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Combined immunosuppression & radiotherapy in thyroid eye disease (CIRTED): a multi-centre, factorial randomised controlled trial

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Abstract

Background

Thyroid eye disease is a disabling inflammatory orbital condition causing visual dysfunction and psychological morbidity. The additional benefit of concomitant orbital radiotherapy and antiproliferative immunosuppression is unclear.

Methods

Participants all received a 24 week course of oral prednisolone and were also randomised to receive radiotherapy or sham-radiotherapy, and azathioprine or placebo, in a 2x2 factorial design. The primary outcomes were a binary composite clinical outcome score and opthalmopathy index at 48 weeks and clinical activity score at 12 weeks. (ISRCTN 22471573).

Findings

126 patients were randomized of which 103 (82%) provided outcome data. In those providing data 39 (80)% of these randomised to radiotherapy remained in the study long enough to complete it. Pre-specified intention-to-treat analysis of improvement in the binary clinical composite outcome measure was observed for azathioprine $OR_{(adj)}$=2·56 (95%CI 0·98, 6·66; p=0·05) but not radiotherapy $OR_{(adj)}$=0·89 (95%CI 0·36, 2·23; p=0·80). In a post hoc analysis of patients completing their allocated therapy, improvement was more frequent on azathioprine ($OR_{(adj)}$=6·83; 95%CI 1·66, 28.1; p=0·008 than radiotherapy ($OR_{(adj)}$=0·71; 95%CI 0·26, 1·95; p=0·50). The opthalmopathy index, clinical activity score and also number of adverse events (azathioprine N=161, radiotherapy N=156) did not differ between treament groups.

Interpretation

In patients receiving oral prednisolone for 24 weeks, the addition of radiotherapy was not beneficial. Regarding azathioprine, our conclusions are limited by a high number of withdrawals from treatment. However, these results suggest that disease severity at 48 weeks was reduced in participants who completed azathioprine treatment.

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Research in Context

Active moderate-to-severe thyroid eye disease is currently treated with systemic corticosteroids, but outcomes are often sub-optimal. Corticosteroids are most effective when administered intravenously, but this is inconvenient, and oral administration remains common in global clinical practice. However, uncertainty remains about the additional benefit of orbital radiotherapy and antiproliferative immunosuppressive drugs.

Evidence before this study

Previous retrospective case series have reported that the antiproliferative immunosuppressive drug azathioprine reduces disease severity and the need for rehabilitative surgery, but no prior RCTs have been completed. The evidence base for orbital radiotherapy is stronger, but conflicting, especially in the context of systemic corticosteroid treatment.

Added value of this study

Eighty per cent of subjects completed radiotherapy, but no significant short (12 week) or longterm (48 week) benefit resulted over and above the improvement seen with a 24-week tapering course of oral corticosteroids. Less strong conclusions can be drawn with regard to azathioprine, as many patients did not complete treatment due to abnormalities in monitoring blood tests or side-effects, but those that continued azathioprine for more than 24 weeks benefitted, predominantly due to a prevention of deterioration after the end of corticosteroid treatment.

Implications of all the available evidence

These results do not support the use of radiotherapy in thyroid eye disease in patients also treated with systemic corticosteroids. They also provide evidence in favour of the use of anti-proliferative immunosuppressive agents such as azathioprine beyond the period of corticosteroid therapy to improve long-term clinical outcomes.

Introduction

Active moderate-to-severe thyroid eye disease, also known as Graves' orbitopathy or thyroid associated orbitopathy) occurs in 5-10% of cases of Graves' disease(1). It can be both visually disabling and cosmetically disfiguring and substantially impairs quality of life(1-3). The aim of treatment is to suppress orbital inflammation and reduce consequent tissue re-modelling in extraocular muscles, orbital fat and other periocular soft tissues(4, 5). Immunosuppressive therapies, in particular corticosteroids(1, 4, 6), are the mainstay of treatment for active moderate-to-severe thyroid eye disease (1). However, they are typically withdrawn after 24 weeks of treatment to limit cumulative toxicity regardless of whether they are administered via the oral or intravenous route(7), and given that active disease lasts 1–2 years, recurrence at the time of withdrawal often occurs(1, 7-9).

Consequently, the avoidance of corticosteroid side-effects, improvement in treatment efficacy and maintenance of long-term disease control are major goals for the field of thyroid eye disease as a whole. However, efforts to use monoclonal antibody therapies to more selectively suppress disease are still either early in their route to market(10), or have failed to demonstrate definitive treatment benefit(11, 12). Hence, given the proven short-term efficacy of corticosteroids in the treatment of active moderate-to-severe thyroid eye disease , it is likely that they will remain the gold-standard first-line treatment for several years to come, and the need to find adjunctive therapies to augment and sustain their benefit remains very real.

