End of Study Report

Title:

Short title: The Role of Avastin and 5-fluorouracil in Trabeculectomy Surgery (RAFTS)

Long title: The use of bevacizumab as a modulator of wound healing following trabeculectomy surgery: A Single Centre Randomised Controlled Phase III Pilot Study

Background and objectives

Filtration surgery remains the most effective surgical strategy to control intraocular pressure in glaucoma. It however lacks the high level of predictability that we expect from other types of ocular surgery, such as phacoemulsification. The predictability in the outcome of this procedure is hampered by the body's natural tendency to heal. Tenon's fibroblasts are the main effector cells in the initiation and mediation of wound healing after trabeculectomy surgery.

During the process of wound healing, fibrosis develops in the conjunctiva and episclera because of progressive fibroblast migration, proliferation and collagen deposition at the filtration site, as well as angiogenesis.

Pharmacological enhancement of trabeculectomy with adjunctive use of antifibrotic agents such as 5-fluorouracil (5-FU) and mitomycin-C (MMC) has significantly improved the success rate of filtration surgery in terms of IOP control, but at the expense of poorer filtration bleb and conjunctival morphology. Current practice is to perform standard trabeculectomy and augment with one of the above antifibrotic agents (5-FU or MMC). The non-specific mechanisms of action of 5-FU and MMC, particularly MMC, can cause widespread cell death and apoptosis, leading to bleb leaks and thin cystic blebs. Potentially sight-threatening complications such as severe post-operative hypotony, blebitis and endophthalmitis may result. Thus alternative wound-healing modulators with fibroblast- specific activity and predictability are needed.

VEGF has a pivotal role in the wound healing response. It has been shown to be a mediator in the signal transduction cascade leading to fibroblast migration and proliferation. It is also a powerful inducer of angiogenesis, which allows early migration of inflammatory cells and fibroblasts.

In 1994, Wong observed that angiogenesis inhibitors had marked inhibitory effects on both human tenon's fibroblast proliferation and migration in vitro.[3]

The purpose of this trial is to look at the wound healing response modification by bevacizumab after trabeculectomy surgery.

More predictable modulators of wound healing are required. Changes in technique have led to improvements in outcomes with lower rates of bleb related infections and bleb leaks reported in results of modern trabeculectomy technique[6]. Despite this, some patients still mount aggressive scarring responses and additional antifibrotic agents, such as 5-FU are injected subconjunctivally in the post-operative period to reduce scar formation. There is concern that these agents are potentially toxic and may result in side effects such as keratitis and are also painful for the patient. These remain the current, recognized care for post trabeculectomy eyes suspected of mounting a scarring response[7]. For those patients that are showing clinical evidence of future scar formation, a more predictable and less toxic modulator of wound healing is desirable in the post-operative period.

Vascular endothelial growth factor (VEGF) has been associated with angiogenesis in numerous pathological situations, including tumour growth, proliferative retinopathy, and rheumatoid arthritis. The original description of VEGF as a potent vascular permeability factor led authors to suggest it contributed to tumour metastases.

Increased vascular permeability and vasodilation, associated with VEGF, has since been shown to occur after challenge with VEGF and during the early phases of wound repair, theoretically allowing deposition of the fibrin-rich matrix necessary for cellular migration. The identification of increased vascular permeability concomitant with increased VEGF production in skin wounds provided evidence for a role for VEGF in wound repair.

Neutralization of VEGF greatly decreases the angiogenic and endothelial cell chemotactic activity of surgical wound fluid and VEGF levels in wound fluid rises steadily through the first week after injury. The primary sources of surgical wound VEGF are the fibroblast and the macrophage. Both cell types have been described to produce VEGF in vitro and are implicated in post- trabeculectomy wound healing.

New vessel formation in healing wounds follows a similar time course with newly formed vessels first evident 2 to 3 days after injury with maximal evidence at 1 to 2 weeks. It has also been shown to be important in epithelialization and collagen deposition.

