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Abstract: Background

Thyroid eye disease is a disabling inflammatory orbital condition which causes visual dysfunction and psychological morbidity. Standard treatment is with systemic corticosteroids, but the additional benefit of 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 ophthalmopathy index at 48 weeks and clinical activity score at 12 weeks. (ISRCTN 22471573).

Findings

126 adults with active moderate-to-severe thyroid eye disease were randomised. 103 provided outcome data, of which 84 completed their allocated treatment of radiotherapy or sham-radiotherapy, and 57 continued to take azathioprine or placebo until 48 weeks. Pre-specified intention-to-treat analysis of the binary clinical composite outcome measure revealed an odds of improvement for azathioprine of OR(adj)=2·56 (95%CI 0·98, 6·66; p=0·05) and for radiotherapy of 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 ophthalmopathy index, clinical activity score and number of adverse events (azathioprine N=161, radiotherapy N=156) did not differ between treatment groups.

Interpretation
In patients receiving oral prednisolone for 24 weeks, the addition of radiotherapy was not beneficial. With regard to 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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National Eye Research Centre, Above and Beyond and Moorfields Eye Charity, supported by infrastructural investment from the National Institute for Health Research
Combined immunosuppression & radiotherapy in thyroid eye disease (CIRTED): a multi-centre, factorial randomised controlled trial

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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 long-term (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 remodelling 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 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 aged 20-75 years 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). Trial registration was assigned retrospectively on 1 February 2006
(ISRCTN22471573) following regulatory permissions, but prior to starting recruitment.

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’ eligibility for the study was assessed by the ophthalmic investigators at each trial
centre. Allocation to treatment groups was by remote computerised randomisation and
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 enrollment, previous corticosteroid use, gender, disease
severity, study centre, disease duration, age greater than 60 years and disease activity.
Patients, clinicians (both ophthalmic and endocrine) and data analysts were all masked. Only
the trial co-ordinators (who monitored trial subjects blood results), pharmacists and
radiographers were unmasked. The success of masking for ophthalmic investigators and patients was assessed at study completion or withdrawal by asking them to declare which treatments they thought had been administered.

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, and the conduct of the trial was subject to independent Data Safety Monitoring Committee and Trial Steering Committee review for the duration of the study.

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 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

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)
(Supplementary Table 3) as an additional assessment of disease severity, patient-reported Graves’ Ophthalmopathy Quality of Life score and health economic indices.

**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, ophthalmopathy 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). The secondary outcome measures of Total Eye Score and Graves ophthalmopathy quality-of-life score were also compared across treatment groups, however patient-reported health economic analyses were 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, Above and Beyond 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 (PNT RH CMD and RL had access to the raw data).

Results

Study Population

126 people were recruited and randomised 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 at 48 weeks was provided by 103 participants, and these were analysed after data-lock (which included separate 3 year assessments on a minority of trial subjects) on 7th October 2016. 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 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 ophthalmopathy 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 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; $p < 0.001$), but this was not affected by the addition of either azathioprine or orbital radiotherapy (Supplementary Table 6).

Graves Ophthalmopathy Quality of Life (secondary outcome)

Across all subjects, mean Graves ophthalmopathy 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). Graves ophthalmopathy quality of life 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 ophthalmopathy quality of life visual function ($B = 17.9 - 95\%$ CI 7.07, 28.6; $p < 0.001$) and a higher Graves ophthalmopathy 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 radiotherapy with regard to long-term Graves ophthalmopathy quality of life visual function or visual appearance (Supplementary Table 7, Supplementary Figure 3).

As-per-protocol analysis

Sixty individuals did not withdraw from study treatment before 48 weeks, completed their therapy period as allocated and were included in the as-per-protocol 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-radiotherapy and placebo. Individuals in the as-per-protocol 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 as-per-protocol 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 patients 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 total eye score (B(adj)= -3·23, 95%CI -6·42, 0·03; p=0·05, Supplementary Table 6). However, the as-per-protocol analysis did not reveal any benefit in ophthalmopathy index, clinical activity score or Graves ophthalmopathy 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 their 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 azathioprine and 34 withdrawals in those randomised to receive orbital radiotherapy. Overall, 103 participants provided outcome data, of which 84 completed their allocated treatment of radiotherapy or sham-radiotherapy, and 57 continued to take azathioprine or
placebo until 48 weeks. 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 receiving 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 (azathioprine N=161, radiotherapy N=156). (Supplementary Table 16 and Supplementary Table 17).

Masking
Of the 69 patients and 71 doctors who recorded their perceived trial allocation for azathioprine or placebo on study completion or withdrawal, 30 patients (43%) and 29 doctors (41%) were incorrect. For radiotherapy and sham-radiotherapy, of the 70 patients and 67 doctors, 23 patients (33%) and 33 doctors (49%) were incorrect.
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 ophthalmopathy quality-of-life score) 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); 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 recognised role of azathioprine as a steroid-sparing agent, used to prevent relapse in other autoimmune conditions, and this is further reinforced by the findings of the MINGO study using an alternative antiproliferative agent (mycophenolate sodium) in thyroid eye disease. 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 ophthalmopathy 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 success of our extensive efforts 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 corticosteroid 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 prescribed in many regions of the world 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.
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Table 2  Intention to treat analysis Binary Composite Clinical Outcome Measure, Ophthalmopathy Index and Change in Clinical Activity Score
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 Activity 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
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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)
The authors wish to thank all the participants involved in this study. We would also like to thank the Trial Steering Committee comprised of Professor Maarten Mourits, Professor John Lazarus, Professor John Sparrow; and the Data Monitoring Committee: Dr Roberto Melotti, Dr Irene Stratton, Professor John Forrester, Miss Gill Adams. The study was designed with the help of Dr Alan Montgomery (Department of Community Based Medicine, University of Bristol) and Dr Rosemary Greenwood (Research and Development Support Unit, United Bristol Healthcare Trust) who gave statistical support and advice on trial design. Research oversight was led on behalf of the Sponsor by Dr Birgit Whitman, Head of Research Governance, University of Bristol. This study was also supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, UK. The views expressed are those of the authors and not necessarily those of the National Health Service, the NIHR or the Department of Health.

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University Hospital of Wales: Pranav Kumar, Anna Scholz and Praneet Bolusani

Gartnavel General Hospital: Maria Elena Gregory and Anna Tarantini
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Analysis and interpretation of data: Taylor, Hills, Dayan, Lee, Li, Bunce, Rajendram, Uddin, Jackson

Drafting of the Manuscript: Taylor, Dayan, Lee, Rajendram, Wilson

Critical revision of the manuscript: Lee, Dayan, Taylor, Rajendram, Uddin, Wilson

All authors approved the final manuscript.

Declaration of Interest

The authors report no declarations of interest
References


Table 1 Characteristics of the Four Trial Groups

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<th>Sham + Aza</th>
<th>RT + Placebo</th>
<th>Sham + Placebo</th>
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<td>32</td>
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<td>51·4 (9·53)</td>
<td>46·1 (11·5)</td>
<td>49·2 (11·7)</td>
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<td>12·9</td>
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<td>15·6</td>
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<td>Disease Duration (months)</td>
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<td>5·01 (4·39)</td>
<td>5·50 (9·41)</td>
<td>7·29 (12·6)</td>
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<tr>
<td>Duration &gt; 6 months</td>
<td>35·5</td>
<td>22·6</td>
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<td>40·6</td>
<td>34·4</td>
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<td>% Smoker</td>
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<td>48·4</td>
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<tr>
<td>Previous steroid use (%</td>
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<td>6·5</td>
<td>12·5</td>
<td>15·6</td>
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<tr>
<td>Study Centre (%)</td>
<td>83·9</td>
<td>83·9</td>
<td>81·3</td>
<td>84·4</td>
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<td>Moorfields or Bristol vs the other centres)</td>
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<td>CAS Score</td>
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<td>2-3</td>
<td>17·9</td>
<td>16·7</td>
<td>24·1</td>
<td>9·4</td>
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<tr>
<td>4-5</td>
<td>53·6</td>
<td>70·0</td>
<td>55·2</td>
<td>68·9</td>
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<td>6-7</td>
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<td>TES Score</td>
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<td>&lt;22</td>
<td>77·4</td>
<td>83·3</td>
<td>81·3</td>
<td>90·6</td>
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<tr>
<td>&gt;22</td>
<td>22·5</td>
<td>16·7</td>
<td>18·8</td>
<td>9·4</td>
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</table>

Aza = Azathioprine, RT = Radiotherapy
CAS = Clinical Activity Score TES = Thyroid Eye Score
### Table 2 Intention to treat analysis Binary Composite Clinical Outcome, Ophthalmopathy Index and Change in Clinical Activity Score

<table>
<thead>
<tr>
<th>Outcome</th>
<th>OR</th>
<th>B</th>
<th>95% CI</th>
<th>P</th>
<th>OR*</th>
<th>B*</th>
<th>95% CI*</th>
<th>P*</th>
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</thead>
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<tr>
<td>Aza Binary Clinical Composite Outcome (N=103)</td>
<td>1·99</td>
<td>-</td>
<td>(0·88, 4·51)</td>
<td>0·10</td>
<td>2·56</td>
<td>0·98, 6·66</td>
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<td>RT</td>
<td>1·07</td>
<td>-</td>
<td>(0·47, 2·39)</td>
<td>0·87</td>
<td>0·89</td>
<td>0·36, 2·23</td>
<td>0·80</td>
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<tr>
<td>AzaRT (N=107)</td>
<td>2·16</td>
<td>-</td>
<td>(0·85, 5·47)</td>
<td>0·11</td>
<td>2·52</td>
<td>0·87, 7·29</td>
<td>0·09</td>
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<tr>
<td>Aza Ophthalmopathy Index</td>
<td>-</td>
<td>0·50</td>
<td>(-1·00, 2·00)</td>
<td>0·51</td>
<td>-</td>
<td>0·46, -1·04, 1·95</td>
<td>0·55</td>
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<tr>
<td>RT</td>
<td>-</td>
<td>-0·41</td>
<td>(-1·91, 1·09)</td>
<td>0·59</td>
<td>-</td>
<td>-0·89, -2·34, 0·56</td>
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<td>AzaRT (N=109)</td>
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<td>-0·43</td>
<td>(-2·21, 1·35)</td>
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<td>-</td>
<td>-0·78, -2·52, 0·96</td>
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<tr>
<td>Aza Change in Clinical Activity Score (N=107)</td>
<td>-</td>
<td>-0·48</td>
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<td>-</td>
<td>-0·54, -1·25, 0·16</td>
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<td>RT</td>
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<td>(-0·78, 0·62)</td>
<td>0·82</td>
<td>-</td>
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<tr>
<td>AzaRT (N=107)</td>
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<td>-0·71</td>
<td>(-1·52, 0·10)</td>
<td>0·09</td>
<td>-</td>
<td>-0·64, -1·46, 0·18</td>
<td>0·13</td>
<td></td>
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</tbody>
</table>

* Adjusted for age group, ethnicity, smoking status, gender, thyroid state, disease duration, study centre, recent steroid use, baseline CAS score, baseline TES score. For Azathioprine and Radiotherapy, this analysis is also adjusted for the other treatment option (but not the combined Azathioprine-Radiotherapy group)

OR= Odds ratio, B = Beta Coefficient
95% CI = 95% confidence interval
p = p value against the null hypothesis of no association
Aza = Randomised to Azathioprine, RT = Randomised to Radiotherapy,
AZART = Randomised to Azathioprine and Radiotherapy

1 6 individuals with last data carried forward
2 8 individuals with last data carried forward
3 10 individuals with last data carried forward

†Absolute values 22/50 patients who received azathioprine improved versus 16/54 who did not receive azathioprine. 19/50 patients who received orbital radiotherapy improved vs 19/54 who did not receive orbital radiotherapy.
Table 3 As per protocol analysis Binary Composite Clinical Outcome, Ophthalmopathy Index and Change in Clinical Activity Score

<table>
<thead>
<tr>
<th>Outcome</th>
<th>OR/B</th>
<th>95% CI</th>
<th>P</th>
<th>OR/B*#</th>
<th>95% CI*</th>
<th>P*</th>
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</thead>
<tbody>
<tr>
<td>Aza</td>
<td>5.21</td>
<td>(1.62, 16.8)</td>
<td>0.006</td>
<td>6.83</td>
<td>(1.66, 28.1)</td>
<td>0.008</td>
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<tr>
<td>RT</td>
<td>1.40</td>
<td>(0.53, 4.21)</td>
<td>0.45</td>
<td>1.32</td>
<td>(0.36, 4.84)</td>
<td>0.67</td>
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<tr>
<td>AzaRT</td>
<td>7.24</td>
<td>(1.40, 37.4)</td>
<td>0.02</td>
<td>16.1</td>
<td>(2.03, 127.6)</td>
<td>0.009</td>
</tr>
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<td>Aza</td>
<td>-0.16</td>
<td>(-2.12, 1.80)</td>
<td>0.87</td>
<td>-0.85</td>
<td>(-2.65, 0.95)</td>
<td>0.35</td>
</tr>
<tr>
<td>RT</td>
<td>-0.20</td>
<td>(-2.10, 1.69)</td>
<td>0.83</td>
<td>-0.79</td>
<td>(-2.52, 0.94)</td>
<td>0.36</td>
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<tr>
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<td>(-3.66, 0.99)</td>
<td>0.26</td>
<td>-2.02</td>
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<td>Aza</td>
<td>-0.63</td>
<td>(-1.37, 0.12)</td>
<td>0.10</td>
<td>-0.54</td>
<td>(-1.29, 0.20)</td>
<td>0.15</td>
</tr>
<tr>
<td>RT</td>
<td>-0.03</td>
<td>(-0.79, 0.73)</td>
<td>0.94</td>
<td>0.10</td>
<td>(-0.66, 0.85)</td>
<td>0.80</td>
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<tr>
<td>AzaRT</td>
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<td>(-1.73, 0.002)</td>
<td>0.05</td>
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<td>(-1.55, 0.22)</td>
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</table>