To date, the only non-corticosteroid conventional immunosuppressant drug to have been evaluated in RCTs is cyclosporine A(13, 14), which was found to be beneficial, but its use has not been widely adopted because of concerns about side-effects(6). An alternative strategy is to use an antiproliferative agent such as azathioprine as it is better tolerated than cyclosporine A(15, 16) and although ineffective as monotherapy(17), retrospective data indicates that in combination with corticosteroids it reduces disease severity and the need for rehabilitative surgery(18). In addition to immunosuppression, non-pharmaceutical treatment

of active thyroid eye disease with orbital radiotherapy has been advocated for decades, and older RCTs demonstrated that this was more effective when used in combination with corticosteroids(19, 20). . However, subsequent studies either questioned the role of orbital radiotherapy or concluded that its benefit was limited to improvement in oculomotility(21-23). This has generated significant controversy, in particular due to concerns about the entry criteria, trial design and radiotherapy administration in Gorman et al's paper(22), which has led to disparity in practice. Orbital radiotherapy has now been largely abandoned in North America, whereas in European centres, including the UK, it is still routinely used(6, 23-25). As it is administered daily over 2-3 weeks and patients are typically of working age, this also has significant implications for the use of healthcare resources and patients' time. Furthermore, only two relatively small studies have evaluated the additional effect of radiotherapy when combined with a high-dose course of systemic corticosteroids(19, 20), and clinical outcomes beyond 24 weeks have rarely been reported for any intervention in thyroid eye disease. We therefore sought to evaluate the long-term benefit of orbital radiotherapy and low-cost antiproliferative immunosuppression with azathioprine in the context of sustained systemic corticosteroid treatment for active moderate-to-severe thyroid eye disease .

Methods

Study design and participants

We undertook this factorial design multicentre RCT in 6 centres in the UK. Patients were recruited to receive either azathioprine or placebo, *plus* either orbital radiotherapy or sham-radiotherapy, in *combination* with a standardised 24-week tapering oral prednisolone regime (**Supplementary Table 1 and Supplementary Figure 1**). In brief, all patients received an initial oral prednisolone dose of 80mg / day, which reduced to 20mg / day by 6 weeks, 10mg / day by 15 weeks and 5mg / day by 21 weeks. In accordance with the factorial design, study recruits were then randomly allocated into 4 groups 2 weeks after starting corticosteroids: azathioprine plus orbital radiotherapy, azathioprine plus sham-radiotherapy, placebo plus orbital radiotherapy, or placebo plus sham-radiotherapy. Full protocol details, including pre-

specified primary and secondary outcome measures and statistical analyses, have been previously peer-reviewed, published and are openly available(26).

Eligible patients had a clinical activity score(27) ≥ 4 (worst eye) OR ≥ 2 (worst eye) with a history of proptosis or motility restriction of less than 6 months duration. They were also required to have a past or present history of abnormal thyroid function or a clinical diagnosis of thyroid eye disease made and confirmed by ≥2 muscle involvement on computed tomography or magnetic resonance imaging scan. The clinical activity score was scored out of 7 at the enrolment visit as its last 3 items (decreasing proptosis, decreasing visual acuity and decreasing eye movement) require a change in consecutive measurements to be calculated. This therefore cannot be done at the first assessment, but at all subsequent visits clinical activity score was scored out of 10. If study recruits *either* had a < 6 month history of thyroid eye disease (defined as time since first symptom) *or* an improvement in any item of clinical activity score 2 weeks after starting the trial prednisolone regime, they were considered to have active disease and were randomised at the second trial visit. Key exclusion criteria included age <20 or >75 years, dysthyroid optic neuropathy, abnormal thiopurine methyltransferase activity and use of radioiodine or any immunomodulatory or cytotoxic drugs within the last 3 months (thyroidectomy was permitted).

Randomisation and masking

Patients were allocated to treatment groups by remote computerised randomization. Minimisation was used to reduce baseline disparities in potential confounding variables between trial interventions. These included smoking status at the time of thyroid eye disease diagnosis, thyroid status on enrolment, previous corticosteroid use, gender, disease severity, study centre, disease duration, age greater than 60 years and disease activity.