In addition to vascular endothelial growth factor’s (VEGF) effect on angiogenesis, it plays a pivotal role in ocular wound healing. It mediates the signal transduction cascade leading to tenon’s fibroblast migration and proliferation, as well as collagen gel contraction.

In summary, VEGF contributes to the angiogenic stimulus in wounds either by direct

effects on proliferating and migrating fibroblast cells or indirectly by effecting persistent vascular permeability and vasodilation at the level of existing microvessels.

Vessels with increased permeability are typically tortuous and dilated and this is the clinical appearance within the conjunctiva suggestive of future excessive wound healing following trabeculectomy. Early interventions such as subconjunctival injections of 5FU are therefore often considered when these clinical findings are apparent, in order to modify the course of wound healing.

The pilot study was proposed to look at the effect of serial injections of bevacizumab on modifying the wound healing response in patients showing early signs of future failure. The purpose of the pilot was also to gather outcome data and information relating to safety and recruitment with a view to powering a definitive study addressing this issue.

Aims and Objectives

The aim of the pilot study was to assess acceptability of the proposed treatment to patients, evaluate trial SOPs such as randomisation and masking, and to gather information on the efficacy and safety of the new treatment combination which would be used to design a definitive randomized controlled study.

1) To gather information on different outcome measures (IOP, bleb morphology, number of post-operative interventions).

2) To gather information on the safety profile of the proposed therapy (hypotony, limbal leaks).

3) To assess recruitment rates

Trial Design

This is a non-commercial trial for an unlicensed use of bevacizumab. The trial is a randomized control pilot study and was been chosen to provide the highest level of evidence to assess the effectiveness of the intervention. The purpose of the pilot was to inform a larger randomized controlled study.

30 adult patients who had undergone standard trabeculectomy surgery and showing signs of an aggressive wound healing response and increased bleb vascularity in the first 6 weeks post-operatively, were to be randomized to one of 2 treatment arms. The study was an observer masked study, as the patients were to be seen by a masked observer post- operatively. The photographic bleb graders and trial statistician were

also be masked to the intervention.

Participants

Eligibility criteria for participants

Selection of Subjects

Adult patients, with primary open angle glaucoma, pseudoexfoliative glaucoma, and pigment dispersion glaucoma, with uncontrolled IOP, visual field and optic disc changes characteristic of glaucoma progression, taking maximum tolerated anti-glaucoma medication and having undergone uncomplicated primary trabeculectomy augmented with mitomycin C. In the event that both eyes meet the eligibility criteria, the 1st eye to be operated on will be included in the study and this will be termed “the study eye”. The eye not included in the study (“the non-study eye”) will receive standard clinical care.

Pseudophakic patients with uncomplicated cataract surgery, who are a minimum of 6 months post cataract surgery can also be included in the trial.

Inclusion criteria

1.Age 18 to 85 years, inclusive

2. Patient must have undergone standard trabeculectomy augmented with Mitomycin-C, within the past 4-6 weeks.

3. Patients who in the clinician’s opinion are mounting an aggressive wound healing response and demonstrate objective increase in bleb vascularity (moderate or severe on MBGS). Bleb function still needs to be maintained in the clinician’s opinion and flat, scarred blebs are not to be included.

Exclusion criteria

If any of the following exclusion criteria are present, the patient will not be entered into the study.

1. Unwilling or unable to give consent, unwilling to accept randomization, or unable to return for scheduled protocol visits.

2. Pregnant or nursing women.

3. A history of cardiovascular or cerebrovascular events in the previous 6 months, such as angina, arrhythmia, Transient Ischaemic Attack, strokes, myocardial infarction.