** Adjusted for age group, ethnicity, smoking status, gender, thyroid state, disease duration, study centre, recent steroid use, baseline CAS score, baseline TES score. For Azathioprine and Radiotherapy, this analysis is also adjusted for the other treatment option (but not the combined Azathioprine-Radiotherapy group)

OR= Odds ratio, B = Beta Coefficient
95% CI = 95% confidence interval
p = p value against the null hypothesis of no association
Aza = Randomized to Azathioprine, RT = Randomized to Radiotherapy,
AZART = Randomized to Azathioprine and Radiotherapy

†Absolute Values 15/21 patients who completed protocol on azathioprine improved vs 12/37 who did not receive azathioprine
14/27 patients who completed protocol on orbital radiotherapy improved vs 13/31 who did not receive orbital radiotherapy
131 patients ineligible
- 69 - Patient is not interested in participating in research
- 20 - Not active enough
- 15 - Miscellaneous
- 6 - Abnormal TPMT
- 6 - Optic neuropathy
- 3 - Pregnant, or planning pregnancy
- 2 - Abnormal liver function tests
- 2 - Recent steroid use
- 1 - Waiting corneal graft
- 1 - Not GO
- 1 - HIV+
- 1 - TB
- 1 - Patient does not want to take steroids
- 1 - Endocrinologist wants to start radioiodine
- 1 - History of carcinoma
- 1 - Has had radio iodine within last 3 months

41 patients consented but ineligible to proceed with randomisation
- 19 - Abnormal blood test results
- 10 - Lack of efficacy of treatment
- 5 - Patient is not interested in participating in research
- 3 - Not active enough clinically
- 2 - Contraindication to prednisolone
- 1 - Excluded due to Amblyopia
- 1 - GP recommended patient should not be in the study

167 enrolled

126 patients randomized

Radiotherapy and Azathioprine (N=31)
- 20† withdrew
- 11 remained

Sham Radiotherapy and Azathioprine (N=31)
- 21†† withdrew
- 10 remained

Radiotherapy and Placebo (N=32)
- 16† withdrew
- 16 remained

Sham Radiotherapy and Placebo (N=32)
- 12†† withdrew
- 20 remained

16 provided FU data
10 provided FU data
7 provided FU data

103 patients provided data on both primary endpoints at week 48

† 1 patient provided data for OI but not BCCOM
†† 2 patients provided data for OI but not BCCOM
Figure 2A Odds ratio of having an improved BCCOM score by treatment and duration in study

Figure 2A
Figure 2B Boxplot of CAS at baseline, week 12 and week 48 by whether a participant was randomised to azathioprine

Did not receive Azathioprine  
Received Azathioprine

CAS Baseline  CAS Week 12  CAS Week 48
Figure 2C Boxplot of CAS at baseline, week 12 and week 48 by whether a participant was randomised to radiotherapy.

Did not receive Radiotherapy

Received Radiotherapy

- CAS Baseline
- CAS Week 12
- CAS Week 48

Statistical significance:
- Did not receive Radiotherapy: (p<0.0001)
- Received Radiotherapy: (p<0.0001)

Annotations:
- (p<0.0001)
- (p=0.001)
- (p<0.0001)
- (p=0.02)
Figure 3A Kaplan Meier showing withdrawals from treatment (all reasons)

Number at risk

<table>
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<tr>
<th>Group</th>
<th>Week 0</th>
<th>Week 0.25</th>
<th>Week 0.50</th>
<th>Week 1.00</th>
<th>Week 1.25</th>
<th>Week 2.00</th>
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<td>RT and AZA</td>
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<td>20</td>
<td>15</td>
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<td>23</td>
<td>23</td>
<td>20</td>
<td>16</td>
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</tr>
<tr>
<td>Sham and AZA</td>
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<td>22</td>
<td>15</td>
<td>12</td>
<td>10</td>
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<tr>
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<td>29</td>
<td>26</td>
<td>23</td>
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Proportion completed

Withdrawal Overall

Time following randomisation (weeks)
Figure 3B Kaplan Meier showing withdrawals from treatment (side effects and abnormal blood test results)

Withdrawal Side Effects

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Time following randomisation (weeks)

Number at risk

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<tr>
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<th>24</th>
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<td>23</td>
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<tr>
<td>Sham and Placebo</td>
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<td>29</td>
<td>26</td>
<td>23</td>
<td>21</td>
</tr>
</tbody>
</table>

Legend:
- RT and AZA
- RT and Placebo
- Sham and AZA
- Sham and Placebo
Figure 3C Kaplan Meier showing withdrawals from treatment (deterioration)

Number at risk

- RT and AZA: 31, 23, 20, 15, 13
- RT and Placebo: 32, 23, 23, 20, 16
- Sham and AZA: 31, 22, 15, 12, 10
- Sham and Placebo: 32, 29, 26, 23, 21

Proportion completed

Time following randomisation (weeks)
Supplementary Text

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

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Abstract

Background
Thyroid eye disease (TED) is a disabling inflammatory orbital condition which causes visual dysfunction and psychological morbidity. Current evidence suggests standard treatment is conflicting about the benefit of systemic corticosteroids, but the additional benefit of orbital radiotherapy and antiprofiterative immunosuppression in addition to systemic corticosteroid treatment. The aim of this study was to evaluate the efficacy of orbital radiotherapy (RT) and the antiprofiterative drug azathioprine (AZA) in combination with oral corticosteroids for patients with active moderate to severe TED is unclear.

Methods
Participants all received a standardised tapering 6 month 24 week course of oral prednisolone and were also randomly allocated to receive RT, or sham-RT, and AZAazathioprine or placebo, in a 2x2 factorial design using minimisation to reduce baseline discrepancies between treatment groups. The primary outcome measure was improvement in disease severity assessed without outcomes were a binary composite clinical outcome score and ophthalmopathy index at 48 weeks. This study reports the main trial analysis using both intention to treat and as per protocol analyses and clinical activity score at 12 weeks. (ISRCTN 22471573).

Findings
126 subjects with active moderate to severe thyroid eye disease were randomised and complete primary 103 provided outcome data were available in 101 (80%). Sixty six (52%) withdrew from their assigned treatment, of which 84 completed their allocated treatment allocation beyond the period of of radiotherapy/ or sham-radiotherapy but before the primary end point (61% in AZA, 40% in RT). Withdrawal due to abnormal blood tests or side effects was more frequent with AZA (adjusted odds ratio ORadj=0.10; 95%CI 2.60, 21.9; p=0.001). In an exploratory analysis of patients completing their allocated therapy, improvement was more frequent on AZAazathioprine (ORadj=2.56; 95%CI 0.98, 6.66; p=0.05) for AZA and for radiotherapy of ORadj=0.89 (95%CI 0.36, 2.23; p=0.80 for RT). In a post hoc analysis of patients completing their allocated therapy, improvement was more frequent on AZAazathioprine (ORadj=6.83; 95%CI 1.66, 28.1; p=0.008) than RT (ORadj=1.320.71; 95%CI 0.46, 8.42; 1.95; p=0.675—50). The ophthalmopathy index, clinical activity score and number of adverse events (azathioprine N=161, radiotherapy N=156) did not differ between treatment groups.

Interpretation
In patients receiving a 24 week course of oral prednisolone, no additional treatment benefit for 24 weeks, the addition of radiotherapy was seen with RT not beneficial. With regard to AZAazathioprine, our conclusions are limited by a high number of withdrawals from treatment. However, our results suggest that patients who completed AZA therapy had a
significant reduction in their disease severity at 48 weeks was reduced in participants who completed azathioprine treatment.

Funding

The National Eye Research Centre, Above and Beyond, and Moorfields Eye Charity, supported by infrastructural investment to the host institutions from the National Institute for Health Research.
Moderate-to-severe thyroid eye disease is currently treated with systemic corticosteroids, but outcomes are often suboptimal. Corticosteroids are most effective when administered intravenously, but oral administration remains common in global clinical practice. 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 long-term (48 week) benefit resulted over and above the improvement seen with a 24-week tapering course of oral corticosteroids. Many patients did not complete the high-dose antiproliferative drug 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 the steroid course.

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 provide evidence in favour of the use of antiproliferative immunosuppressive agents such as AZA beyond the period of steroid therapy to improve long-term outcomes.
Introduction

Active moderate-to-severe thyroid eye disease (TED, 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 TED\(^1\), 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 TED 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 TED 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...
In addition to immunosuppression, non-pharmaceutical treatment of active TED has been advocated for decades, and older RCTs demonstrated that this was more effective when used in combination with corticosteroids. However, subsequent studies either questioned the role of orbital radiotherapy or concluded that its benefit was limited to improvement in oculomotility. 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, 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. 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, and clinical outcomes beyond 24 weeks have rarely been reported for any intervention in TED. We therefore sought to evaluate the long-term benefit of low-cost orbital radiotherapy and antiproliferative immunosuppression and orbital radiotherapy with azathioprine in the context of sustained systemic corticosteroid treatment for active moderate-to-severe TED.

**Methods**

**Study design and participants**

We undertook this factorial design multicentre, double masked RCT in 6 centres in the UK. Patients aged 20-75 years 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\textsuperscript{(26)}. Trial registration was assigned retrospectively on 1 February, 2006 (ISRCTN22471573) following regulatory permissions, but prior to starting recruitment.

Eligible patients had a clinical activity score\textsuperscript{(27)} (CAS) ≥ 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 TED thyroid eye disease made and confirmed by ≥2 muscle involvement on computed tomography or magnetic resonance imaging scan. CAS 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 CAS clinical activity score was scored out of 10. If study recruits either had a < 6 month history of TED thyroid eye disease (defined as time since first symptom) or an improvement in any item of CAS 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 (TPMT) activity and use of radioiodine or any immunomodulatory or cytotoxic drugs within the last 3 months (thyroidectomy was permitted).

Randomisation and masking

Patients’ eligibility for the study was assessed by the ophthalmic investigators at each trial centre. Allocation of trial participants
Patients were allocated to treatment groups by remote computerised randomisation. 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. Patients, clinicians (both ophthalmic and endocrine) and data analysts were all masked. Only the trial co-ordinators (who monitored trial subjects blood results), pharmacists and radiographers were unmasked. The success of masking for ophthalmic investigators and patients was assessed at study completion or withdrawal by asking them to declare which treatments they thought had been administered.

Interventions

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 a-sham therapy, including use of a noise emitting device to simulate treatment administration (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

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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 CAS clinical activity score or development of optic neuropathy) and sustained blood test abnormalities (leucopenia, lymphopenia or abnormal liver function tests despite dose adjustment of AZA 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, and the conduct of the trial was subject to independent Data Safety Monitoring Committee and Trial Steering Committee review for the duration of the study.

**Outcomes**

Outcome measures...
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 composite outcome measure [BCCOM] and Ophthalmopathy Index [OI]) were selected to quantify the change in ocular deformity and visual dysfunction. An early, 12-week, assessment of disease activity using the CAS clinical activity score was given lower priority and designated as a co-primary outcome (we expected that all participants would have a significant improvement in disease by 48 weeks in accordance with the natural history of the
disease). Secondary outcome measures included Total Eye Score (TES) as an additional assessment of disease severity, and the patient reported Graves’ Ophthalmopathy Quality of Life (GO-QoL) score. Secondary outcome measures included Total Eye Score (Supplementary Table 3) as an additional assessment of disease severity.
severity, patient-reported Graves’ Ophthalmopathy Quality of Life score and health economic indices.

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, ophthalmopathy 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 ITT-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 secondary post-hoc as-per-protocol (APP) 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 BCCOM primary 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). The secondary outcome measures of Total Eye Score and Graves ophthalmopathy quality-of-life score were also compared across treatment groups, however patient-reported health economic analyses were 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, Above and Beyond 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. (PNT RH CMD and RL had access to the raw data).