Procedures

Orbital radiotherapy

Twenty gray (Gy) of radiation was administered to the retrobulbar orbit in 10-12 fractions over 2 to 3 weeks. Subjects receiving sham-radiotherapy also attended and underwent all the same procedures other than no radiation being delivered. Extensive effort was used across trial centres to ensure participants were unable to identify if they were receiving sham therapy, including use of a noise emitting device to simulate treatment administration(26) (for details of the radiotherapy procedures at each trial centre see **Supplementary Text 2)**

Azathioprine

Treatment dose varied between 100mg and 200mg daily (dispensed as 50 mg tablets), depending on body weight. Matched placebo tablets and packaging were used and the dose was adjusted according to a standard algorithm dependent on patients' blood test results. Again, extensive effort was taken to ensure participants were unaware if they were receiving placebo, including identical blood tests and random placebo dose adjustments. To reduce the risk of serious adverse events, patients with abnormal thiopurine methyltransferase activity who are at increased risk of developing bone marrow suppression (low activity) or hepatotoxicity (high activity) with azathioprine were not enrolled.

Follow-up and withdrawals

Follow-up continued for a minimum of 48 weeks. Withdrawn subjects were returned to their referring ophthalmologist, however they were invited to attend assessment visits at the early (co-primary) and late (primary) outcome measure assessment times of 12 and 48 weeks to obtain data in accordance with the planned intention-to-treat analyses. Withdrawal criteria included worsening of disease (defined as a 2 point increase in clinical activity score or development of optic neuropathy) and sustained blood test abnormalities (leucopenia, lymphopenia or abnormal liver function tests despite dose adjustment of azathioprine or placebo).

Ethical approval and Trial Oversight

The trial protocol was given a favourable opinion by the UK's National Health Service South West Central Bristol Research Ethics Committee (REC reference: 05/Q2006/62). Clinical Trial Authorisation was given by the Medicines and Healthcare products Regulatory Agency (MHRA, reference: 03299/0003/001-0001; ISRCTN22471573) with the University of Bristol acting as the legal sponsor. Research governance and local Research and Development approvals were obtained across all sites prior to the start of recruitment. All participants gave written informed consent.

Outcomes

As the principle objective of the trial was to evaluate treatment success and failure at the late time-point of 48 weeks, our primary outcome measures of disease severity binary clinical composite outcome measure (**BOX 1)** and Ophthalmopathy Index **(Supplementary Table 2**) were selected to quantify the change in ocular deformity and visual dysfunction. An early, 12-week, assessment of disease activity using the clinical activity score score was given lower priority and designated as a co-primary outcome (we expected that all participants would have a significant improvement in clinical activity score by 48 weeks in accordance with the natural history of the disease(28)). Secondary outcome measures included Total Eye Score (**Supplementary Table 3**) as an additional assessment of disease severity, and the patient-reported Graves' Ophthalmopathy Quality of Life score.

Box 1 Calculation of the Binary Clinical Composite Outcome Measure

Major Criteria

- An improvement of ≥ 1 grade in diplopia score
- An improvement of >8 degrees of eye movement in any direction
- A reduction of ≥ 2 mm in proptosis

Minor Criteria

- A reduction of ≥ 2 mm in lid aperture
- An improvement of ≥ 1 grade in soft tissue involvement
- An improvement in best-corrected visual acuity of ≥ 1 line on the Snellen chart
- Subjective improvement

All items refer to the worst eye

Response to treatment is calculated as follows

Improved = improvement in ≥1 major criteria or ≥2 minor criteria

No Change = improvement or deterioration in ≤1 minor criterion

Worse = deterioration in ≥1 major or ≥2 minor criteria (even if other criteria improve)

Statistical analyses

Planned statistical analyses were pre-specified in our protocol paper, based on a sample size of 100 complete datasets at 48 weeks(26). These were undertaken according to CONSORT guidelines for RCTs. As required by the factorial design, the primary intention-to-treat analysis (ITT) combined the treatment groups to compare radiotherapy versus sham-radiotherapy and azathioprine versus placebo for each of the two primary outcomes at 48 weeks follow up. This analysis was made using multivariable regression models, adjusting for minimisation variables, the factorial design, and the value of the outcome variable at baseline. Statistical significance was defined in advance as a p-value of <0.05. Patients who had no outcome data for the primary analyses had data imputed using last observation carried forward if they had data available between 24-48 weeks. Analysis was performed for all primary outcomes (binary clinical composite outcome, Opthalmopathy Index and Clinical Activity Score) Patients who withdrew from treatment due to side-effects, disease progression or personal preference, were encouraged to continue to attend for follow-up assessments and their data included in the intention-to-treat analyses. Since there were a large number of withdrawals from treatment (although most trial subjects still returned for assessment at the primary endpoint visit), a post-hoc as-per-protocol analysis was conducted including only patients who had not withdrawn and continued to receive their assigned treatment. Testing for interaction was performed using likelihood ratio tests. Additional sensitivity analyses were performed for the binary clinical composite outcome measure including recoding those who withdrew due to deterioration, irrespective of their final status at 48 weeks (as they may have received alternative rescue therapy). Secondary patient-reported health economic analyses were planned but not completed due to insufficient data. All statistical analyses were undertaken using STATA version 12 (STATACORP, College Station, TX, USA).