4. Uncontrolled hypertension defined as systolic blood pressure >160mmHg or diastolic blood pressure >90mmHg

5. Subject hypersensitive to bevacizumab, 5-FU, and mitomycin-C and its excipients

6. Failed trabeculectomy bleb

7. Persistent wound leak following trabeculectomy at the time of randomisation

The following exclusions apply to the study eye only (i.e. they may be present for the non study eye

8. No light perception.

9. Aphakia

10. Previous, or planned, ocular surgery: vitreo-retinal, conjunctival surgery, etc considered likely to interfere with trabeculectomy outcome

11. Complicated cataract surgery

12. Cataract surgery less than 6 months in duration

13. Secondary glaucoma, other than PDS and PXF

14. Ocular trauma within the past 3 months

15. Active iris neovascularization or active proliferative retinopathy.

16. Severe posterior blepharitis.

17. Unwilling to discontinue contact lens use after surgery.

18. Current or recent (<3months) use of bevacizumab into the study eye.

Settings and Locations where the data were collected

Subjects were to be recruited when they attend regular post-operative outpatient appointments in the Glaucoma clinics at Moorfields, within 4-6 weeks after trabeculectomy surgery. All doctors working in Glaucoma clinics at Moorfields Eye Hospital, City Road or any of its outreach clinics were informed about the trial and could identify patients who they felt would potentially benefit, as well as meet the inclusion criteria. The patient was informed about the trial and if in agreement, was managed in the Clinical Research Unit at Moorfields Eye Hospital, City Road.

Interventions

Patients will be randomised to 1 of 2 treatment arms:

Group 1: subconjunctival injections of dexamethasone and 5-FU as required for 4 consecutive weeks after entry into the trial – current standard treatment.

Group 2: subconjunctival injections of dexamethasone and bevacizumab given for up to 4 consecutive weeks from time of entry into trial.

Only one eye per patient was included in the study (the study eye). The 1st eye operated on was included in the study and was termed “the study eye”. The eye not included in the study (“the non-study eye”) received usual clinical care.

The patients were recruited from the glaucoma clinic. In order to aid recruitment, the trial was publicised at monthly departmental meetings, teaching and weekly by the recruitment coordinators within the glaucoma clinics. Incentives were also given for the highest recruiters.

Statistical Analysis Plan:

Baseline characteristics of the two groups were to be compared to assess the adequacy of randomisation.

Primary outcome analysis

The median time to recruitment reported with the interquartile range. The proportion of patients who agree to randomisation with 95 % confidence intervals computed by the exact binomial method. Loss to follow up or withdrawal rates.

Recruitment was assessed by the time taken to recruit the target set of 30 subjects (from start of study), the proportion of patients who are eligible for randomization who agree to participate and the proportion of patients who are lost to follow up or withdraw from the study prior to the planned final study visit.

Secondary outcome analysis

Summary statistics for all secondary outcomes. Means and standard deviations used for normally distributed (approximately) continuous variables and medians and interquartile ranges used for non normal continuous variables.

Categorical variables as numbers and percentages.

IOP was measured using Goldmann tonometry. IOP measurements were not measured at the same time of day at each visit. The number of topical ocular hypotensive medications at 3 months and 18 months were recorded.

Bleb morphology was assessed objectively by a reading centre as determined by the Moorfields Bleb Grading system. Number of post-operative interventions with subconjunctival 5-fluorouracil (5-FU) and subconjunctival dexamethasone, following the 4 week trial intervention period; and number of bleb needling (received between surgery and 18 months of follow up).

<u>Outcomes</u>

Time taken to recruit 30 subjects (from start of study)

Proportion of patients who are eligible who agree to be randomized

Proportion of patients who are lost to follow up or who withdraw from the study

Secondary outcomes

1. Intraocular pressure

2. Bleb morphology (as determined by Moorfields Bleb Grading system)

3. Number of additional postoperative interventions with 5-fluorouracil (5-FU) and subconjunctival dexamethasone, following the 4-week trial intervention period; bleb needling. (received between surgery and 18 months of follow up)

4. Time to failure

5. Number of topical ocular hypotensive medications at 3 months and 18 months

6. The incidence of the following complications will be recorded: hypotony (IOP < 6mmHg at 2 consecutive post-operative visits after 3 months), hypotony maculopathy, wound leakage and bleb leakage, bleb-related infections (blebitis, bleb-related endophthalmitis).

Failure is defined as any of the following occurring in the study eye:

- IOP ≥18 mmHg and not reduced by 20% below listing IOP on two consecutive follow-up visits after randomisation. IOP below 6mmHg on two consecutive follow-up visits, three months after randomisation.