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 Gartnave 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 at 48 weeks was provided by 101 participants, and these were analysed after data-lock (which included separate 3 year assessments on a minority of trial subjects) on 7th October 2016. 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 (ITT) analysis

Binary Clinical Composite Outcome (BCCOM) Measure (primary outcome)

The difference in BCCOM 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 BCCOM’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 indication of improvement with orbital radiotherapy (OR\textsubscript{(adj)} =0·89, 95%CI 0·36, 2·23, p=0·80). With regard to the factorial design, there was no evidence of interaction between azathioprine and radiotherapy (p\textsubscript{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 BCCOM of 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 BCCOM outcomes regardless of their status at 48 weeks enhanced rather than lessened the improvement observed with azathioprine treatment (OR\textsubscript{adj} \textsubscript{adj} 3·65; 95%CI 1·34, 9·86; p=0·01) (**Supplementary Table 4**).

**Ophthalmopathy Index (OI) (primary outcome)**

Analysis of all patients revealed that the OI 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\textsubscript{(adj)}) of 0·46 (95%CI -1·04, 1·95; p=0·55) and in those randomised to orbital radiotherapy B\textsubscript{(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 OI ophthalmopathy index (p\textsubscript{int} = 0·51).

**Clinical Activity Score (CAS) (co-primary outcome)**

Across all subjects, substantial improvement in median CAS 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 CAS clinical activity score by week 12 and 96 (98%) of the 98 patients with CAS clinical activity score data at 48 weeks showed improvement in their CAS clinical activity score versus baseline. No difference in the change in CAS 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 $B_{\text{adj}} = -0.01$ (95%CI -0.69, 0.68; $p=0.99$ – Table 2). There was no evidence of interaction between azathioprine and radiotherapy in their effect on CAS-clinical activity score ($p_{\text{int}} = 0.48$). There was also no evidence for substantial benefit from azathioprine or orbital radiotherapy on improving CAS-improved clinical activity score at week 48 (Supplementary Table 5).

There was no evidence of interaction between azathioprine and radiotherapy in their effect on CAS-clinical activity score ($p_{\text{int}} = 0.48$). There was also no evidence for substantial benefit from that azathioprine or orbital radiotherapy on improving CAS-improved clinical activity score at week 48 (Supplementary Table 5).

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<th>ThyroidTotal Eye Score (TES) (secondary outcome)</th>
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|TESTotal Eye Score| improved considerably over the study period with a mean TES at baseline of 15.1 (95%CI 13.8, 16.3) falling to a mean TES 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 Ophthalmopathy Quality of Life (GO-QoL) (secondary outcome)

Across all subjects, mean GO-QoL Graves ophthalmopathy 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 Graves ophthalmopathy quality of life 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 BCCOM outcome binary clinical composite measure at week 48 had a higher GO-QoL Graves ophthalmopathy quality of life visual function ($B = 17.9 - 95\%\text{CI} 7.07, 28.6; p=0.001$) and a higher GO-QoL Graves ophthalmopathy quality of life visual appearance ($B_{\text{adj}} = 11.5 - 95\%\text{CI} 0.60, 23.6; p=0.06$). There was no clear benefit from the addition of either azathioprine or orbital radiotherapy with regard to long-term Graves ophthalmopathy quality of life visual function – or visual appearance (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-as-per-protocol 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-radiotherapy and placebo. Individuals in the APP-as-per-protocol 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-as-per-protocol 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 BCCOM 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 patients 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 6 and Supplementary Figure 2A). Individuals receiving azathioprine also had a modest improvement in TES total eye score (B(adj)=-3.23, 95%CI -6.42, 0.03; p=0.05, Supplementary Table 6). However, the APP-as-per-protocol analysis did not reveal any benefit in OI-CASophthalmopathy index, clinical activity score or GO-QoLGraves ophthalmopathy quality of life of being randomised to receive either azathioprine or orbital radiotherapy (Table 3).

Withdrawals from the study

In the study there was a high number of patients who withdrew from their 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...
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 azathioprine and 34 withdrawals in those randomised to receive orbital radiotherapy. Overall, 103 participants provided outcome data, of which 84 completed their allocated treatment of radiotherapy or sham-radiotherapy, and 57 continued to take azathioprine or placebo until 48 weeks. 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 9). 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 10). However, unlike patients receiving 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 11). There was no evidence of bias between treatment groups with regard to failure to provide data at 48 weeks (Supplementary Table 12 and Supplementary Table 13).

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 14). 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 (azathioprine N=161, radiotherapy N=156) (Supplementary Table 15 and Supplementary Table 16).
Masking

Of the 69 patients and 71 doctors who recorded their perceived trial allocation for azathioprine or placebo on study completion or withdrawal, 30 patients (43%) and 29 doctors (41%) were incorrect. For radiotherapy and sham-radiotherapy, of the 70 patients and 67 doctors, 23 patients (33%) and 33 doctors (49%) were incorrect.

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 (BCCOM, CAS and GO-QoL: binary clinical composite outcome measure, clinical activity score and Graves ophthalmopathy quality-of-life score) 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). Since radiotherapy was delivered early in the treatment (before 12 weeks†), 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 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 49). 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 TED: 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 recognised role of azathioprine as a steroid-sparing agent, used to prevent relapse in other autoimmune conditions, and this is further reinforced by the findings of the MINGO study using an alternative antiproliferative agent (mycophenolate sodium) in thyroid eye disease. Furthermore, this view is supported by analysis of the BCCOM binary clinical composite outcome measure components indicating that azathioprine did not increase major improvement rates overall but did reduce major deterioration in the BCCOM 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 the ITT 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, this was because of the lack of fully validated long-term disease severity measures in TED, (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 \textit{BCCOM} binary clinical composite outcome measure has since become the preferred end-point for \textit{TED} thyroid eye disease studies as it is more sensitive to change\cite{21,23}. We have therefore focused on this rather than the \textit{Ophthalmopathy 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 success of our 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 corticosteroid 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 prescribed in many regions of the world including North America \cite{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 they indicate that, if tolerated, azathioprine improves 48-week clinical outcomes in patients with active moderate-to-severe \textit{TED} 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.
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Declaration of Interest

The authors report no declarations of interest
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Combined Immunosuppression and Radiotherapy in Thyroid Eye Disease (CIRTED)

PROTOCOL v5.3

Confidential
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2 BACKGROUND INFORMATION

Thyroid Eye Disease (TED) is an inflammatory autoimmune condition of the orbit which is clinically apparent in 25-50% of people with Graves' hyperthyroidism. The majority of patients report only mild symptoms, but 5% of the UK's 400 000 TED sufferers (many of them young women in the age group 20-50) have severe TED, characterised by swollen eyelids, protrusion of the eyes (proptosis) and double vision (diplopia).

2.1 Pathophysiology

The key pathological features of TED are orbital inflammation, orbital fat proliferation and extra-ocular muscle enlargement. The size of the bony space to accommodate these expanding tissues is fixed, causing the intra-orbital pressure to rise and the globe to propose forward. Rising orbital pressure also alters the local circulation of lymph and blood: outflow is obstructed while inflow continues, and a vicious cycle of further tissue engorgement ensues. In extreme cases this leads to optic nerve compression and results in loss of vision. Individuals with tight eyelid ligaments, who are unable to “autodecompress” by proptosis, are at particular risk of this.

The exact pathogenesis of TED is uncertain, but it is known to be an autoimmune process caused by cross-reactivity between proteins in the orbit and thyroid gland. Its natural history is classically described in three phases; an initial ‘active’ inflammatory phase, a subsequent regressing phase, and (after 1 – 3 years) a final ‘inactive’ ('burnt out') phase. Recovery is variable, and the residual disfigurement and visual disability is permanent. Unlike most other autoimmune diseases, TED rarely reactivates.

2.2 Treatment

There is great controversy about the best treatment for TED. Traditionally, it was managed conservatively during the inflammatory phase, and the residual deformity was then corrected surgically (including orbital decompression, strabismus correction and eyelid repositioning) when the disease had burnt out. In addition to the surgical burden for patients and ophthalmologists, this approach did not address the underlying pathology and outcomes were frequently imperfect.

Systemic immunosuppression and radiotherapy, directed at the initial inflammatory response in the active phase of the disease, might be expected to modify the subsequent disease course and reduce the severity of residual orbital fibrosis; thereby improving long-term function and reducing the need for rehabilitative surgery. However, confusion has arisen over the role of these treatments for the following reasons:
1. Limitations in the quality of evidence:
   
a. The majority of previous reports on radiotherapy in TED are retrospective and / or uncontrolled.

b. Most studies have involved small sample sizes.

c. Few trials have evaluated long-term results (1 year or more)

d. Several studies have not distinguished between inactive and active disease in their analysis of treatment outcome.

2. Recent randomised controlled trials on radiotherapy in TED have produced conflicting results.

3. Concern about the side-effects caused by systemic immunosuppressants.

**2.3 Review of the Literature - the Need for a Trial**

**2.3.1 Glucocorticoids and Ciclosporin**

Several studies have shown that glucocorticoids are effective in the management of severe TED. However, treatment is typically discontinued after 3 to 5 months because of the side-effects associated with long-term steroid use, and subsequent disease reactivation is a common problem. This recurrence can be prevented by the concomitant use of ciclosporin (which continues after steroid treatment stops), and combinations of ciclosporin and prednisolone also achieve a better initial treatment response than either agent alone. However, the routine use of such second-line immunosuppressive drugs in the management of severe TED has been limited by fears about their potential toxicity, and glucocorticoid monotherapy remains the mainstay of conventional treatment.

**2.3.2 Orbital Radiotherapy**

Radiotherapy has been used to treat TED for more than 60 years, and until recently it had an established place in the management of the disease. Its use was supported by the results of a prospective blind randomised control trial (RCT) which reported (in 1993) that orbital radiotherapy was as effective as oral prednisolone. However, the same authors subsequently found that radiotherapy was no better than placebo (except in a subgroup of patients with motility impairment), and a recent trial from the Mayo Clinic in the USA also could not demonstrate a beneficial therapeutic effect. As a result, many clinicians, particularly in North America, have abandoned its use.

The Mayo study has been widely criticised, and European groups argue that the balance of evidence remains in favour of radiotherapy. In support of this, the most recent randomised control trial (in patients with relatively mild disease) found that radiotherapy was better than placebo when outcome was assessed by clinical measures. However, this was not associated with quality-of-life or health economic

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gain, and the role of radiotherapy as monotherapy for TED remains the subject of heated debate\textsuperscript{20-29}.

Older RCTs have shown that radiotherapy is more effective in combination with steroids than when either agent is used alone\textsuperscript{30 31}. However, this distinction between monotherapy and combination therapy is rarely highlighted, and the confusion which has resulted from the inconsistent recent evidence has caused many ophthalmologists to abandon radiotherapy as part of a combined treatment regime. Consequently there is an urgent need for a definitive study to establish radiotherapy’s role in combination with steroids.

### 2.3.3 Combined Immunosuppression and Azathioprine

The data supporting the use of ciclosporin combined with steroids\textsuperscript{8 12}, and radiotherapy combined with steroids\textsuperscript{30 31} are not surprising given current knowledge about the benefits of combination therapies in other autoimmune ocular and systemic conditions (such as uveitis and rheumatoid arthritis)\textsuperscript{32 33}. Manipulation of an autoimmune response is more effective when more than one mechanism of immunosuppression is used, and this can be especially so early in the disease\textsuperscript{32}. Each drug can also often be given in a lower dose than with monotherapy, improving safety. Combined drug treatments can also be continued long-term, preventing the disease reactivation which is commonly seen at the end of typical short-term steroid monotherapy regimes, and enabling the duration of an individual’s treatment to be tailored to the length of their disease.

Azathioprine is a low-cost second-line immunosuppressive agent which is commonly used in the management of other autoimmune conditions. It is better tolerated than ciclosporin\textsuperscript{34} and does not cause the renal toxicity and cardiovascular side-effects\textsuperscript{35} (including hypertension and hypercholesterolaemia) associated with ciclosporin. However, it can cause bone marrow suppression\textsuperscript{36} and hepatotoxicity\textsuperscript{37}. This risk has been greatly reduced with the advent of a new laboratory assay for the enzyme thiopurine methyltransferase (TPMT), which regulates a key step in azathioprine metabolism. Now individuals with low TPMT activity, who would otherwise be at particular risk of azathioprine toxicity, can be identified and their treatment modified or withheld\textsuperscript{38}. Hence, azathioprine has safety and tolerability advantages over ciclosporin.

Retrospective data from Bristol Eye Hospital suggests that azathioprine, when used in combination with oral prednisolone and radiotherapy, can significantly reduce long-term TED severity and the need for rehabilitative surgery\textsuperscript{39}. A recent publication from the Royal College of Ophthalmologists has recommended this regime\textsuperscript{40}, however the use of azathioprine for TED remains highly controversial; in part because it has previously been proven ineffective as monotherapy\textsuperscript{41}. As with radiotherapy, there is no clear evidence base and clinical practice consequently varies widely.
2.4 Trial Summary

This randomised controlled trial of azathioprine and radiotherapy (with prednisolone) will be the first to establish the role of these interventions as part of a *long-term, combination* immunosuppressive treatment regime for TED. Such a definitive study is required to resolve the debate which currently surrounds these treatments and to provide evidence for authoritative management guidelines. Only patients with active orbital inflammation will be enrolled and follow-up will continue for a minimum of 1 year. A factorial design is used to allow the efficacy of both interventions to be assessed in a single study with efficient use of patients; and the establishment of a multi-centre network of trial sites will ensure adequate patient numbers for statistical power. Health economic and carefully designed quality of life/disfigurement analyses in collaboration with the Division of Primary Care (University of Bristol) and the Centre for Appearance Research (University of West of England) will accompany the trial.