Study Sponsor and role of the funding source

The study sponsor was the University of Bristol. Funding was provided by the UK's National Eye Research Centre and Moorfields Eye Charity supported by infrastructural investment from the National Institute for Health Research. The sponsor and funders had no role in the study design, in the collection, analysis, and interpretation of data, in the writing of the report or in the decision to submit the paper for publication. In addition, the corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Study Population

126 people were recruited and randomised in this study between February 2006 and October 2013 (71 patients from Moorfields Eye Hospital, 34 from Bristol Eye Hospital, 7 from Manchester Eye Hospital, 5 from the Western Eye Hospital, 4 from University College London Hospital, 4 from Gartnavel General Hospital and 1 from the University Hospital of Wales). The flow of study participants is shown in **Figure 1.** Data on both the primary outcomes was provided by 103 participants. Baseline characteristics of the minimisation variables by group are shown in **Table 1.** Individuals allocated to azathioprine had a relatively lower proportion of non-caucasian patients (not a criterion used for minimisation).

Intention-to-treat analysis

Binary Clinical Composite Outcome Measure (primary outcome)

The difference in the binary clinical composite outcome measure between individuals randomised to azathioprine versus placebo tablets was on the threshold of our pre-specified significant p-value of <0.05, but did not meet this (the adjusted odds ratio [OR_{adj}] of the binary clinical composite outcome measure's improvement on azathioprine was 2·56; 95%CI 0·98, 6·66; p=0·05, **Table 2 Figure 2A**). In contrast, there was no improvement with orbital radiotherapy ($OR_{(adj)}$ =0·89, 95%CI 0·36, 2·23, p=0·80). Also with regard to the factorial design, there was no evidence of interaction between azathioprine and radiotherapy (p_{int} = 0·86) and the combination of azathioprine and orbital radiotherapy did not offer additional advantage over azathioprine alone. An overview of the impact on the binary clinical

composite outcome measure of azathioprine and orbital radiotherapy is shown in **Supplementary Figure 2A+2B**. Furthermore, additional sensitivity analyses in which withdrawn patients were coded to unfavourable outcomes regardless of their status at 48 weeks enhanced rather than lessened the improvement observed with azathioprine treatment (OR_{adj} 3·65; 95%CI 1·34, 9·86; p=0·01, **Supplementary Table 4).**

Ophthalmopathy Index (primary outcome)

Analysis of all patients revealed that the ophthalmopathy index fell between week 12 (mean 9.15, SD 0.39) and week 48 (mean 8.43, SD 0.38, p=0.04). No additional benefits were seen with either azathioprine or orbital radiotherapy. Individuals randomised to azathioprine had an adjusted Beta $(B)_{(adj)}$ of 0·46 (95%CI -1·04, 1·95; p=0·55) and in those randomised to orbital radiotherapy $B_{(adj)}$ was -0·89 (95%CI -2·34, 0·56; p=0·23) (**Table 2**). There was also no evidence of an interaction between azathioprine and radiotherapy in their effect on opthalmpathy index (p_{int} = 0·51).

Clinical Activity Score (co-primary outcome)

Across all subjects, substantial improvement in median clinical activity score was seen over the study period from 5 (IQR 4 - 5) at baseline to 3 (IQR 2- 4; p<0·0001) at week 12, and 2 (IQR 1-3; p<0·0001) at week 48 **(Figure 2B, 2C)**. The majority of patients n=97 (70·0%) improved their clinical activity score by week 12 and 96 (98%) of the 98 patients with clinical activity score data at 48 weeks showed improvement in their clinical activity score versus baseline. No difference in the change in clinical activity score at 12 weeks was observed between individuals who received treatment with azathioprine versus not receiving azathioprine, or who received radiotherapy versus sham radiotherapy $B_{(adj)}$= -0·01 (95%CI -0·69, 0·68; p=0·99 – **Table 2**). There was no interaction between azathioprine and radiotherapy in their effect on clinical activity score (p_{int}= 0·48). There was also no evidence that azathioprine or orbital radiotherapy improved clinical activity score score at week 48 (**Supplementary Table 5)**.

Total Eye Score (secondary outcome)

Total eye score improved considerably over the study period with a mean at baseline of 15·1 (95%CI 13·8, 16·3) falling to a mean of 9·36 (95%CI 8·12, 10·6; p <0·0001), but this was not affected by the addition of either azathioprine or orbital radiotherapy (**Supplementary Table 6)**.