- loss of vision to no perception of light

- requiring additional glaucoma surgery. except bleb needling. Any patient that does not fail, between the time of surgery and final follow-up at 18 months after randomisation, post-operatively will be either a complete or partial success based on the following criteria.

Patients whose surgery did not fail, between the time of surgery and final follow-up at 18 months were categorised as complete or partial success based on the following criteria.

Complete success was defined as an IOP < 18mmHg and ≥6mmHg without anti-glaucoma medications 18 months after randomization and at least a 20% reduction from baseline IOP.

Qualified Success was defined as an IOP of <18mmHg and ≥6mmHg with anti-glaucoma medication(s) or needling 18 months after randomisation.

Adverse events include but are not limited to hypotony, hypotony maculopathy, early and late post-operative leakage, bleb-related infections (blebitis, bleb-related

endophthalmitis). Hypotony was defined as IOP < 6mmHg at two consecutive post-operative visits 3 months after trabeculectomy surgery.

Sample Size

No formal sample size calculation was done as priori. Thirty participants was deemed to be the minimum number to allow estimates of recruitment times, loss to follow up and eligibility to be made.

Randomisation

Patients were randomised to study arms with 1:1 ratio. A randomisation allocation list using permuted blocks with varying sizes was prepared by the senior data manager in Moorfields Eye Hospital Research & Development department, remote from the patient and medic using STATA software. The list containing study ID and treatment allocation was given to the trial pharmacist to dispense the drugs (either 5FU or bevacizumab).

Masking

Post-operative clinical assessors, graders of bleb photographs and trial statistician were masked to allocation during the study.

Interventions in both treatment arms are delivered by a subconjunctival injection, however vials of the medication were not altered. Patients in both arms of the study received the same number of follow up visits.

