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Comparison of the use of 5-FU and bevacizumab in primary trabeculectomy: results at one year

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Short running title: Trabeculectomy with bevacizumab

ABSTRACT

Background: The present study compared the effects of adjuvant bevacizumab and 5-fluorouracil (5-FU) on the efficacy and safety of trabeculectomy.
Design: A nonrandomized, prospective, interventional case study.
Participants: 62 patients in 2 groups undergoing primary trabeculectomy.
Methods: In Group 1 (21 POAG, 9 PEXG), trabeculectomy was performed with an adjuvant 5% solution of 5-FU administered for 4 min. intraoperatively. In Group 2 (21 POAG, 11 PEXG), trabeculectomy was enhanced with 1.25 mg of bevacizumab applied subconjunctivally immediately before and after surgery and again 1 and 7 days after surgery.
Main outcome measures: IOP, BCVA, VFI, bleb morphology, cornel endothelial cell count.
Results. Mean IOP was 28.0±8.0 mmHg before 5-FU-augmented trabeculectomy and 27.8±9.5 mmHg before bevacizumab-augmented trabeculectomy. After 6 months, mean IOP was 13.4±4.6 mmHg in the 5-FU group and 13.7±5.2 mmHg in the bevacizumab group. After 12 months, mean IOP was 13.6±4.4 mmHg in the 5-FU group and 14.7±4.7 mmHg in the bevacizumab group. A 30% reduction of initial IOP was attained in 86.7% of patients in the 5-FU group and 78.1% of patients in the bevacizumab group at the end of follow-up. No significant differences were noted between the 2 studied groups with respect to corneal endothelial density, visual field indices and postoperative complications.
Conclusions. The twelve-month IOP results showed no significant differences between the 2 groups of patients after bevacizumab or 5-FU to augment trabeculectomy. However, to obtain successful IOP control more patients in bevacizumab group needed medical therapy.

Key words: trabeculectomy, bevacizumab, 5-fluorouracil, glaucoma

INTRODUCTION

Trabeculectomy has been widely applied for surgical treatment of glaucoma since its use was pioneered by Cairns in 1968 [1]. The result of the surgery depends on its success in forming a functional fistula which enables aqueous humour outflow. The key factor in trabeculectomy failure is postoperative scarring in the filtrating tract and bleb [2].

Pharmacological techniques, especially antiproliferative agents, have drawn attention as a means of avoiding bleb insufficiency, which may ensue from enhanced healing due to fibroblast proliferation and subconjunctival scarification. Most of the currently used adjuncts for moderating the wound healing process attempt to modify fibroblast activity.

5-Fluorouracil (5-FU) and mitomycin C (MMC) are the most frequently used antimetabolites in this situation. Although intraoperative MMC is the most widely used adjunctive antifibrotic agent in trabeculectomy, a scant body of evidence exists to support the use of MMC instead of intraoperative 5-FU [3].

However, the adjunctive therapy increases the risk of adverse effects such as corneal endothelial cytotoxicity, chronic hypotony, bleb infection, endophthalmitis associated with prolonged bleb leakage, and formation of cystic avascular blebs [4]. To minimize the risk of these potentially devastating events, new therapeutic approaches involving wound healing processes are currently undergoing experimental and clinical study. However, a clinical trial with one of the most promising agents, namely an antibody against transforming growth factor □2, failed unexpectedly [5].

Genetic engineering is currently producing new agents − like bevacizumab – which, by blocking vascular endothelial growth factor, show promise as a potent means of moderating scarring after filtration surgery. Bevacizumab's pharmacological activity includes inhibition of tumour vasculature. It precludes the activation of VEGF and its receptor VGFR-2-dependent intracellular pathways, thus diminishing choriocapillary permeability, endothelial proliferation and the formation of new vessel branches [6]. Increased vascular permeability is a pivotal stage of angiogenesis in connection with e.g. wound healing [7,8]. Bevacizumab is commonly administered in ophthalmology, mainly to treat endovascular retinal disorders [9,10] and corneal vascularisation [11,12].

The aim of the present study was to compare the efficacy and safety of bevacizumab and 5-fluorouracil in trabeculectomy.