Graves Opthalmopathy Quality of Life (secondary outcome)

Across all subjects, mean Graves opthalmopathy quality of life visual function was higher (improved) at 12 weeks than at baseline (71·5 - 95%CI 66·1, 76·9 vs 64·1 - 95%CI 58·5, 70·0; p=0·002), and at week 48 (75·5 - 95%CI 70·3, 80·7; p<0·001 versus baseline). GO-QoL visual appearance was also higher at 12 weeks than at baseline (58·0 - 95%CI 52·5, 63·5 vs 53·2 - 95%CI 47·9, 58·6; p=0·007) and at week 48 (61·3 - 95%CI 55·6, 67·1; p=0·001 versus baseline). Individuals who had an improvement in the binary clinical composite measure at week 48 had a higher Graves opthalmopathy quality of life visual function (B=17·9 - 95%CI 7·07, 28·6; p<0·001) and a higher Graves opthalmopathy quality of life visual appearance ($B_{(adj)=}$11·5 - 95%CI 0·60, 23·6; p=0·06). There was no clear benefit from the addition of either azathioprine or orbital radtiotherapy with regard to long-term Graves opthalmopathy quality of life visual function or visual appearence (**Supplementary Table 7, Supplementary Figure 3**).

As-per-protocol (APP) analysis

Sixty individuals did not withdraw from study treatment before 48 weeks, completed their therapy period as allocated and were included in the APP analysis. Ten of these patients were randomised to azathioprine and sham-radiotherapy, 17 were randomised to orbital radiotherapy and placebo alone, 12 were randomised to azathioprine and orbital radiotherapy and 21 were randomised to sham-radiothearpy and placebo. Individuals in the APP analysis appeared similar at baseline to those who were withdrawn from study treatment, although there was a higher percentage of non-caucasians in those recruited from the larger study centres (**Supplementary Table 8)**.

In the APP analysis, individuals randomised to receive azathioprine (n=22) had a higher odds ratio of improvement in their disease severity measured by the primary binary clinical composite outcome measure at 48 weeks ($OR_{(adj)}$=6·83, 95%CI 1·66, 28·1; p=0·008). No benefit was seen in individuals randomised to receive orbital radiotherapy ($OR_{(adj)}$ 1·32, 95%CI 0·36, 4·84; p=0·67, **Table 3 Figure 2A**). To assess the effect of the duration of exposure to azathioprine we also conducted a comparative analysis of pateints who continued to receive their allocated treatments at 12 weeks (n=84), 24 weeks (n= 79) and 36 weeks (n=68). This indicated that benefit was observed with ≥24 weeks of azathioprine exposure (**Figure 2A, Supplementary Table 9 and Supplementary Figure 2A).** Individuals receiving azathioprine also had a modest improvement in TES ($B_{(adj)}$= -3·23, 95%CI -6·42, 0·03; p=0·05, **Supplementary Table 6**). However, the APP analysis did not reveal any benefit in opthalmopathy index, clinical acitivity score or Graves opthalmopathy quality of life of being randomised to receive either azathioprine or orbital radiotherapy (**Table 3**).

Withdrawals from the study

There was a high number of patients who withdrew from there allocated treatment (n=66, 52·4%) (**Figure 1**), but the majority of these (n=45, 68.2%) returned for primary outcome evaluation. Twenty-five withdrawals were within the first 12 weeks (**Figure 3**). Withdrawals were less in non-caucasians and in participants at two of the study centres (Moorfields and Bristol Eye Hospitals). Before 48 weeks there were 40 withdrawals in those randomised to receive azathiporine and 34 withdrawls in those randomised to receive orbital radiotherapy. Overall, participants randomised to receive azathioprine had increased odds of withdrawal compared to those who did not $OR_{(adj)}$=2·82 (95%CI 1·23, 6·45) p=0·01 (**Supplementary Table 10**). The reasons for withdrawal are presented in **Supplementary Figure 4**. Patients receiving azathioprine had an increased odds of withdrawal due to precautionary blood test abnormalities or side effects OR=9·10 (95%CI 2·60, 31·9) p=0.001 (**Supplementary Table 11**). However, unlike patients receiveing placebo, patients taking azathioprine did not withdraw due to deterioration following cessation of steroid treatment at 24 weeks (**Figure**

3C). No baseline characteristics predicted withdrawal due to either azathioprine or orbital radiotherapy although the highest odds of withdrawal for disease deterioration was in the sham-radiotherapy and placebo group (**Supplementary Table 12**). There was no evidence of bias between treatment groups with regard to failure to provide data at 48 weeks (**Supplementary Table 13** and **Supplementary Table 14**).