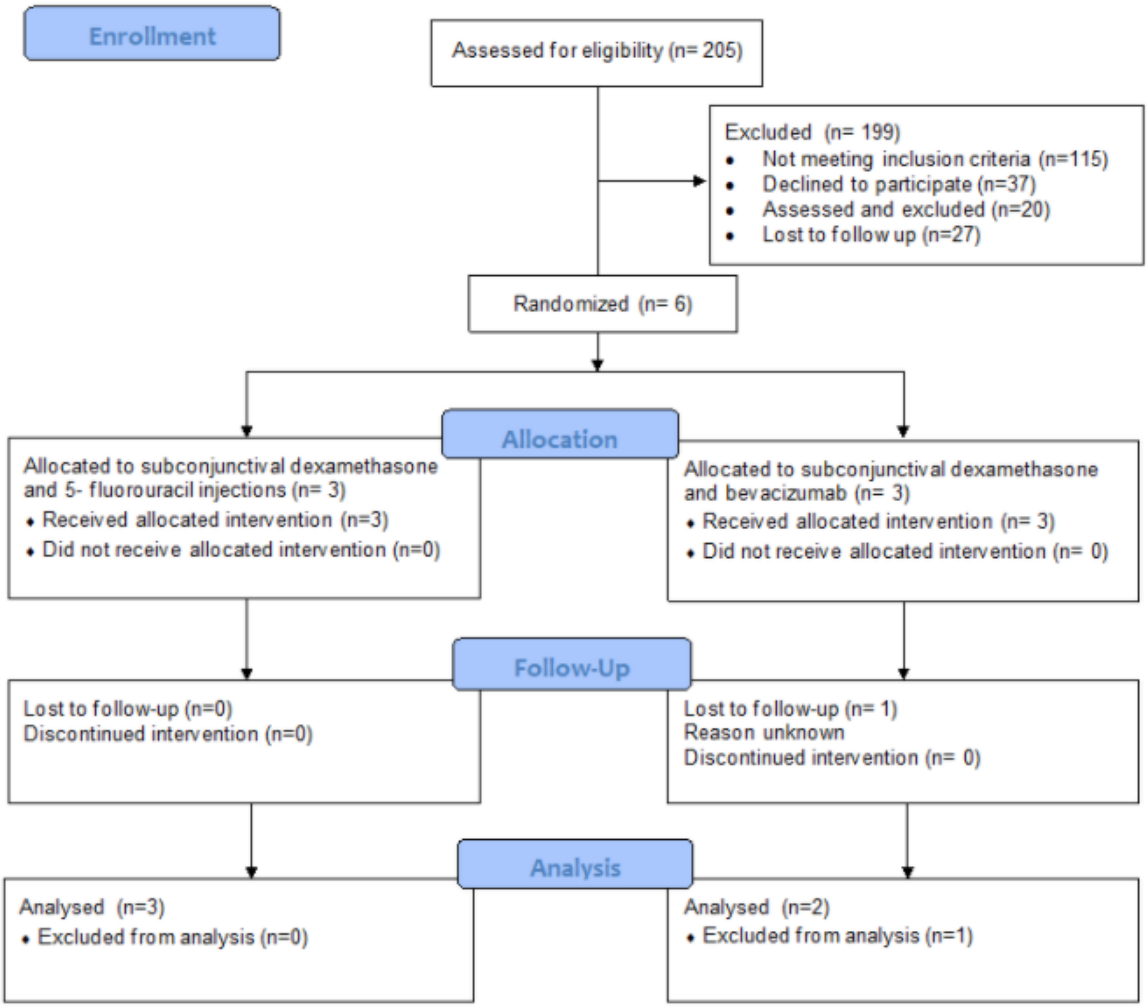
Analytical methods

Data collected was analysed based on the intention to treat principle. Descriptive statistical methods used to compare groups. Summary statistics for the primary and all secondary endpoints are presented using mean and standard deviation for continuous (approximate) normally distributed variables, medians and interquartile ranges for non-normally distributed variables, and frequencies and percentages for categorical variables. No subgroup or sensitivity analysis was performed.

Results

Recruitment was initially intended to be 6-month period, however it was extended to 18-month period due to surgeons' reluctance to refer patients with high risk blebs to the study. Participants once enrolled in the study were followed up for 18 months. Of the 6 adults referred to the study and screened, all were eligible and they all participated in the study before the study was stopped. One participant allocated to group 2 withdrew from the study after visit 5 (a withdrawal rate of 17%) see Figure 1. The pilot study was stopped due to a very slow recruitment times.

Figure 1. Consort flow chart


Enrollment
Assessed for eligibility (n= 205)
Excluded (n= 199)
Not meeting inclusion criteria (n=115)
Declined to participate (n=37)
Assessed and excluded (n=20)
Lost to follow up (n=27)
Randomized (n= 6)
Allocation
Allocated to subconjunctival dexamethasone and 5- fluorouracil injections (n= 3)
Received allocated intervention (n=3)
Did not receive allocated intervention (n=0)
Allocated to subconjunctival dexamethasone and bevacizumab (n= 3)
Received allocated intervention (n= 3)
Did not receive allocated intervention (n= 0)
Follow-Up
Lost to follow-up (n=0)
Discontinued intervention (n=0)
Lost to follow-up (n= 1)
Reason unknown
Discontinued intervention (n= 0)
Analysis
Analysed (n=3)
Excluded from analysis (n=0)
Analysed (n=2)
Excluded from analysis (n=1)

Baseline data

Tables 1 and 2 show baseline demographic and clinical characteristics for each group. Baseline characteristics of the two groups was compared to assess the adequacy of randomisation….

Table 1. Baseline characteristics

	5-Fluorouracil (N=3)	**Avastin (bevacizumab) (N=3)**
Number of patients (eyes), n (%)	3(50%)	3 (50%)
Male, n (%)	3 (100%)	2 (67%)
Age (years), Mean(SD)/Median(IQR)	70 (±10.4)/ 75 (IQR 9.5)	59 (±9.8)/62 (IQR 9.5)
Ethnicity, n (%): - White - Asian - Mixed - Other	3 (100%) 0 0 0	1 (33%) 0 0 2 (67%) Black Caribbean and Black African
Smoke, n (%): - Yes - No	0 (0) 3 (100%)	1 (33%) 2 (67%)
Diabetes, n (%): - Yes - No	0 (0) 3 (100%)	1 (33%) 2 (67%)
Duration of Diabetes (years), Mean (SD)/Median (IQR)	N/A	17 years