2.5 Trial Publications

3 OBJECTIVES

3.1 Primary
Test the hypotheses that in patients being treated with prednisolone for active TED:

1. **RADIOTHERAPY** (compared with placebo) induces early remission and reduces long-term disease severity.

2. **COMBINED SYSTEMIC IMMUNOSUPPRESSION WITH ORAL AZATHIOPRINE** (compared with placebo) reduces long-term disease severity.

3.2 Secondary

1. To test the hypotheses that in patients being treated with prednisolone for active TED, radiotherapy and azathioprine improve health-related quality of life, and are cost-effective.

2. To validate the use of the GO-QoL (a new TED specific quality of life score) in the UK population.

3. To improve understanding of the extent and type of psychosocial distress experienced by TED patients.

4. To conduct an economic evaluation of the cost of TED and its treatment to patients, the National Health Service (NHS) and Society.

5. To report the safety and tolerability of radiotherapy and azathioprine in the study cohort.
4 TRIAL DESIGN

4.1 Factorial Design

Factorial randomised trials allow two interventions to be evaluated in a single study. This has particular advantages for the assessment of combined treatment regimes, especially when considering relatively uncommon conditions with a limited number of potential recruits\textsuperscript{43, 44}, such as TED. They are the most valid means of establishing whether the combination of two or more therapies achieves incremental benefits\textsuperscript{43}.

4.1.1 Application to CIRTED.

![TRIAL ARMS Diagram]

Trial participants will be separately randomised to receive radiotherapy or sham-radiotherapy, and azathioprine or placebo (illustrated above).

4.1.2 Discussion of the Risks Associated with a Factorial Design

When factorial design is used in the analysis of very large samples it can determine the interaction between interventions. For example, to assess whether the effect of Treatment A plus Treatment B is greater (or less) than the sum of Treatment A’s effect alone and Treatment B’s effect alone. This can give special insight into the benefits, or otherwise, of multiple therapies. However, if the two interventions under
consideration interact, and the sample size is too small to reveal this interaction, their calculated independent effects may be incorrect. Hence, when using factorial design to establish the effect attributable to individual components of a combination treatment regime it is important to be sure there is no significant interaction between interventions\textsuperscript{43}.

In CIRTED the sample size will not be large enough to draw any meaningful conclusions about the degree of interaction between treatments, as the confidence intervals from this analysis will be too large. Consequently we have assessed the risk of potential 'biological interactions' and 'statistical interactions' which might lead to erroneous results in the analysis of individual treatment effects.

Radiotherapy and azathioprine are both thought to reduce the inflammatory response in TED through their effects on T-lymphocytes in the orbit, but each works by an entirely different biological mechanism. Their common goal of reducing the T-lymphocyte response means that they are subject to the 'law of diminishing returns'. That is, once the T-lymphocytes have been 'hit' by radiotherapy there will be a limit to the further suppression which can be achieved with the addition of azathioprine. However, we would not expect radiotherapy to affect the mechanism by which azathioprine works, and in this sense they should not 'interact'.

Taking all the above into account, the statisticians who have advised on the CIRTED trial consider the potential for an interaction between our interventions having a 'clinically significant' effect on the interpretation of the trial data is very low (approximately 5%), and factorial randomisation has been recommended as the most appropriate trial design.
4.1.3 Principles of Analysis

The following table is an alternative representation of the treatment groups in CIRTED which can be used to illustrate the method of factorial trial analysis.

<table>
<thead>
<tr>
<th>RADIOTHERAPY + AZATHIOPRINE</th>
<th>SHAM – RADIOTHERAPY + AZATHIOPRINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RADIOTHERAPY + PLACEBO</td>
<td>SHAM – RADIOTHERAPY + PLACEBO</td>
</tr>
</tbody>
</table>

Factorial analyses can be conducted ‘inside the table’ or ‘at the margins’. Both will be calculated, but the examination of the interaction between the effects of azathioprine and radiotherapy ‘inside the table’ will be imprecise with our sample size.

The effects of radiotherapy and azathioprine will be determined separately by combining and comparing the cells of the table ‘at the margins’ as illustrated overleaf:
**RADIOTHERAPY vs SHAM-RADIOTHERAPY**

Treatment response will be compared between patients receiving *either* radiotherapy plus azathioprine or radiotherapy plus placebo *WITH* patients receiving *either* sham-radiotherapy plus azathioprine or sham-radiotherapy plus placebo.

**AZATHIOPRINE vs PLACEBO**

Treatment response will be compared between patients receiving *either* azathioprine plus radiotherapy or azathioprine plus sham-radiotherapy *WITH* patients receiving *either* placebo plus radiotherapy or placebo plus sham-radiotherapy.
4.2 Minimizing Bias

4.2.1 Treatment allocation

Allocation of patients to the 4 arms of the study will be done using minimisation. This is a process that allocates patients based on (a) their own characteristics, and (b) the allocations of patients with the same characteristics already in the trial. The process gains its name by minimising the imbalance in numbers in each of the trial arms with respect to a range of important prognostic variables simultaneously. It is a dynamic process and a randomisation schedule is therefore not drawn up in advance. The variables used to minimise patients are:

- Smoker at time of TED diagnosis (Y/N)
- Thyroid status on enrolment (hyperthyroid / hypothyroid / euthyroid)
- Previous Steroid use - defined as ≥ 20mg prednisolone for ≥ 1 month in the previous 6 months (Y/N)
- Gender (M/F)
- Disease severity (Total Eye Score < 22 / Total Eye Score ≥ 22)
- Study Centre
- Disease Duration (<6 months / ≥ 6 months)
- Age (< 60 yrs / ≥ 60 yrs)
- Disease Activity (Clinical Activity Score 2 – 3 / 4 – 5 / 6 – 7)

The 4 trial arms to which minimisation will be applied in this study are: (A) sham-radiotherapy and azathioprine (B) radiotherapy and azathioprine (C) sham-radiotherapy and placebo, and (D) radiotherapy and placebo.

The Trial pharmacists at each study centre, who are unmasked, will contact the Telephone Randomisation Service provided by Moorfields Eye Hospital Clinical Trials Unit to obtain the treatment allocation for each recruit. This is confirmed by email, which also states the patient’s radiotherapy group – a copy of which is sent to the Trial Radiotherapy Centre radiographers and the un-masked trial co-ordinator.

4.2.2 Masking

Masked individuals:

1. Patients
2. Clinical Investigators (Ophthalmologists, Research orthoptists, Masked research nurses, Psychosocial investigators, Health economic analysts and clinical oncologists)
3. Data Analysts

**Un-masked individuals:**

1. Unmasked trial co-ordinators and research nurses (responsible for reviewing blood tests to monitor for azathioprine related adverse events)
2. Trial Pharmacists
3. Radiographers
4. Ophthalmic Clinical Investigators will be masked to the treatments received by patients in their own Centre, but they may be un-masked to the treatment group the patients in the other Centre in order to give medical advice on the appropriate action following abnormal blood test results or to give prescribing instructions

Patients and the ophthalmic / endocrine teams responsible for their care, will be informed of their treatment allocation on trial exit or completion.

4.2.3 Other Measures for Maintaining Masking and Protecting Against Bias

All trial recruits, whether allocated to the azathioprine or placebo arms of the trial, will have regular blood tests to monitor for the potential adverse effects of azathioprine treatment; otherwise this would break masking for both the patient and investigators.

**Repeat Blood Tests and Azathioprine Dose Adjustment**

It is estimated that less than 5% of azathioprine treated trial subjects will have blood test abnormalities for which they will be recalled\(^{45}\). In order to maintain masking we also plan to randomly recall ≤5% of the placebo treated group.

On recall, these trial subjects will have repeat blood tests and / or their drug dose adjusted. For azathioprine-treated patients this dose adjustment will be titrated to their blood test results. The dose range is 50mg – 200mg (ie 1 - 4 x 50mg Azathioprine tablets). Recalled placebo-treated patients will also have the number of tablets they take randomly changed (ie 1 - 4 x placebo).

**Standard Patient Assessments**

A standard ‘atlas’ of disease severity measures will be used to reduce inter-observer error in patient assessment\(^{46}\)
4.3 Determination Of Sample Size

4.3.1 Primary and Co-Primary Outcome Measures

There are two primary outcomes, a binary composite variable rating success or failure\(^{10, 16, 19}\) and the Ophthalmopathy Index\(^{47}\). Both will be measured at 12 months after randomisation:

- **Binary composite variable rating success or failure**: Success rates in the placebo arms compared with the treated arms have been estimated to be 60% and 87% based on figures available in the literature\(^{48}\) and allowing for a possible placebo effect. A two group continuity corrected chi-squared test with a 0.050 two-sided significance level will have 80% power to detect the difference between a success rate in the placebo arm of 60% and success rate in the treated arm of 87% (odds ratio of 4.462) when the sample size in each group is 48. It is therefore necessary for there to be around 100 subjects in the final data set equally distributed to the 4 randomisation codes. However, the power of the study to detect clinically significant differences will improve with increasing numbers of subjects.

- **Ophthalmopathy Index**: The power for continuous outcome measures is more favourable. A total sample size of 100 patients will yield 92% power to detect a difference of 2.7 in the Ophthalmic Index with two-sided 5% alpha, assuming a standard deviation of 3.3.

The Clinical Activity Score\(^{49}\) measured at 3 months after randomisation is defined as a co-primary outcome. This outcome does not have the same status as the two primary outcomes, in that it is not considered as important as the primary outcomes in terms of influencing future clinical practice. However it is of greater interest and importance than the secondary outcome variables, and as such, the power of the trial to detect a clinically important difference is presented here. Assuming a standard deviation of between 1.2 and 2.0, a total sample of 100 patients will yield between 71% and 99% to detect a difference of 1 point with 5% two-sided alpha.

4.3.2 Secondary Outcome Measures

- The Total Eye Score (TES)\(^{8}\)
- The Graves Ophthalmopathy Quality of Life Score (GO-QoL)\(^{42, 50}\), a disease specific quality of life score

4.3.3 Predicted Enrolment

Extrapolating recruitment across all trial centres from Jan 2006 until May 2008 it would take until Dec 2012 to complete the trial (total 6yrs recruitment plus 1yr follow-up). Hence, in order to hit our revised end date of Dec 2011 (see section 4.4) the number of trial centres has been increased.
4.3.3.1 Compliance and loss to follow-up

Of the 42 patients enrolled in Gorman et al's sham-radiotherapy trial, only 2 did not complete the protocol; and of the 60 patients in Mourits et al's sham-radiotherapy study, only one did not complete the protocol. Prummel et al's trial of combined immunosuppression with cyclosporine and prednisolone in 36 patients also reported only one loss to follow-up. Consequently, compliance is expected to be excellent once patients are enrolled in the study.

Using these figures as a basis for estimating recruitment and drop-out:

- 33% of these will choose not to take part in the study\(^\text{16}\);
- 11% of these will be excluded because of TPMT inactivity\(^\text{51}\);
- 10% of the azathioprine treated group will be intolerant of therapy, or suffer an adverse event necessitating withdrawal from the trial\(^\text{52}\);
- 5% will drop-out for other reasons.

4.4 Timescales

4.4.1 Proposed Trial Start

January 2006

4.4.2 Projected Trial Completion

December 2011

4.4.3 Trial Duration

6 Years

4.4.4 Duration of Each Patient’s Participation

1 year (plus a single additional review 2 yrs after trial completion for patients participating in the optional long-term follow-up study)

4.4.5 Frequency of Follow-up

Trial subjects are seen and randomised (if eligible) 2 weeks after enrolment. Their treatment response and tolerance is then assessed 4 weeks later, just prior to starting radiotherapy / sham-radiotherapy (see section 6.1). The short-term outcome assessment is then conducted at week 12, and trial recruits are subsequently seen 3-monthly until they exit the trial 11 months post-randomisation. Regular blood tests will also be required after randomisation to monitor the potential adverse effects of azathioprine treatment. Further reviews can be arranged at the trial centres for any
reason at the patient or investigators’ request. In addition, study recruits will be invited to have an optional long-term outcome assessment 2yrs after completing the trial.

4.5 Quality Control

4.5.1 Accountability for Investigational Products

- Active Azathioprine tablets are being sourced via the NHS.
- St Thomas’ Hospital NHS Trust’s Drug Manufacturing Unit are producing matched placebo tablets. In addition, they are responsible for packaging both the active Azathioprine and its placebo as Investigational Medicinal Products for use in the trial.