Rescue therapy (including surgery) and adverse events

Twenty-one (47%) of the trial subjects who withdrew from study treatment but provided outcome data were documented to have received additional therapy **(Supplementary Table 15)**. In most cases this was additional steroid therapy continuing until the endpoint of the study (week 48). Surgery was however required in 5 individuals, 3 of whom were in the azathioprine group (3 orbital decompressions, 1 lid surgery and 1 strabismus correction). The number of individuals experiencing an adverse event did not differ across the treatment groups (**Supplementary Table 16 and Supplementary Table 17)**.

Discussion

CIRTED fulfilled its target sample size, with more than 100 complete data sets at 48 weeks. Improvement in our primary, co-primary and secondary outcome measures (binary clinical composite outcome measure, clinical activity score and Graves opthalmopathy quality of life) across all groups confirmed the previously reported benefits of high dose systemic corticosteroid therapy in active moderate-to-severe thyroid eye disease (**Figures 2B and 2C).** In this context, orbital radiotherapy did not confer additional patient benefit in any pre-specified outcome measure either in the short (12-week) or longer term (48-week). Radiotherapy was delivered early in the treatment (before 12 weeks), and 80.2% (101 subjects) remained in the study up to this point; hence it is unlikely that this result is significantly confounded by the high withdrawal rate later in the treatment course.

Less strong conclusions can be drawn with regard to azathioprine as comparatively few patients completed the full course of treatment. Nonetheless, the improvement in the binary

clinical composite outcome measure observed in the azathioprine-treated group of subjects that was on the threshold of statistical significance in our intention-to-treat analysis (p=0.05) is likely to be real as the effect was sustained or enhanced in our sensitivity analyses (**Supplementary Table 4, Supplementary Table 9**). This is reinforced by the post-hoc as-per-protocol analysis results which showed substantial benefit in favour of azathioprine ($OR_{(adj)}$=6·83 p=0·008). Of note, patient outcomes improved particularly in those receiving azathioprine for 24 weeks or more (figure 3A). Since steroid therapy was stopped at 24 weeks (as is common practice in thyroid eye disease), this suggests that the key benefit of azathioprine is to prevent relapse after withdrawal of steroids. This observation is consistent with the generally recognsied role of azathioprine as a steroid-sparing agent, used to prevent relapse in other autoimmune conditions and the findings of the MINGO study using an alternative antiproliferative agent (mycophenylate sodium) (REF). Furthermore, this view is supported by analysis of the binary clinical composite outcome measure components indicating that azathioprine did not increase major improvement rates overall but did reduce major deterioration in the binary clinical composite outcome measure (p=0.004, **Supplementary Figure 2A**), plus the observation that late withdrawal (after 24 weeks) due to deterioration was not seen in patients treated with azathioprine (**Figure 3C**).

A major feature of this study was the high rate of withdrawal from patients' allocated treatment. In all study groups, early withdrawals (before 24 weeks) due to disease deterioration were seen as the steroid dose was reduced and this was not mitigated by orbital radiotherapy (**Figure 3C**). Our masked protocol necessarily set strict thresholds for withdrawal due to abnormal monitoring blood tests (white cell counts and liver function), which together with treatment side-effects led to more common withdrawals in those allocated to azathioprine (**Figure 3B**). Hence, it is likely that in usual clinical practice azathioprine treatment would be continued in a higher percentage of patients. Importantly, many of those withdrawing from treatment still completed their study follow-up visits until the primary endpoint (48 weeks), resulting in the outcomes for over 80% of randomised subjects being available for our intention-to-treat analysis.

The other key methodological point to consider is our use of two primary outcome measures at 48 weeks. As we have previously published(26), this was because of the lack of fully validated long-term disease severity measures in thyroid eye disease. We also wished to mitigate the theoretical limitations of composite binary scoring systems, in particular with regard to baseline variability between treatment groups, by using a continuous variable with regression analyses in mind. However, our minimisation strategy was successful in balancing baseline features across trial arms and the binary clinical composite outcome measure has since become the preferred end-point for thyroid eye disease studies as it is more sensitive to change(21, 23). We have therefore focused on this rather than the opthalmopathy index which has not been a primary endpoint in other recent trials.

The key strengths of this RCT include the use of minimisation, low rates of loss-to-follow-up (including of withdrawn patients) and the extensive efforts that were made to mask both azathioprine and radiotherapy treatment allocation to both the patients and clinicians (including the use of sham radiotherapy). In addition, we observed no evidence of interaction between the two interventions (radiotherapy and azathioprine), which is supportive of our choice of a factorial design. Conversely, a major limitation of our study was the high withdrawal rate, particularly for those randomised to receive azathioprine. Therefore our conclusions with regard to the efficacy of this treatment need to be interpreted with caution. We also permitted patients to enrol in the trial and start systemic corticosteroid therapy before their thyroid function tests were normalised. This potentially confounds the interpretation of our data with the benefit of returning to euthyroidism, but we judged intervening with immunosuppression in the early active phase of disease to outweigh this risk. Furthermore, given that demonstration of clinical improvement following a 2 week course of high-dose oral steroids was a key entry criterion, our results cannot be extrapolated to infer the value of radiotherapy or azathioprine in patients with steroid refractory disease. Oral steroid therapy was used in this study and given to all study participants as this was the

standard of care in the study centres at the time of trial initiation and remains commonly prescibed in many regions including North America(29).