METHODS

The present study was a nonrandomized, prospective, interventional case series study. The study's protocol was approved by the local ethics committee and adhered to the tenets of the Declaration of Helsinki. Sixty-two eyes of 62 Caucasian patients with uncomplicated open-angle glaucoma (primary open-angle glaucoma and pseudoexfoliative glaucoma) were recruited for primary trabeculectomy with either 5-fluorouracil (50 mg/ml, EBEWE Pharma, Austria) or bevacizumab (Avastin®, 25 mg/ml, Roche Pharma AG, Germany) as an adjunct. The patients who fulfilled the criteria for inclusion were assigned consecutively either to the group treated with 5-FU or bevacizumab. Observer-masking was impossible because of the differences in drug application. Intraocular pressure (IOP) before surgery was at least 22 mmHg in all patients. The patients were currently on medication for treatment of glaucoma and demonstrated a progression of the disease with variable combinations of optic disc cupping, visual field deterioration, and elevated IOP. According to the anamnesis, patients were treated for glaucoma for 5.1 years in group 1 and 5.7 in group 2 (p=0.85).

Standard surgery was performed as follows: Group 1 = 30 eyes with 5-FU, and Group 2 = 32 eyes with adjunctive bevacizumab between January and June 2007. All patients underwent a complete preoperative ophthalmic examination, including measurement of corrected visual acuity, applanation tonometry, measurement of central corneal thickness, slit lamp examination, ophthalmoscopy, gonioscopy, visual field examination (Humphrey perimeter, SITA STANDARD central 30-2 program), and specular microscopy (Konan NONCON Robo). Written informed consent was obtained beforehand from each patient. Of the 30 eyes in Group 1 (19 women and 11 men), 21 were classified as POAG and 9 as PEXG. In Group 2 (16 women and 16 men), 21 of the 32 eyes were classified as POAG and 11 as PEXG. There were no significant demographic differences between the two groups [Table 1].

The surgical procedure was similar in both groups. All operations and injections were performed by the same surgeon (JJ). After peribulbar anaesthesia, a fornix-based conjunctival flap was prepared. Haemostasis was achieved with wet field cautery. A rectangular scleral flap, measuring 4 x 3 mm was then dissected to a depth of one-half the scleral thickness until the entire corneoscleral limbus was exposed. In the 5-FU group, two small 4 x 3 mm rectangular fragments of surgical sponge were saturated with a 5% solution of 5-fluorouracil, then one was placed under the scleral flap and the other one over the scleral flap under Tenon's capsule for 4 minutes. In the bevacizumab group, 0.1 ml (1.25 mg) of commercially available Avastin solution was administered subconjunctivally immediately before and after surgery, then again 1 day and 7 days after surgery [5]. Trabeculectomy was performed using

1-2 bites with a Pierce punch, after which peripheral iridectomy was performed. The scleral flap was closed with 2 fixed and two releasable 10-0 nylon sutures in both groups in the same way. Tenon's capsule and the conjunctiva were then each tightly closed with a single 10-0 nylon suture.

Topical dexamethasone/gentamycin was used ≥4 times a day for a minimum of 4 weeks, the same schedule was used in both groups. Postoperative physical manoeuvres such as bleb massage, suture release or lysis to lower IOP were also performed when considered necessary.

Postoperative follow-up was carried out 1 day, 1 week, 1 month, 3 months, 6 months and 12 months after surgery. The postoperative data collected included best corrected visual acuity (BCVA), IOP, clinical bleb appearance, visual field (12 months after surgery), and corneal endothelial cell count (12 months after surgery). The treatment's effectiveness in terms of successful trabeculectomy was defined as at least a 30% reduction from baseline IOP.

Bleb morphology was evaluated by the same clinician (JJ) using the MBGS (Moorfields Blebs Grading System) scale, which assesses the bleb's area (A), height (H), and vascularisation (V). In this case, the bleb area is described as central (Aa) and maximal (Ab) and is divided into 1-5 grades depending on the bleb area in relation to the area of the superior conjunctiva (1= 0%; 2= 25%; 3= 50%; 4= 75%; 5= 100%). Bleb height is assessed on a scale of 1 to 4. Bleb vascularisation is divided into 5 stages (1 = avascular; 2 = vascularisation similar to that of the conjunctiva; 3 = mild hyperaemia; 4 = moderate hyperaemia; 5 = severe hyperaemia) [13].

The number of patients in the study was chosen after a calculation performed with GraphPad StatMate2, which can detect IOP differences of approx. 3.5-3.8 mmHg (a 95% accuracy of the test was assumed). Data analysis was performed using STATISTICA v. 8.0 (StatSoft, Poland), and $p \leq 0.05$ was considered to be statistically significant.