(See Clinical Trials Authorisation for further details)

4.5.2 Procedures for monitoring subject compliance

- Tablets will be counted by the Trial Pharmacists at each visit.
- The patient will be asked about treatment compliance by the Clinical Investigators at each trial visit.

4.5.3 Site Monitoring

This is the responsibility of the Trial Sponsor, who will have direct access to source data.

4.6 Safety

4.6.1 Data Monitoring Committee (DMC) – also see section 1.4.1

4.6.1.1 Interim analyses

No formal interim analyses are planned, although this may be requested by the DMC, who will meet every 12-18 months to review a masked report of trial progress and adverse events.

4.6.1.2 Adverse Events

Adverse Event reports will also be presented to the DMC (although it is unlikely that any new safety data on the trial interventions will be revealed. Radiotherapy has been used to treat TED and Azathioprine has been used as a systemic immunosuppressant for decades.
### 4.6.1.3 Procedures for breaking randomisation codes

Randomisation codes may be broken prior to trial completion at the request of the DMC.

Randomisation codes for individual patients may be broken for withdrawn subjects.

### 4.6.1.4 Criteria for termination of the trial

The trial will only be terminated if the p value of an interim analysis is smaller than 0.0001, and a DMC meeting to review all the evidence decides that this is the best course of action to take.

### 4.6.2 Trial Steering Committee – also see section 1.4.2

The Trial Steering Committee will meet on a 12 – 18 monthly basis. Their principal responsibilities will be to:

1. Review the protocol and comment on any major errors in the trial design that they feel would prevent it addressing the primary objectives.
2. Review progress reports on the trial and provide advice if problems arise.
3. Comment on protocol amendments.
4. Ensure that any new information on the trial interventions which becomes available after the start of the trial is properly considered.
5. Protect the interests of patients should safety issues arise (although this will primarily be the responsibility of the Data Monitoring Committee).

## 5 SELECTION AND WITHDRAWAL OF SUBJECTS

### 5.1 Selection

#### 5.1.1 Subject Inclusion Criteria

1. Mourits’ Clinical Activity Score ≥ 4 out of 7 (worst eye) and a history of proptosis* or motility restriction†
   
   OR
   
   Mourits’ Clinical Activity Score 2 or 3 out of 7 (worst eye) with a history of proptosis* or motility restriction† of less than 6 months duration

2. Past or present history of abnormal Thyroid Gland Function OR a clinical diagnosis of TED made and confirmed by ≥ 2 muscle involvement on CT or MRI scan
Definition of proptosis = EITHER subjective unilateral proptosis confirmed by asymmetry in exophthalmometry of > 2mm OR subjective bilateral proptosis.

\[^{\text{\textsuperscript{\textdagger}}}\text{Definition of motility restriction = intermittent, inconstant or constant diplopia grade}^{54}\]

5.1.2 Subject Exclusion Criteria

- Age <20 or >75 yrs
- Optic neuropathy
- Pre-existing glaucoma with a characteristic optic disc appearance and associated visual field defect
- Use of radioiodine within the last 3 months
- Pre-existing Diabetes Mellitus (not simply steroid induced disease from recent therapy)
- Previous adverse event associated with, or contraindication to, either prednisolone or azathioprine
- Within 6/12 of pregnancy, women planning pregnancy
- Lactation
- Haemoglobin Concentration > 1 g/dl below the local laboratory's reference range
- Platelet Count < 130 × 10\(^9\)/L
- Total White Cell Count below the local laboratory's reference range
- Abnormal Thiopurine Methyltransferase (TPMT) activity
- Lymphocyte Count < 0.8 × 10\(^9\) / L
- Abnormal renal function (estimated Glomerular Filtration Rate (eGFR) < 60 ml/min/1.73 m\(^2\))
- Abnormal liver function, specifically: bilirubin, alanine aminotransferase or alkaline phosphatase concentrations > 1.5× above the local laboratory's reference range (excluding Gilbert’s syndrome\(^*\))
- Malignant or pre-malignant (dysplastic) condition within the past 5 years
- Previous tuberculosis
- Shingles within the past 3/12
- HIV / AIDS
- Concurrent use of
  - other immunosuppressive or cytotoxic agents
  - allopurinol
- Live vaccines within the past 3 months
- Previous orbital irradiation

**NB** Trial subjects with a disease duration of > 6 months who do not improve with steroid treatment in the first 2 weeks post enrolment will withdraw from the trial and not be randomised (see section 5.3.1 Exit Criteria)

*Gilbert’s syndrome is a common hereditary, harmless cause of raised bilirubin which is not a contraindication to azathioprine treatment.

## 5.2 Recruitment and Identification of Eligible subjects

### 5.2.1 Advertising

The CIRTED trial will be formally announced at academic and professional meetings to encourage referral of eligible patients to the Trial Centres. Literature publicising the trial will also be circulated to Ophthalmologists in the region of each Trial Centre and a website for health-care professionals and patients to access information about the trial has been set up (www.cirted.org). The trial protocol has also been published (see section 2.5)

Patient groups are already aware of this study and a summary of the trial’s goals and structure has been presented at meetings of the Thyroid Eye Disease Charitable Trust (TEDct). An introduction to the trial has also been published in the TEDct newsletter.

### 5.2.2 Screening

*Identification of Trial Subjects and Pre-visit Screening*

All referral letters to the Trial Centres for patients with active Thyroid Eye Disease will be reviewed by the Investigators.

*Contacting Patients*

**Who are already aware of the Trial (ie they have been specifically referred for the study)**

Patients specifically referred for trial screening are contacted by phone to arrange an appointment. Their patient information is then sent out by email or post (whichever they prefer).

**Who are unaware of the Trial, and have just been referred for assessment of their TED**

Patients who are potentially suitable for the trial are contacted by letter and sent a Patient Information Sheet prior to their out-patient appointment. If the patient returns
the study reply slip they will be phoned by a member of the research team to check their eligibility. This phone call will:

- Explain that the Eye Hospital / Clinic to which the patient has been referred for specialist management of their TED is involved in a research study to investigate the best treatment for this condition, and that all new referrals are being assessed for their eligibility to participate.
- Highlight that there is no expectation or requirement that the patient will take part in the clinical trial.
- Clarify whether the patient is potentially eligible to participate
- Offer the opportunity to answer any questions they may have about the study after reading the patient information sheet or website.

**Contacting the Patient's General Practitioner, Endocrinologist or Local Biochemistry Laboratory**

If required, this will be done to clarify:

- The results of all Thyroid Function Tests within the past 3 months.

### 5.2.3 Clinical Assessment of New Referrals and Trial Enrolment

Potentially eligible patients will be assessed by one of the Clinical Investigators. If this consultation is in a NHS out-patient clinic, subjects fitting the clinical eligibility criteria will be invited back for a formal trial enrolment visit in the Centre’s Clinical Research Unit.

If the patient wishes to start steroids at their first hospital appointment, or there is any clinical urgency and:

- *They want to take part in the trial*, they will be enrolled at this first visit
- *They are unclear whether or not they wish to take part in the trial*, or it is impractical / inconvenient for them to complete the trial enrolment assessments (including questionnaires, tear and blood tests) at this visit, they will start the steroid treatment protocol (which is standard whether or not the patient takes part in the study). Their next appointment will then be arranged 2 weeks later in the Trial Centre’s Clinical Research Unit, at which time they can be simultaneously enrolled and randomised should they wish to take part in the study.

**Enrolment Visit**

*Purpose: to confirm eligibility, further discuss the pros and cons of taking part in the study, conduct the trial baseline assessments and obtain consent if the patient wishes to take part*
A modified version of the Initial Assessment Proforma (Appendix 2) published in 2002 by the European Group on Graves Orbitopathy (EUGOGO) will be used for recording the case history and examination features of each potential Trial Subject. The key components of the screening visit are:

**Consultation**

To document the:

- Duration and severity of TED symptoms
- TED treatment to date
- Prior and current thyroid status and treatments
- Ocular Co-morbidity
- General Past Medical History
- Current medications
- Smoking History

**Ocular Examination**

This will be conducted according to the trial's standard operating procedures to assess:

- Snellen Visual Acuity
- Eyelid redness and swelling
- Conjunctival redness and chemosis
- Caruncular swelling
- Palpebral aperture
- Corneal integrity
- Proptosis (using an Oculus © exophthalmometer)
- Oculomotility, including
  - Monocular Ductions in six directions of gaze
  - Gorman’s Diplopia score
- Intraocular pressure in primary position and upgaze
  - Optic Nerve Function, including
    - Colour Vision (Ishihara assessment)
    - Relative Afferent Pupillary Defect
    - Visual Fields
- Optic disc assessment

**General Examination**
- Weight / height
- Blood Pressure
- Urinalysis

**Investigation results review**
- Blood tests:
  - Full Blood Count (FBC)
  - Thyroid Function Tests (TSH, FT3, FT4)
  - Liver Function Tests (LFTs)
  - Urea, Creatinine and Electrolytes (U&Es)
  - Random Glucose (Glu)
  - Thiopurine methyltransferase (TPMT) Activity

**Enrolment**
Eligible subjects who have received and read the Patient Information Sheet prior to their review will be asked to enrol and start the standard oral steroid treatment regime.

**5.3 Withdrawal**

**5.3.1 Exit Criteria**

**AT ANY TIME**
- Development of Dysthyroid-associated Optic Neuropathy (DON), defined as
  - a significant visual field defect
  - a relative afferent pupillary defect
  OR
  - an unexplained best corrected visual acuity of less than 6/12 (with or without a swollen optic disc)
- Patient withdrawal of consent

**BEFORE RANDOMISATION**
Non-response to Steroids

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- Trial subjects who have had TED for more than 6 months and whose Clinical Activity Score (CAS) has not improved by at least one point 2 weeks after starting high-dose steroids, are unlikely to benefit from Radiotherapy and/or Azathioprine. They will be withdrawn from the trial – ie they will not be randomised.

**AFTER RANDOMISATION**

**Worsening TED severity**
- Increasing Clinical Activity Score (CAS) by ≥2 points, confirmed on repeat examination (within 14 days).

**Azathioprine toxicity**

(Full blood count & liver function tests will be measured every week for the first 4 weeks and then 8 weekly until completion of trial)
- Fall in any of the following measures below the local laboratory’s reference range:
  - Total White Cell Count
  - Neutrophil Count
- A persistent fall in lymphocyte count to < 0.5 x 10⁹ / L despite a reduction in azathioprine dose (see section 6.1.3 – oral azathioprine treatment protocol)
- A persistent Alanine Aminotransferase (ALT) rise to >120 IU/L (ie >3x the upper limit of normal) despite a reduction in azathioprine dose (see section 6.1.3 - oral azathioprine treatment protocol)
- A persistent Alkaline Phosphatase (ALP) rise to > 360 IU / L (ie >3x the upper limit of normal) despite a reduction in azathioprine dose (see section 6.1.3 - oral azathioprine treatment protocol)
- A rise in bilirubin to > 40 µmol / L
- Any other adverse event necessitating withdrawal of azathioprine, eg patient intolerance (eg lethargy and nausea) or pancreatitis.

**Other**
- A Serious Adverse Event potentially attributable to radiotherapy or azathioprine.

### 5.3.2 Treatment of Withdrawn Subjects

The patient, Consultant Ophthalmologist and Consultant Radiotherapist responsible for their care, will be informed of their treatment allocation on withdrawal from the trial. If the patient received sham-radiotherapy or placebo tablets (or both) they will be offered the active treatments (if appropriate). Withdrawn patients will also have access to alternative therapies, such as other immunosuppressive agents or surgery.

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5.3.3 Follow-up of Withdrawn Subjects

Care for withdrawn subjects will return to their referring ophthalmologist, however they will also be invited to attend assessment visits at 3, 12 and 36 months, to obtain outcome data in accordance with the planned intention-to-treat analysis.

5.3.4 Management of Data from Withdrawn Subjects

All withdrawn patients will be invited to produce outcome data for the purposes of the planned intention-to-treat analysis (see 5.3.3). Withdrawn patients who have no outcome data will have data imputed using the principle of last observation carried forward. They will not be 'replaced'.