Systolic Blood Pressure (mmHg), Mean (SD)/Median(IQR)	139 (±19)/ 144 (IQR 18)	131 (±23) / 124 (IQR 22)
Diastolic Blood Pressure (mmHg), Mean (SD)/Median(IQR)	79 (±7) / 79 (IQR 7)	78 (±6) / 78 (IQR 6)

Table 1. Ocular Baseline Characteristics

	5-Fluorouracil (N=3)	**Avastin (N=3)**
Study Eye, n (%) - Right - Left	1 (33%) 2 (67%)	2 (67%) 1 (33%)
ETDRS BCVA, Mean (SD)	0.5 (±0.44)	0.40 (±0.26)
ETDRS BCVA, n (%): - CF - HM - PL - NPL	0 (0) 0 (0) 0 (0) 0 (0)	0 (0) 0 (0) 0 (0) 0 (0)
IOP (mmHg), Mean (SD)	15 (±4.5)	14 (±3.6)
Glaucoma Type, n (%) - POAG - PXFG - PDG	3 (100%) 0 (0) 0 (0)	3 (100%) 0 (0) 0 (0)
Anterior Segment, n (%) - Deep - Shallow - Flat	3 (100%) 0 (0) 0 (0)	3 (100%) 0 (0) 0 (0)

Ocular comorbidity, n (%): - Diabetic retinopathy - AMD - Other	0 (0) 0 (0) 1 (33%) High myopia	0 (0) 0 (0) 1 (33%) High myopia
Lens status, n (%): - Phakic - Pseudophakic	3 (100%) 0 (0)	3 (100%) 0 (0)

BCVA = best corrected visual acuity; IOP = intraocular pressure; mmHg = millimetre of mercury; CF = counting fingers; HM = hand motion; PL = perception of light; NPL = no perception of light; AMD = age-related macular degeneration

Table 2. Primary outcome measures

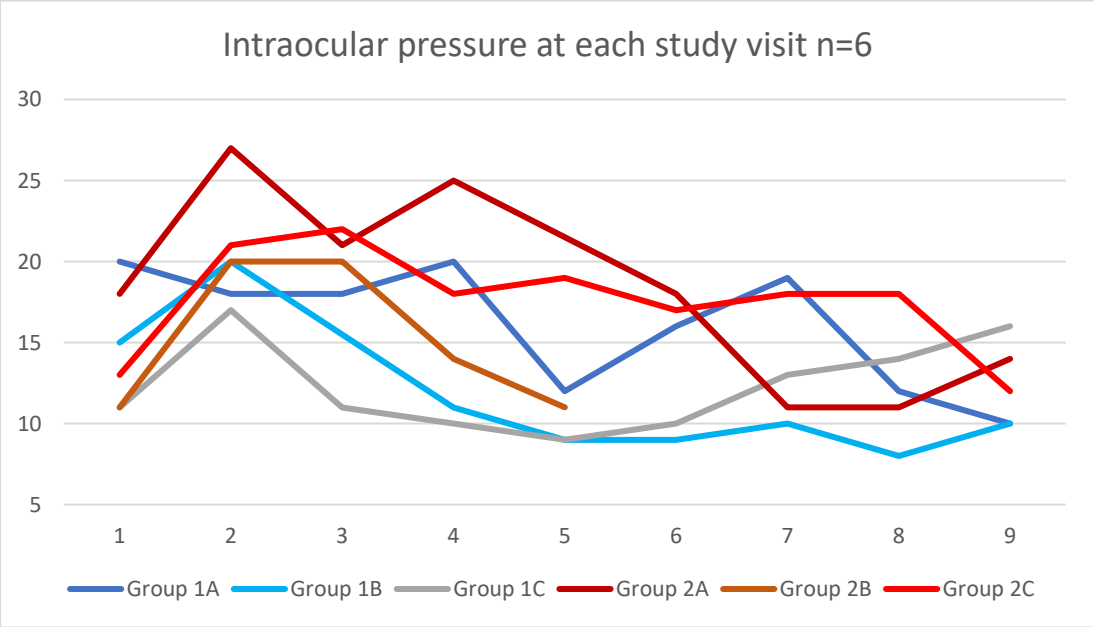
Time taken to recruit 30 subjects (months), Median (IQR)	N/A
Time taken to recruit 6 subjects (months), Median (IQR)	13 months Median 12 (IQR 5.5)
Proportion of patients who are eligible and agree to be randomised, n (%)	6 (100%)
Proportion of patients who are lost to follow-up or early withdraw from the study, n (%)	1 (16.7%)