In summary, our results suggest that low-dose orbital radiotherapy confers no additional short or long-term treatment benefit when combined with a six-month reducing course of oral corticosteroids. Our findings with regard to azathioprine are less definitive, but taken together indicate that, if tolerated, azathioprine improves 48-week clinical outcomes in patients with active moderate-to-severe thyroid eye disease. This supports the use of long-term antiproliferative treatments in combination with systemic corticosteroids for the treatment of active moderate-to-severe thyroid eye disease, consistent with established practice in other autoimmune conditions.

Table and figure headings

Table 1	Characteristics of the 4 trial groups
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Table 3	As per protocol analysis Binary Composite Clinical Outcome Measure, Ophthalmopathy Index and Change in Clinical Activity Score
Figure 1	Consort Diagram
Figure 2A	Odds ratio of having an improved Binary Composite Clinical Outcome Measure score by treatment and duration in study
Figure 2B	Boxplot of Clinical Acitvity Score at baseline, week 12 and week 48 by whether a participant was randomised to azathioprine
Figure 2C	Boxplot of Clinical Activity Score at baseline, week 12 and week 48 by whether a participant was randomised to radiotherapy
Figure 3A	Kaplan Meier survival showing withdrawals from treatment (all reasons)
Figure 3B	Kaplan Meier survival showing withdrawals from treatment (side effects and abnormal blood results)
Figure 3C	Kaplan Meier survival showing withdrawals from treatment (deterioration)

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Declaration of Interest

The authors report no declarations of interest

References

1. Bartalena L, Pinchera A, Marcocci C. Management of Graves' ophthalmopathy: reality and perspectives. Endocr Rev. 2000;21(2):168-99.
2. Wiersinga WM. Advances in treatment of active, moderate-to-severe Graves' ophthalmopathy. The lancet Diabetes & endocrinology. 2017;5(2):134-42.
3. Terwee C, Wakelkamp I, Tan S, Dekker F, Prummel MF, Wiersinga W. Long-term effects of Graves' ophthalmopathy on health-related quality of life. European journal of endocrinology / European Federation of Endocrine Societies. 2002;146(6):751-7.
4. Wiersinga WM. Advances in treatment of active, moderate-to-severe Graves' ophthalmopathy. The Lancet Diabetes & Endocrinology.
5. Salvi M, Campi I. Medical Treatment of Graves' Orbitopathy. Horm Metab Res. 2015;47(10):779-88.
6. Bartalena L, Baldeschi L, Boboridis K, Eckstein A, Kahaly GJ, Marcocci C, et al. The 2016 European Thyroid Association/European Group on Graves' Orbitopathy Guidelines for the Management of Graves' Orbitopathy. Eur Thyroid J. 2016;5(1):9-26.
7. Marcocci C, Bartalena L, Tanda ML, Manetti L, Dell'Unto E, Rocchi R, et al. Comparison of the effectiveness and tolerability of intravenous or oral glucocorticoids associated with orbital radiotherapy in the management of severe Graves' ophthalmopathy: results of a prospective, single-blind, randomized study. J Clin Endocrinol Metab. 2001;86(8):3562-7.
8. Kahaly GJ, Pitz S, Hommel G, Dittmar M. Randomized, single blind trial of intravenous versus oral steroid monotherapy in Graves' orbitopathy. J Clin Endocrinol Metab. 2005;90(9):5234-40.
9. Bartalena L, Krassas GE, Wiersinga W, Marcocci C, Salvi M, Daumerie C, et al. Efficacy and safety of three different cumulative doses of intravenous methylprednisolone for moderate to severe and active Graves' orbitopathy. J Clin Endocrinol Metab. 2012;97(12):4454-63.
10. Smith TJ, Kahaly GJ, Ezra DG, Fleming JC, Dailey RA, Tang RA, et al. Teprotumumab for Thyroid-Associated Ophthalmopathy. N Engl J Med. 2017;376(18):1748-61.
11. Stan MN, Garrity JA, Carranza Leon BG, Prabin T, Bradley EA, Bahn RS. Randomized controlled trial of rituximab in patients with Graves' orbitopathy. J Clin Endocrinol Metab. 2015;100(2):432-41.
12. Salvi M, Vannucchi G, Curro N, Campi I, Covelli D, Dazzi D, et al. Efficacy of B-cell targeted therapy with rituximab in patients with active moderate to severe Graves' orbitopathy: a randomized controlled study. J Clin Endocrinol Metab. 2015;100(2):422-31.
13. Kahaly G, Schrezenmeir J, Krause U, Schweikert B, Meuer S, Muller W, et al. Ciclosporin and prednisone v. prednisone in treatment of Graves' ophthalmopathy: a