6 TRIAL TREATMENTS

6.1 Trial Interventions

6.1.1 Radiotherapy Protocols

ST BARTHOLOMEW’S HOSPITAL (LONDON), THE CHRISTIE HOSPITAL (MANCHESTER), AND THE BEATSON ONCOLOGY CENTRE (GLASGOW)

1. Planning

- Orbital CT scan
- The lens, orbit and pituitary are outlined. The post orbital cone should be encompassed by the 95% isodose and the maximum dose to the lens is < 10%. A dosimetric plan is produced and “signed off” by the appropriate Clinical Oncologist.
- The plan is verified prior to starting treatment

2. Immobilisation

- Full head shell (individually made for each patient)
3. Treatment

- Prior to starting treatment appropriate data checks are completed
- **Field Arrangement**
  Generally a 5 x 5cm lateral field planned to lens and orbit position (fields may not be truly opposed if lens dose > 10%)
- **Dose**
  20 Gy to the retrobulbar orbit
- **Fractionation**
  12 Fractions over 2.5 – 3 weeks

**BRISTOL ONCOLOGY CENTRE**

1. **Planning**

- The patient is marked up on the treatment machine.
- The patient lies supine with an appropriate firm head-rest such that the outer canthus and the tragus lie on a vertical line. The distance from chin to the supra-sternal notch is measured and recorded

2. **Immobilisation**

- SP12 with tape across the forehead

3. **Apparatus**

- 6-MV linear accelerator
4. **Treatment**

- **Isodose Planning & Verification**
  
  Isodose plans are not produced as a routine. Electronic portal imaging devices (EPIDs) will be used on both fields on day 1, and checked by the Clinical Oncologist.

- **Field Arrangement**
  
  4cm x 4cm lateral opposed fields (using asymmetric jaws to eliminate divergence towards the contra-lateral lens)

- **Setting-up Details**
  
  - Lasers are used to ensure symmetry as follows:
    - Lt → Rt. = Patient midline
    - Sup → Inf. = Through outer canthus
  
  - The patient is planned using a 6 cm wide field with the asymmetric jaws set as below:
    - Post independent Jaw = 4cm
    - Ant independent Jaw = 2cm
    - Gantry = 90º
    - Diaphragm = 90º
  
  - The light beam is set to **just** skim the surface of the ipsi-lateral cornea, by adjusting the couch height as necessary. The post "T" junction and centre (point A) are marked with felt tip pen.
  
  - The gantry is rotated to 270º, and the diaphragm to 270º. Minor adjustments are made to the patient's head and the couch positioned to ensure the light beam **just** skims the surface of the cornea on this side whilst keeping the first centre correct. The post "T" junction and centre (point A) are marked with felt tip pen as before.
  
  - The head is taped, and the alignment of the marks is checked
  
  - The separation is measured at the field centre
  
  - The anterior independent jaw is set to zero

- **Dose**
  
  20 Gy to the retro-bulbar orbit (prescribed as mid-plane dose)

- **Fractionation**
  
  12 Fractions over 2.5 weeks
Although Barts and Bristol Oncology Centre have different planning, immobilisation and treatment protocols (and differences in apparatus mean that this cannot be standardised), the dose, fractionation and field orientation is essentially identical in each Centre.

6.1.2 Sham-radiotherapy Protocols

ST BARTHOLOMEW'S HOSPITAL (LONDON), THE CHRISTIE HOSPITAL (MANCHESTER), AND THE BEATSON ONCOLOGY CENTRE (GLASGOW)

1. Planning, Immobilisation and Apparatus

- Patients in the sham-radiotherapy arm of the study will also have a sham planning CT scan, so that they are not exposed to any ionising radiation.
- Otherwise their planning will be the same as for ‘radiotherapy’ except a generic dosimetric plan will be used as no megavoltage dose is to be received, with downloaded monitor units equal to 0.

2. Treatment

- As for ‘radiotherapy’ except the dose will be zero Gy because the apparatus will not be turned on. Instead, a noise-emitting electronic device will be placed above the gantry of the machine and the patient will hear a similar sound to that which accompanies normal treatment.

BRISTOL ONCOLOGY CENTRE

1. Planning, Immobilisation and Apparatus

- As for ‘radiotherapy’

  (NB There is no ionising radiation exposure in planning)
2. Treatment

- As for ‘radiotherapy’ except the dose will be zero Gy because the apparatus will not be turned on. Instead, a noise-emitting electronic device will be placed above the gantry of the machine and the patient will hear a similar sound to that which accompanies normal treatment.

Any patients starting radiotherapy more than 9 weeks after randomisation will be considered to be outside the protocol, and therefore will not be allowed to proceed with trial treatment.

6.1.3 Oral Azathioprine Treatment Protocol

AZATHIOPRINE STARTING DOSE (2-3mg / kg):

<table>
<thead>
<tr>
<th>BODY MASS</th>
<th>AZATHIOPRINE STARTING DOSE (Once Daily)</th>
<th>NUMBER OF AZATHIOPRINE 50mg TABLETS (per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50 kg</td>
<td>100 mg</td>
<td>2</td>
</tr>
<tr>
<td>50 – 79kg</td>
<td>150 mg</td>
<td>3</td>
</tr>
<tr>
<td>≥ 80 kg</td>
<td>200 mg</td>
<td>4</td>
</tr>
</tbody>
</table>
MONITORING FOR ADVERSE EVENTS

- Full Blood Count (FBC) weekly for the first 4 weeks of treatment, then every 8 weeks
- Liver Function Tests (LFTs) at week 4, then every 8 weeks.

DOSE ADJUSTMENT IN RESPONSE TO LFT CHANGE

Alanine aminotransferase (ALT)

1. If ALT >120 IU/L (ie >3x the upper limit of normal), repeat in 2 weeks and see if upwards or downwards trend
2. If repeat ALT 2 weeks later is ≤ the last value:
   a. Continue treatment and do not change Azathioprine dose.
   b. Re-check ALT in 8 weeks
      If this is:
         i. <120 IU/L: continue at same dose and repeat LFT in 8 weeks (when return to 1)
         ii. ≥120 IU/L: Go straight back to 1
3. If repeat ALT 2 weeks later is > the last value:
   a. Reduce Azathioprine dose by 50mg/day
   b. Re-check LFTs in 2 weeks
      If repeat ALT 2 weeks after this dose change is ≤ the last value:
         i. Continue treatment at the reduced dose.
         ii. Re-check LFTs in 8 weeks (when return to 1)
      If repeat ALT 2 weeks after this dose change is > the last value:
         iii. Stop azathioprine and exit the trial
4. If ALT >275IU/L at any point, stop azathioprine and exit the trial
5. If ALT ≥120 when returning to 1 for a second time, discuss with unmasked investigator

Alkaline Phosphatase (ALP)

6. If ALP > 360 IU/L (ie >3x the upper limit of normal), repeat in 2 weeks and see if upwards or downwards trend

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7. If repeat ALP 2 weeks later is ≤ the last value:
   a. Continue treatment and do not change Azathioprine dose
   b. Re-check ALP in 8 weeks
      If this is:
      i. <360 IU/L: continue at same dose and repeat LFT in 8 weeks (when return to 6)
      ii. ≥360 IU/L: Go straight back to 6

8. If repeat ALP 2 weeks later is > the last value:
   c. Reduce Azathioprine dose by 50mg/day.
   d. Re-check LFTs in 2 weeks
      If repeat ALP 2 weeks after this dose change is ≤ the last value:
      i. Continue treatment at the reduced dose
      ii. Re-check LFTs in 8 weeks (when return to 6)
      If repeat ALP 2 weeks after this dose change is > the last value:
      iii. Stop azathioprine and exit the trial

9. If ALP > 630 IU/L at any point, stop azathioprine and exit the trial

10. If ALP > 360 IU/L when returning to 6 for a second time, discuss with unmasked investigator.

Bilirubin

11. If bilirubin > 40 µmol/L, stop azathioprine and exit the trial

DOSE ADJUSTMENT IN RESPONSE TO LYMPHOPENIA

12. If lymphocyte count < 0.5x10^9/L reduce azathioprine dose by 50mg/day and repeat FBC in 2 weeks

13. If repeat lymphocyte count ≥ 0.5x10^9/L
   a. Repeat FBC in 2 weeks
      i. If repeat lymphocyte count remains ≥ 0.5x10^9/L continue treatment at reduced dose and repeat FBC in 8 weeks (when return to 12)
      ii. If repeat lymphocyte count < 0.5x10^9/L, stop azathioprine and exit the trial

14. If repeat lymphocyte count < 0.5 x 10^9/L, stop azathioprine and exit the trial

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15. If repeat lymphocyte count <0.5x10^9/L when returning to 12 for a second time, discuss with unmasked investigator
6.1.4 Placebo

PLACEBO STARTING ‘DOSE’

<table>
<thead>
<tr>
<th>BODY MASS</th>
<th>NUMBER OF PLACEBO TABLETS (per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50 kg</td>
<td>2</td>
</tr>
<tr>
<td>50 – 79kg</td>
<td>3</td>
</tr>
<tr>
<td>≥ 80 kg</td>
<td>4</td>
</tr>
</tbody>
</table>

Hence the number of placebo tablets matches the number of Azathioprine (50mg) tablets given to a patient of equivalent body mass in the active treatment group.

BLOOD MONITORING AND TABLET NUMBER ADJUSTMENT

Patients randomised to receive placebo tablets will have the same blood tests as the azathioprine treated group.

≤5% of the placebo treated group will be randomly selected and recalled for repeat blood tests (to match the management of azathioprine treated patients). The number of placebo tablets given to these trial subjects will also vary (to mimic the azathioprine dose titration)

6.2 Standardised Treatments (Common to all trial arms)

On enrolment all patients will receive a standard combination of oral steroid, bisphosphonate and proton-pump inhibitor (PPI) treatment.

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6.2.1 Oral Prednisolone

Non-enteric coated preparations will be used unless the patient suffers indigestion with this form of medication despite PPI therapy. All patients will receive a course of prednisolone according to the protocol below.

<table>
<thead>
<tr>
<th>TIME AFTER ENROLMENT</th>
<th>PREDNISOLONE DOSE (mg per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 3 days</td>
<td>80</td>
</tr>
<tr>
<td>4 – 7 days</td>
<td>60</td>
</tr>
<tr>
<td>2\textsuperscript{nd} week</td>
<td>40</td>
</tr>
<tr>
<td>3 – 6 weeks</td>
<td>30</td>
</tr>
<tr>
<td>7 – 10 weeks</td>
<td>20</td>
</tr>
<tr>
<td>11 – 14 weeks</td>
<td>15</td>
</tr>
<tr>
<td>15 – 18 weeks</td>
<td>10</td>
</tr>
<tr>
<td>19 – 20 weeks</td>
<td>7.5</td>
</tr>
<tr>
<td>21 – 22 weeks</td>
<td>5</td>
</tr>
<tr>
<td>23 – 24 weeks</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\textbf{NB} Intravenous methyl-prednisolone will not be used (to maximise recruitment of patients from a wide geographical area around the Trial Centres and facilitate their management on an outpatient basis).
6.2.2 Treatments to prevent steroid-associated side-effects

- **Bisphosphonates**
  
  Either:
  - *Risedronate Sodium (Actonel®)* 35mg po once weekly
  - *Alendroic Acid (Fosamax®)* 70mg po once weekly

  To continue for the duration of oral prednisolone treatment

- **Proton pump inhibitors:**
  
  To be prescribed if patients have a previous history of upper-gastrointestinal inflammation (with or without steroid use), or report indigestion on taking oral prednisolone.

  Either:
  - *Lansoprazole (Zoton®)* 15mg po OD (for prophylaxis), or 30mg po OD (if symptomatic)
  - *Omeprazole* 10mg po OD (for prophylaxis), or 20mg po OD (if symptomatic)

  To continue for the duration of oral prednisolone treatment or to use as required.
6.3 Endocrine Management

**Hyperthyroid Patients** will be treated with carbimazole 40mg daily until euthyroid and then transferred to block-replace treatment (carbimazole 40mg and thyroxine 100µg daily).

**Hypothyroid Patients** will be treated with thyroxine, and the dose adjusted to normalise TSH levels.

**Previously Hyperthyroid Patients** (treated in the last 1 year) will also receive block-replace therapy (as above) to ensure stable thyroid function.

**Previously Hypothyroid Patients** will have their dose adjusted (if required) according to their TSH levels.

**Euthyroid Patients** (5% of patients with TED have normal thyroid hormone levels) will not require any treatment for thyroid disease, but their Thyroid Function Tests (TSH, FT3, FT4) will be monitored at 3 monthly intervals and treated (as above) if changes develop.

### ENDOCRINE MANAGEMENT ALGORITHM

- **TSH, FT4+ FT3 in reference range**
  - **No – FT4 low**
    - 
    - **Or TSH > 4µu/l**
      - Begin or increase dose of thyroxine by 50µg
      - Recheck TSH, FT4, FT3 in 6 weeks
  - **No but TSH not raised And FT4/FT3 in Reference range**
    - If on thyroxine or no treatment - No change in endocrine treatment
    - If on carbimazole alone, ensure dose is 40mg and add thyroxine 100ug/day
    - Recheck TSH, FT4, FT3 in 6 weeks
  - **Yes – TSH suppressed AND FT3 or FT4 raised**
    - If on no Rx, begin carbimazole\(^1\) 40mg/day
    - If on thyroxine, reduce dose by 25-50ug/day
    - If on carbimazole and thyroxine, reduce thyroxine by 25ug/day
    - If on carbimazole alone\(^2\) – increase/ensure dose to/is 40mg
    - Recheck TSH, FT4, FT3 in 3 months

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\(^1\)If intolerant of carbimazole or on PTU, discuss with trial endocrinologist

\(^2\)If this has already been done once, and patient remains thyrotoxic, discuss with trial endocrinologist

NB IF ON CARBIMAZOLE AND PT DEVELOPS SORES IN MOUTH WITHOLD MEDICATION + CHECK WCC within 24 hours – IF NEUTS< 1.0 discuss with trial endocrinologist within 24 hours
6.3.1 Responsibility for Endocrine Care

Patients will either transfer responsibility for their Endocrine care to the trial team (under the supervision of the Endocrine Chief Investigator) for the duration of their participation in the study, or if they prefer to remain under the care of their own Endocrinologist the trial team will liaise closely with them in order to ensure that the patient is rendered euthyroid and maintained euthyroid for the duration of the trial.