Table 3. Secondary outcome measures

	5-Fluorouracil (N = 3)	**Avastin (N = 3)**
Success (Intraocular pressure), n (%) at 18 months		

- Complete success - Qualified success - Failure	1 (33%) 1 (33%%) 1(33%)	0 (0) 2 (67%) 1 (33%)
Bleb Morphology: bleb needling received between randomisation and 18 months follow-up, Patients n (%)	0 (0)	1 (33%) The patient who withdrew from study after visit 5 one session of bleb needling
Number of additional postoperative interventions, Median (IQR)	0 (IQR 1)	0 (IQR 1.5)
Number of topical ocular hypotensive medications, Median (IQR) - 3 months - 18 months	 1 (3.5) 1 (0.5)	 0 (4) 1 (2.5)

Intraocular pressure



Harms outcomes

Hypotony was defined as IOP < 6mmHg at two consecutive post-operative visits 3 months after trabeculectomy surgery.

All adverse events (Table 5)

There were no other unintended effects from the interventions in each group.

Table 5. Incidence of complications

	5-Fluorouracil (N = 3)	**Avastin (N = 3)**
Incidence of Complications, n (%)		
- Hypotony	0 (0)	0 (0)
- Hypotony Maculopathy	0 (0)	0 (0)
- Wound Leakage	0 (0)	0 (0)
- Bleb Leakage	1 (33%)	0 (0)
- Blebitis	0 (0)	0 (0)
- Bleb-related Endophthalmitis	0 (0)	0 (0)
- Cornea Epithelium defect	0 (0)	0 (0)
- Bleb dysaesthesia	0 (0)	1 (33%)
- Foreign body sensation	0 (0)	1 (33%)
- Blepharitis	1 (33%)	0 (0)
- Adenovirus infection	1 (33%)	0 (0)
- SLT to fellow eye	1 (33%)	0 (0)
- Conjunctival suture removal	0 (0)	1 (33%)
- Additional 5-FU injection after visit 4		

- Discomfort following injection	1 (33%) 0 (0)	1 (33%) 1 (33%)
Adverse event number of events	5	4
Extraocular serious adverse events Knee arthroscopy and repair of medial meniscus	0 (0)	1 (33%)