controlled, randomized and prospective study. European journal of clinical investigation. 1986;16(5):415-22.
14. Prummel MF, Mourits MP, Berghout A, Krenning EP, van der Gaag R, Koornneef L, et al. Prednisone and cyclosporine in the treatment of severe Graves' ophthalmopathy. N Engl J Med. 1989;321(20):1353-9.
15. Ahern MJ, Harrison W, Hollingsworth P, Bradley J, Laing B, Bayliss C. A randomised double-blind trial of cyclosporin and azathioprine in refractory rheumatoid arthritis. Australian and New Zealand journal of medicine. 1991;21(6):844-9.
16. MacPhee IA, Bradley JA, Briggs JD, Junor BJ, MacPherson SG, McMillan MA, et al. Long-term outcome of a prospective randomized trial of conversion from cyclosporine to azathioprine treatment one year after renal transplantation. Transplantation. 1998;66(9):1186-92.
17. Perros P, Weightman DR, Crombie AL, Kendall-Taylor P. Azathioprine in the treatment of thyroid-associated ophthalmopathy. Acta endocrinologica. 1990;122(1):8-12.
18. Claridge KG, Ghabrial R, Davis G, Tomlinson M, Goodman S, Harrad RA, et al. Combined radiotherapy and medical immunosuppression in the management of thyroid eye disease. Eye. 1997;11:717-22.
19. Bartalena L, Marcocci C, Chiovato L, Laddaga M, Lepri G, Andreani D, et al. Orbital cobalt irradiation combined with systemic corticosteroids for Graves' ophthalmopathy: comparison with systemic corticosteroids alone. J Clin Endocrinol Metab. 1983;56(6):1139-44.
20. Ng CM, Yuen HK, Choi KL, Chan MK, Yuen KT, Ng YW, et al. Combined orbital irradiation and systemic steroids compared with systemic steroids alone in the management of moderate-to-severe Graves' ophthalmopathy: a preliminary study. Hong Kong medical journal = Xianggang yi xue za zhi. 2005;11(5):322-30.
21. Mourits MP, van Kempen-Harteveld ML, Garcia MB, Koppeschaar HP, Tick L, Terwee CB. Radiotherapy for Graves' orbitopathy: randomised placebo-controlled study. Lancet. 2000;355(9214):1505-9.
22. Gorman CA, Garrity JA, Fatourechi V, Bahn RS, Petersen IA, Stafford SL, et al. A prospective, randomized, double-blind, placebo-controlled study of orbital radiotherapy for Graves' ophthalmopathy. Ophthalmology. 2001;108(9):1523-34.
23. Prummel MF, Terwee CB, Gerding MN, Baldeschi L, Mourits MP, Blank L, et al. A randomized controlled trial of orbital radiotherapy versus sham irradiation in patients with mild Graves' ophthalmopathy. J Clin Endocrinol Metab. 2004;89(1):15-20.
24. Bartalena L, Marcocci C, Pinchera A. Orbital radiotherapy for Graves' ophthalmopathy. J Clin Endocrinol Metab. 2004;89(1):13-4.
25. Rajendram R, Bunce C, Lee RW, Morley AM. Orbital radiotherapy for adult thyroid eye disease. Cochrane database of systematic reviews (Online). 2012(7):Cd007114.
26. Rajendram R, Lee RWJ, Potts MJ, Rose GE, Jain R, Olver JM, et al. Protocol for the combined immunosuppression & radiotherapy in thyroid eye disease (CIRTED) trial: A multi-centre, double-masked, factorial randomised controlled trial. Trials. 2008;9:6-.

608 27. Mourits MP, Prummel MF, Wiersinga WM, Koornneef L. Clinical activity score as a
609 guide in the management of patients with Graves' ophthalmopathy. Clin Endocrinol (Oxf).
610 1997;47(1):9-14.
611 28. Rundle FF, Wilson CW. Development and course of exophthalmos and
612 ophthalmoplegia in Graves' disease with special reference to the effect of thyroidectomy.
613 Clinical science. 1945;5(3-4):177-94.
614 29. Perumal B, Meyer DR. Treatment of severe thyroid eye disease: a survey of the
615 American Society of Ophthalmic Plastic and Reconstructive Surgery (ASOPRS). Ophthalmic
616 plastic and reconstructive surgery. 2015;31(2):127-31.
617