6.4 Smoking advice

All trial subjects will be advised to stop smoking. We will monitor changes in smoking habits.

7 ASSESSMENT OF TREATMENT EFFICACY

Trial subjects will be seen 2 weeks after enrolment to determine their response to, and tolerability of, steroids. If they have had TED for more than 6 months and their Clinical Activity Score (CAS) has not improved in at least one point at this 2 week visit, they are unlikely to benefit from Radiotherapy and / or Azathioprine. Consequently they will be withdrawn from the trial and will not be randomised.

All recruits who either have had TED for less than 6 months, or have improved in at least one CAS item in response to steroids, will be randomised to receive either radiotherapy, azathioprine or their placebos at their visit 2 weeks post enrolment. They will be reviewed again four weeks later to ensure that their TED is not flaring up on steroid taper and that they are tolerating Azathioprine before starting radiotherapy; then again 6 weeks later for the short-term outcome assessment (about 5 weeks after completing radiotherapy), and subsequently every 3 months until the trial exit assessment at 1 year (with an optional long-term follow-up assessment at 3 years).

7.1 Standard Clinical Assessments at each Follow-up Visit

The primary outcome measures for the trial are based on Clinical Measures of TED Severity and Activity. These will be assessed by the Clinical Investigators at each follow-up visit and their measurement recorded on the trial’s Clinical Report Form.

The key components of the follow-up visits are:

Consultation

To establish:

- Changes in the activity and severity of TED symptoms

To record:

- Changes in medications (including thyroid treatments)
- Changes in smoking habit
- Adverse events

**Ocular Examination**
This will be conducted by the Clinical Investigators and Hospital Orthoptists (+/- Clinical Research Nurses) to assess:

- Snellen Visual Acuity
- Eyelid redness and swelling
- Conjunctival redness and chemosis
- Caruncular swelling
- Palpebral aperture
- Corneal integrity
- Proptosis (using an Oculus® exophthalmometer)
- Oculomotility, including
  - Monocular Ductions in six directions of gaze
  - Gorman's Diplopia score
- Intraocular pressure in primary position and upgaze
  - Optic Nerve Function, including
    - Colour Vision (Ishihara assessment)
    - Relative Afferent Pupillary Defect
    - Visual Fields
    - Optic disc assessment

**General Examination**

- Weight / height
- Blood Pressure
- Urinalysis

**Investigations**

- Blood tests:
  - Thyroid Function Tests (TSH, FT4 & FT3) 6 – 12 weekly (see flow chart in 6.3)
Trial subjects will also have blood tests performed by their General Practitioner or the Trial Centre (whichever is more convenient) every week for 4 weeks post randomisation and 2-monthly thereafter (see section 6.1.3) to monitor their tolerance of azathioprine.

7.2 Patient-Centred Measures of Psychosocial Distress, Disease Severity and Treatment Efficacy

It is widely acknowledged that psychological issues related to appearance have particular importance in TED, however the implications of this in terms of anxiety, social avoidance and depression are poorly understood. This assessment seeks to investigate these issues and to evaluate patient’s perceptions of their visual function and appearance in the analysis of treatment efficacy.

Plan of Investigation

All trial subjects will be asked to complete 2 standardised psychosocial measures at recruitment, 3 months and 1yr (trial end):

- Hospital Anxiety and Depression Scale (HADS)
- Derriford Appearance Scale-Short Form measuring appearance-related concerns and social avoidance.
- WHOQoL-Brief Quality of Life Assessment Score

These have been chosen on the basis of a review of the literature to identify the psychological factors most commonly implicated in appearance-related distress, their suitability in the assessment of psychosocial need in people with disfiguring conditions, and for their ease of completion in a clinic setting.

In addition trial recruits will be asked to complete an English-language version of the recently developed Graves’ Ophthalmopathy Quality of Life (GO-QoL) questionnaire; a disease-specific health-related QoL measure.

Recruits will also be asked if they are willing to participate in semi-structured interviews. Of those who are, a subset of patients (ensuring proportional representation of age, gender, trial treatment and trial centre) will be selected for interview on the basis their response to the baseline questionnaires (targeting those with high and low levels of distress). The interviews will explore their individual concerns, strengths and difficulties in social functioning, coping strategies, levels of perceived support and the personal financial consequences of their disease.

Interviews will be either face-to-face or conducted over the telephone. They will be tape recorded and subsequently transcribed, after which the tapes will be erased. Transcriptions will also be destroyed at the end of the study.
7.3 Health Economic Evaluation

The primary intention of the economic evaluation is to compare the costs and benefits of: (i) radiotherapy; and (ii) azathioprine; versus placebo in treating trial subjects.

The different methods of patient care will be evaluated from the viewpoint of the National Health Service (NHS) and patients/carers. We will also attempt to estimate the societal effect of time off work due to TED.

1. Measurement of health outcomes

Evidence for the effectiveness of the different treatments will be collected through the trial's clinical and psychosocial assessments. Costs will be related to the percentage of patients responding to treatment, using the binary long-term primary clinical outcome measure. Incremental cost effectiveness ratios will be formed, which will estimate the extra cost per extra patient responding to (i) radiotherapy; and (ii) dual-therapy at 11 months.

2. Identification of relevant costs / resource use

The cost of monotherapy and dual-therapy with and without radiotherapy will be compared from the point at which patients are randomised, and will include all relevant resources under the control of the NHS, all out-of-pocket expenses incurred by patients and carers, and time off work due to thyroid eye disease.

The costs to the NHS identified as being of relevance in the analysis are:

- Primary care consultations: General Practitioner (face-to-face) General Practitioner (telephone), Practice Nurse/Nurse Practitioner, Out-of-Hours telephone consultation, Out-of-Hours face-to-face consultation, Home visits.
- Walk-in Centre
- NHS Direct
- Secondary care contacts:
  - Out-patient visits, including routine patient monitoring of TED, which involves a complex series of eye movement tests performed by orthoptists and involving specialist equipment. These assessments can take up to 45 mins to perform and patients will also be photographed to obtain an objective record of their appearance. As TED can be severely disfiguring these appointments may also involve a specialist nurse or counsellor.
  - Treatment costs including the cost of radiotherapy and blood tests, and radiological investigations.
- Out-patient visits at an endocrinology clinic to monitor and treat the underlying thyroid disease
- Accident & Emergency visits
- Inpatient stays, including surgical intervention. Patients with increased severity of disease may require ‘emergency’ surgery to prevent optic nerve compression and others may require rehabilitative surgery.

- Prescribed medication. This will include medication to treat TED and also the underlying thyroid disease.
- The cost of prisms. Patients with double vision require prisms to be fitted to their glasses; this may be done by an orthoptist or an optometrist.

The costs to the patients and carers will include:

- Travel costs incurred during visits to health care facilities
- Use of over-the-counter drugs, for example lubricating eye drops and oral analgesics
- Prescription charges
- Expenditure on complementary and alternative therapy, counselling, and support groups, plus associated travel costs
- The cost of glasses / sunglasses
- Cosmetic-related purchases, for example, make-up
- Expenditure on smoking
- Cost of child care and care of other dependents
- Loss of earnings

The costs to society will include:

- Time off work
3. Measurement of resource use (data collection)

Data on resource use associated with the interventions will be collected during the study by patients in their Trial Diary.

*Information collected from the diary will include:*

- Number of visits, where they took place, who seen, and treatment provided for:
  - Intervention treatment
  - Routine monitoring and treatment of TED
  - Routine monitoring and treatment of underlying thyroid disease
  - Primary and secondary non-routine health care relating to TED and underlying thyroid disease
- Prescribed medication: name of preparation, strength, dosage form, quantity
- Use of:
  - Out-of-Hours care
  - Walk-in Centre
  - NHS Direct
- Travel costs for all health care visits: mode of travel and distance, fares paid and car parking charges
- Over-the-counter medication purchased and cost
- Cost of complementary and alternative medication and associated travel costs
- Cost of counselling and/or support group, and associated travel costs
- Cost of glasses, sunglasses, and any cosmetic-related purchases
- Expenditure on smoking
- Cost of childcare and care of other dependants
- Loss of earnings
- Time off work and employment details

4. Valuation of resource use

National data sets such as the Unit Costs of Health and Social Care (http://www.kent.ac.uk/PSSRU/) will be used to value primary care contacts.

Visits to Walk-in Centres and calls to NHS Direct will be valued using information in the National Evaluations.

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The British National Formulary (http://bnf.vhn.net/bnf) will be used to value prescribed medication.

Secondary care use will be valued using the Department of Health National Reference Costs (http://www.doh.gov.uk/nhsexec/refcosts.htm).

5. Discounting
Costs and outcomes will not be discounted, as the study will be limited to a period of 12 months.

6. Uncertainty
Sensitivity analysis will be conducted in areas where there is uncertainty around assumptions about resource use measurement and/or valuation.
7.4 Outcome Measures

7.4.1 Primary

7.4.1.1 Binary Composite Clinical Outcome Measure
Response to treatment will be compared between treatment groups 12 months after enrolment (11 months after randomisation). This will be determined using a system of major and minor criteria modified from others\textsuperscript{10, 16, 19}. Each component of the composite score will be assessed according to the trial's Standard Operating Procedures.

**Major Criteria**
- An improvement of $\geq 1$ grade in diplopia score\textsuperscript{54}
- An improvement of $\geq 8^\circ$ of eye movement in any direction\textsuperscript{53}
- A reduction of $\geq 2$ mm in proptosis

**Minor Criteria**
- A reduction of $\geq 2$ mm in lid aperture
- An improvement of $\geq 1$ grade in soft tissue involvement\textsuperscript{46}
- An improvement in best-corrected visual acuity of $\geq 1$ line on the Snellen chart.
- Subjective improvement\textsuperscript{10}

**Response to Treatment**

- **Very good:** $\geq 2$ major criteria
- **Good:** 1 major criterion
- **Fair:** $\geq 2$ minor criteria
- **No Change:** 1 minor criterion
- **Worse:** Deterioration in at least 1 major or 2 minor criteria.

**Response Rate**

Number of patients with a fair, good or very good response to treatment versus the number of patients who have no change or are worse (expressed as a percentage).
7.4.1.2 Ophthalmopathy Index

Response to treatment will also be compared between treatment groups 12 months after enrolment (11 months after randomisation) with the Ophthalmopathy Index. This measure of disease severity is calculated by summatng the values in the right-hand column of the following table. Each component of the Index will be assessed according to the trial’s Standard Operating Procedures.

<table>
<thead>
<tr>
<th>DEGREE OF INVOLVEMENT</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soft Tissue Inflammation</strong></td>
<td></td>
</tr>
<tr>
<td>Slight</td>
<td>1</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
</tr>
<tr>
<td><strong>Exophthalmos (mm)</strong></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.2</td>
</tr>
<tr>
<td>17</td>
<td>0.4</td>
</tr>
<tr>
<td>18</td>
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<td>21</td>
<td>2</td>
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<td>22</td>
<td>3</td>
</tr>
<tr>
<td>≥23</td>
<td>4</td>
</tr>
<tr>
<td><strong>Palpebral Aperture (mm)</strong></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.15</td>
</tr>
<tr>
<td>9</td>
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<td>0.7</td>
</tr>
<tr>
<td>8</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Diplopia</strong></td>
<td></td>
</tr>
<tr>
<td>Intermittent</td>
<td>1</td>
</tr>
<tr>
<td>Inconstant</td>
<td>2</td>
</tr>
<tr>
<td>Constant</td>
<td>3</td>
</tr>
</tbody>
</table>
## 7.4.2 Co-Primary Outcome Measure

### 7.4.2.1 Clinical Activity Score

The average change in Clinical Activity Score (CAS) between enrolment and the short-term outcome assessment (6 weeks after radiotherapy) will be compared between treatment groups. CAS is calculated by assessing the presence or absence of each of the following clinical features using the trial’s Standard Operating Procedures.

1. **Pain**
   - Pain on eye movement in the last 4 weeks
   - Painful, oppressive feeling on or behind globe in the last 4 weeks

2. **Redness**
   - Conjunctival redness
   - Eyelid redness

3. **Swelling**
   - Chemosis
   - Swollen caruncle
   - Eyelid oedema
   - Increasing proptosis of > 2mm

4. **Impaired Function**
   - Decreasing visual acuity of > 1 snellen line
   - Decreasing eye movement of ≥ 8°

Each feature = one point. The maximum score is 10 at each follow-up visit and 7 at enrolment (because there will be no previous records to determine changing measurements of proptosis, visual acuity and eye movements).