Figure 2. Kaplan Meier (time to failure) by randomised arm

Hazard ratio (Mantel-Haenszel) 19.66 **(95% CI 1.524 to 253.6)**

Gehan-Breslow-Wilcoxon test Chi Squared 4.65 p=0.03

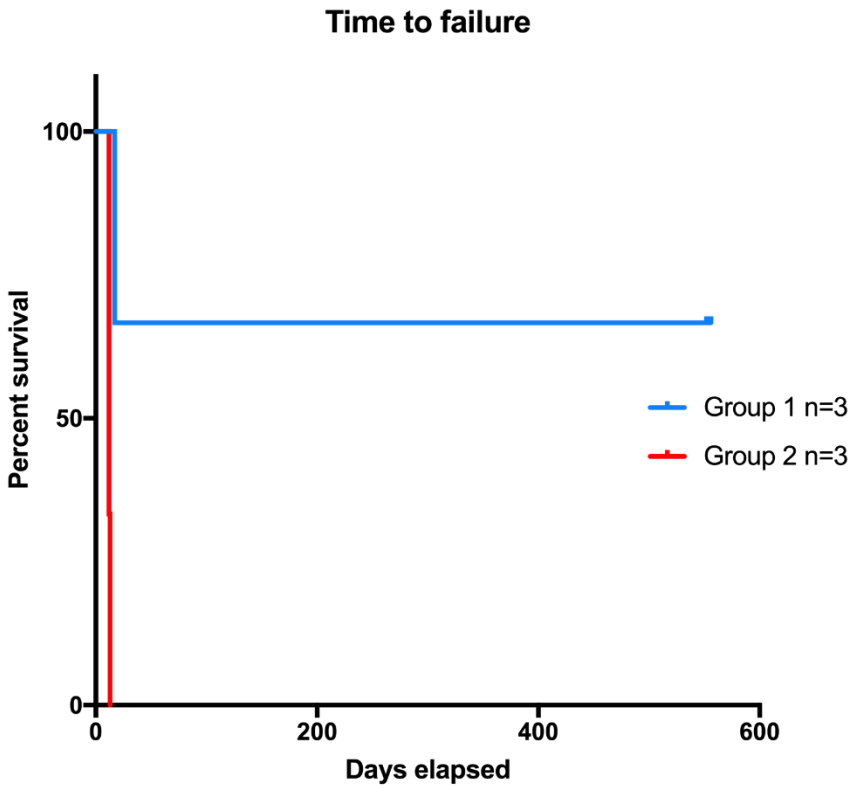


Table 6. **Ocular Characteristics at 18 months (study eye only)**

	5-Fluorouracil (N=3)	**Avastin (N=2)**
ETDRS BCVA, Mean (SD)	0.1 (±0)	0.1 (±0.14)
ETDRS BCVA, n (%): - CF - HM - PL - NPL	 0(0) 0(0) 0(0) 0(0)	 0(0) 0(0) 0(0) 0(0)
IOP (mmHg), Mean (SD)	12 (±3.5)	13 (±1.4)
Anterior Segment, n (%) - Deep - Shallow - Flat	 2(67%) 1(33%) 0(0)	 2(100%) 0(0) 0(0)
Retina Flat, n (%) - Yes - No	 3(100%) 0(0)	 2(100%) 0(0%)
Mean Deviation, Mean (SD)/Median(IQR)	-16.8 (±2.7) / -17.3 (IQR 2.7)	-7.5 (±0.5) / -7.5 (IQR 0.4)
PSD, Mean (SD)/Median(IQR)	13.3 (±2.2) / 13.9 (IQR 2.2)	7.7 (±3.0) / 7.7 (IQR 2.1)

CF = counting fingers; HM = hand motion; PL = perception of light; NPL = no perception of light; AMD = age-related macular degeneration

Limitations, Generalisability and Interpretation

The main study limitation was due to recruitment. The study failed to recruit according to the recruitment plan. After a lot of promotion, the study eventually started to recruit, but unfortunately there was an issue with the 5-FU drug having precipitates, which caused the study to be halted. 5-FU precipitation incident was reported 28.04.2017. The issue was resolved and the MHRA approved the study to start again on 2.06.2017. REC approval was on 26.06.2017 and then HRA approval was on 21.07.2017.

Once this issue had been resolved and the various trial authorities gave approval, we struggled to recruit again. On speaking to several of the consultants, they stated they were reluctant to recruit their patients into a trial at a point when the patient's trabeculectomy surgery was failing and preferred to look after these patients themselves. Due to our failure to recruit, the external trial steering committee and sponsor decided that the trial should be terminated. The trial was terminated on 5.09.2019.

It is difficult to generalise the results from the study, as we only recruited 6 patients.

There were no serious incidents and so from the 6 patients recruited, some of whom received multiple doses of bevacizumab, we can infer that the drug is likely to be relatively safe for ocular use.

Registration, Protocol and Funding

MEH Protocol Number: MATR1001

ClinicalTrials.gov Identifier: NCT02767219

REC Reference Number: 16/LO/01/80

EuDRACT number: 2013-000395-15

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