted for baseline phase, carryover and assessors.
N=Number of patients in the model.

Table 3: Patient-reported ocular side effects.

Ocular side effects			
	Bimatoprost n (%)	Placebo n (%)	Fisher exact P value
Conjunctival hyperemia	10 (32.3)	3 (9.7)	0.029
Eye pruritus	4 (12.9)	1 (3.2)	0.177
Eyelid swelling	3 (9.7)	2 (6.5)	0.500
Visual disturbance	2 (6.5)	0	0.245
Meibomian cyst	2 (6.5)	2 (6.5)	0.694
Burning sensation	1 (3.2)	1 (3.2)	0.754
Eye dryness	1 (3.2)	1 (3.2)	0.500
Eyelid pigmentation	1 (3.2)	0	0.500
Conjunctivitis	1 (3.2)	0	0.500
Foreign body sensation	0	1 (3.2)	0.500
Eye pain	0	1 (3.2)	0.500
Ptosis	0	1 (3.2)	0.500
Difficulty eye opening	0	1 (3.2)	0.500

The percentage was calculated from the total number of patients in the trial (N=31).