---

### Cornea

<table>
<thead>
<tr>
<th>Initial Lesions</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcers</td>
<td>2</td>
</tr>
<tr>
<td>Clouding / Perforation</td>
<td>3</td>
</tr>
</tbody>
</table>

### Optic Neuropathy

<table>
<thead>
<tr>
<th>Abnormal VEP</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA = 0.5 – 0.9</td>
<td>5</td>
</tr>
<tr>
<td>VA = 0.1 – 0.4</td>
<td>7</td>
</tr>
<tr>
<td>VA &lt; 0.1</td>
<td>9</td>
</tr>
</tbody>
</table>
7.4.3 Secondary

7.4.3.1 Clinical Measures

- Total Eye Score (TES) 8

7.4.3.2 Psychosocial Measures

The following will be measured to assess psychosocial effects of TED, and to compare psychosocial responses across treatment groups.

- Hospital Anxiety and Depression Scale (HADS) score57
- Derriford Appearance Scale-Short Form score58
- Graves’ Ophthalmopathy Quality of Life (GO-QoL) score61
- WHOQoL-Brief Quality of Life Assessment Score
- Open-ended responses to interview questions

7.4.3.3 Health Economic Measures

The cost of TED and its treatment will be measured from the perspective of:

- The National Health Service (NHS)
- The patient
- Society

and compared across treatment groups.
8 TREATMENT SAFETY

There is established clinical experience of both trial interventions, and it is unlikely that this trial will contribute significantly to published safety data. However, adverse events will be monitored, reported in the trial publications and to the Trial Sponsor and Medicines and Healthcare products Regulatory Agency (MHRA) if appropriate.

8.1 Adverse Events

An adverse event is any untoward medical occurrence in a patient administered a pharmaceutical product, without regard to the possibility of a causal relationship. These will be recorded for all trial subjects after enrolment.

8.2 Serious Adverse Events

Serious adverse events are:
- Fatal
- Life-threatening
- Disabling or incapacitating
- Hospitalising (or cause prolonged hospitalisation)
- The cause of a congenital abnormality or birth defect

If these occur they will be reported to the Trial Sponsor and Medicines and Healthcare products Regulatory Agency (MHRA).

8.3 Prevention of Adverse Events

The trial exclusion criteria prevent patients especially at risk of developing adverse events from participating in the trial. In particular, patients with abnormal thiopurine methyltransferase TPMT activity who are likely to develop bone marrow suppression (low activity) or hepatotoxicity (high activity) with azathioprine will not be enrolled. Also, diabetic patients who are likely to develop complications with high dose steroid use and are at increased risk of developing retinopathy with radiotherapy will not take part.

8.4 Eliciting Adverse Events

All trial subjects will have an enquiry about co-morbidities, medications and new or unexpected symptoms at each follow-up visit, as well as an ophthalmic and general examination. Blood tests to monitor for azathioprine’s potential adverse effects will be carried out in accordance with published guidance.
9 PLANNED ANALYSES

9.1 Interim

See sections 4.6.1.1 and 4.6.1.4.

9.2 Final

Data analysis will proceed according to CONSORT guidelines for randomised controlled trials. The first stage of the analysis will be to use descriptive statistics to describe the group of individuals recruited to the trial in relation to those eligible, and to investigate comparability of the trial arms at baseline. The primary intention-to-treat analyses will comprise comparisons between radiotherapy versus sham-radiotherapy, and azathioprine versus placebo, for each of the two primary outcomes at 12 months follow up. Outcomes for patients participating in the optional long-term follow-up study will also be compared 3 years post-enrolment. The comparisons will be made using appropriate (that is, logistic or linear) multivariable regression models, adjusting for minimisation variables, the factorial design, and the value of the outcome variable at baseline. Full attention will be paid to the estimates and the confidence intervals for these comparisons as well as the p-values. Secondary analyses will then be conducted using regression models with further adjustment for any prognostic factors that exhibit marked imbalance at baseline. Patients who have no outcome data for the primary analyses will have data imputed using last observation carried forward. Patients who have become non-compliant for any reason will still be invited to produce outcome data such that they can be included in the intention to treat analyses. The assumptions required for the regression models will be investigated using appropriate diagnostic plots, and actions such as transformation of continuous outcome variables taken as necessary.

The co-primary outcome Clinical Activity Score at 3 months follow up and all secondary outcomes will be analysed in the same way, using appropriate (linear or logistic) regression models depending on the nature of the outcome measure. Bonferroni corrections for multiple testing will be considered for the secondary outcomes.

Other secondary analyses will involve: (a) investigation of any interaction between the two interventions for each of the two primary outcomes; (b) pre-planned sub-group analyses to ascertain any differential effects of the interventions according to steroid use versus no steroid use in the six months prior to enrolment. These secondary analyses are readily performed as extensions to the multivariable regression models described above, by simply introducing the appropriate interaction terms. However, the precision of the estimates of interaction is very likely to be too
poor, and high p-values will most likely reflect low power and so cannot be taken as evidence for no interactions.

**9.2.1.1 Health Economic Indices**

Costs will be related to the percentage of patients responding to treatment. Incremental cost effectiveness ratios will be formed, which will estimate the extra cost per extra patient responding to (i) radiotherapy; and (ii) azathioprine 11 months after randomisation, and (for patients participating in the long-term follow-up study) 3 years after enrolment.

A secondary analysis will estimate the cost-of-illness of Thyroid Eye Disease from a societal perspective.

**10 DATA HANDLING AND RECORD KEEPING**

Source documents produced for this trial will be kept in the patient’s hospital records and source data will be transcribed into trial-specific Clinical Record Forms (CRFs) at the end of each patient visit. This will be checked and signed by the Clinical Investigators at each Trial Centre. CRF data will then be transferred onto a bespoke database prior to data analysis. All electronically stored patient information will be anonymised and patients’ personal information will be stored on a paper record in a locked secure location in each Centre’s Clinical Trial Facilities.

**11 ETHICS**

The trial protocol, consent documentation, patient information sheets, standard correspondence with the patient’s general practitioner and ethics application form received a favourable opinion from a UK Main Research Ethics Committee (REC), and the appropriate Local RECs prior to starting recruitment at each Centre.

**12 FINANCIAL AND INSURANCE DETAILS**

Support for this trial has been granted by the following charitable bodies:

- **The National Eye Research Centre:**
  For administrative and clinical research support; Investigational Medicinal Product (IMP) Manufacture (placebo tablets) and packaging (placebo and azathioprine tablets); and radiotherapy technician time

- **Moorfield’s Eye Hospital Special Trustees:**
  For Clinical Research Fellow and administrative support.
▪ **Medical Research Committee of the Charitable Trusts for the United Bristol Hospitals:**
  For the psychosocial outcome assessments and ancillary studies conducted in collaboration with The Centre for Appearance Research at the University of the West of England.

▪ **National Hospital for Neurology and Neurosurgery and Moorfields Special Trustees**
  For the ancillary Magnetic Resonance Imaging Study on London patients (see section 15.5 and Appendix 1).

The University of Bristol has arranged clinical research insurance to cover the legal liability of the University for both negligent and non-negligent harm. In addition the study doctors hold substantive or honorary NHS contracts, giving them the protection of the NHS clinical negligence arrangements.
13 PATIENT INVOLVEMENT

13.1 Thyroid Eye Disease Charitable Trust (TEDct)

The Endocrine Chief Investigator is chairman of the TEDct, an active national organisation with 800 members. The trial was presented and discussed at their meeting at Moorfields Eye Hospital on 12 February 2005 and it received strong support. Their members are very aware of the varying clinical practices for this condition (some had received radiotherapy, others not; some had been on immunosuppression, others not) and because of the impact these treatments had on their lives they were very keen to see a proper evidence base established to inform management guidelines and ensure that future TED sufferers have access to high-quality, standardized care. Our efforts to integrate endocrine and ophthalmic care were also strongly endorsed. A member of the Trust advised on the contents of the trial’s Patient Information Sheets and Introductory Letter.

14 PUBLICATION POLICY

The results of this trial will be submitted for publication in a peer-reviewed medical journal regardless of whether the outcome is in favour of the trial interventions.

15 ANCILLARY STUDIES – Project summaries

Patients will also be invited to participate in the following optional investigations, and may continue to do so after trial withdrawal (even if this is precedes randomisation) or completion.

15.1 Tear Cytokines

New laboratory tests (using flow-cytometric bead arrays) enable the concentrations of inflammatory mediators (cytokines) to be measured in micro-litre volumes of fluid (such as tears).

Pilot data suggests that cytokines released in orbital inflammation, such as TED, may be present in tears. If so, tear cytokine assays may be used to develop a new clinical test to quantify the severity of an individual’s orbital inflammation and its potential to respond to immunosuppressive therapy.

Tear samples may be taken from both eyes of trial participants at each visit using standard clinical techniques. Tear cytokine levels will then be correlated with clinical measures of disease severity and response to immunosuppression.
15.2 Steroid Sensitivity Assays

A new laboratory assay is being developed in Bristol to predict and monitor an individual’s response to steroids and other immunosuppressive drugs. This has the potential to enable drug treatments to be tested and tailored to an individual before they start therapy, which would be of great benefit to patients with TED and other inflammatory conditions.

CIRTED recruits will have a course of high-dose steroids at the start of the trial and the clinical response to this treatment will be prospectively documented using standardized measures of disease severity. Hence, they are an ideal cohort of patients on which to test the predictive power of this new steroid sensitivity assay.

Separate consent will be taken from patients for this optional study. Two large blood samples (50mls each) will be required, one on enrolment and another four weeks after starting steroids.

15.3 Azathioprine Metabolite Assays

There is some evidence that azathioprine metabolite assays can be used to predict disease response in patients with inflammatory bowel disease. If this is true for patients with TED it would enable an individual’s drug dose to be titrated to ensure that the concentrations of active metabolites in their blood were adequate to achieve an optimal disease response. It is currently thought that most patients who fail treatment do so because they have an inadequate drug dose, rather than because they are resistant to therapy. The purpose of this study (which is being performed in collaboration with the Purine Research Laboratory, Guy’s and St Thomas’ Hospital, London) is to establish whether the concentrations of Azathioprine metabolites in CIRTED patients’ blood correlates with their disease severity and response to immunosuppression.

Patients will be asked for a single 5ml blood sample at the short and long-term follow-up assessments (3 and 11 months after randomisation).

15.4 Thyroid Stimulating Antibody Levels

The purpose of this study is to correlate the concentration of Thyroid Stimulating Antibodies (using newly available assays) in the serum of CIRTED patients with their disease severity and response to treatment.

Patients may be asked for a single small blood sample (5mls) at each follow-up visit.

CIRTED Protocol v5.3, 2 June 2011
15.5 Magnetic Resonance Imaging (MRI)

Up to fifty of the patients recruited in London will be invited to participate in this ancillary study, the primary goals of which are to validate the use of MRI as a measure of orbital inflammation to use in future studies of immunosuppressive therapy in Thyroid Eye Disease (TED) and to evaluate the use of a novel MRI technique (using Diffusion and Magnetism Sequences) in grading TED severity. Each MRI study participant will have up to 3 scans - at trial enrolment, 3 months and 12 months (see separate MEH / NHNN TED MRI protocol) with the option of undertaking repeat images if required.

15.6 Future Studies

A small blood sample (5mls) may be taken at each visit and stored in our laboratories for use in future TED research. The exact nature of the tests which will be performed on these samples is not known and they will be anonymised so that they will not be linked to any patient-identifiable information. This means that trial subjects will never be able to find out the results of tests done on these stored samples and the investigators will not be able to trace results back to an individual patient.

Anonymised tear samples may also be stored after the end of the trial.
16 DIAGRAMATIC TRIAL OVERVIEW

Pre-enrolment screening

START ORAL STEROIDS ---- Recruitment ---- 2 weeks

Recruitment ---- Randomisation ---- 0 weeks

AZATHIOPRINE AZATHIOPRINE PLACEBO PLACEBO

AZATHIOPRINE SHAM - RADIOTHERAPY RADIOTHERAPY SHAM - RADIOTHERAPY

RADIOTHERAPY

12 weeks

STOP STEROIDS AFTER 6 MONTHS

Follow-up visits @ 12 weekly intervals

Completion

Optional review

48 weeks

17 ACKNOWLEDGEMENTS

The investigators wish to thank Dr Chris Probert, Professor of Gastroenterology at the University of Bristol and University Hospitals Bristol, for his advice on the Azathioprine prescribing and monitoring protocol for this trial; and Rosemary Greenwood, Medical Statistician, University Hospitals Bristol Research and Development Support Unit for her advice on the initial trial design.
18 REFERENCES


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CIRTED Protocol v5.3, 2 June 2011


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Click here to download Necessary Additional Data: CONSORT_Checklist_CIRTED_R2.doc