For a procedure to be regarded as completely successful, IOP had to drop by at least 30% from baseline without medication (complete success) or further therapy; the same result with medication was graded as qualified success.

RESULTS

Initial mean IOP did not differ significantly between the two groups. The detailed IOP results are put in Table 2. Post operative intraocular pressure was notably lower than

initial IOP upon all scheduled visits to the patients of both study groups and never exceeded 21 mmHg.

Before surgery the mean number of types of antiglaucoma drugs prescribed for the patients in the 5-FU group was 1.67 per person and in the bevacizumab group was 1.81 per person (p=0.43).

A 30% drop in IOP (complete success) was observed in 86.7% of eyes in the 5-FU group and 78.1% in the bevacizumab group 12 months after surgery (p = 0.38). Of these patients, none of those treated with 5-FU needed additional topical treatment (= complete success), whereas 2 patients from the bevacizumab group required the administration of topical beta-blockers (6.2% eyes, = qualified success). Detailed data of total success (complete and qualified) are shown in Figure 1.

In patients with a less than 30% drop from initial IOP, IOP after trabeculectomy never exceeded 21 mmHg: those from the 5-FU group required no additional topical therapy, while 3 from the bevacizumab group required additional topical administration of beta-blockers.

The mean number of various types of antiglaucoma medication required postoperatively was 0.16 per person in the bevacizumab group and 0.0 in the 5-FU group (p=0.02).

None of the patients suffered from hypotonia (IOP<6mmHg) at 12 months after trabeculectomy.

In the early postoperative period mean number of removable sutures removed in 5-FU group was 0.87 whereas in bevacizumab group was 1.0 (p=0.54). All remaining sutures has been removed by 6 weeks after surgery.

In both groups in 6 patients bleb massage was performed (p=0.9).5-FU injections and needling were not performed in any patients.

BCVA remained unchanged during the 12 months after surgery in both groups (p=0.64). BCVA before and after surgery in both groups according to logMAR scale was 0.6±0.2.

Initial visual field parameters did not differ between the study's groups. In the 5-FU group, mean MD was -19.6±7.9 dB and mean PSD 7.9±3.3 dB; in the bevacizumab group, mean MD was -19.3±8.2 dB and mean PSD 8.4±2.6 dB. After 12 months visual field parameters had not changed significantly in either group compared to pre-operative values. In the 5-FU group, mean MD was -19.4±8.5 dB and mean PSD 8.0±3.0 dB; in the bevacizumab group, mean MD was -19.1±8.5 dB and mean PSD 8.1±2.8 dB.

Initial mean corneal endothelial cell density did not differ significantly between the two groups (2013.9±296.1 cell/mm^2 in 5-FU patients and 2108.4±351.3 cell/mm^2 in the bevacizumab group; p=0.13). After 12 months, corneal endothelial cell density had decreased from the preoperative level in the 5-FU patients to 1908.8±313.4 cell/mm^2, i.e. a mean decline of 5.2% ($p<0.05$) vs. the preoperative value and in bevacizumab group to 2026.8±326.1 cell/mm^2, a mean decline of 3.9% ($p<0.05$). However, neither difference was significant (p=0.08) [Table 3].

Bleb morphology 12 months after trabeculectomy was assessed using the MBGS scale. The detailed results are shown in Table 4.

Two patients in the 5-FU group developed a cyst in Tenon's capsule. In one case, topical beta-blocker treatment successfully diminished IOP to acceptable levels. In the second case, bleb revision was performed twice (1 month and 5 months after trabeculectomy) and was successful in reducing IOP to acceptable levels.

In two patients, leakage from the bleb lasted for 3 weeks. It ceased spontaneously in one case, whereas in the second case a shallow anterior chamber was observed. After additional conjunctival suturing the chamber deepened, but a cataract developed and was treated operatively 6 months after trabeculectomy.

Three bevacizumab patients suffered from a Tenon's capsule cyst; in one case, topical beta-blockers proved adequate for treatment, while two required bleb revision (2 months after trabeculectomy in the one, 6 months after trabeculectomy in the other). In two patients bleb leakage lasting 3 weeks was observed. In both cases, this was remedied with additional conjunctival sutures; one of the two also required anterior chamber reformation [Table 5].

DISCUSSION

Injections of bevacizumab were first tested as a treatment for neovascular glaucoma (NVG): Jonas successfully brought intraocular pressure under control with intravitreal injections of 1.5 mg of bevacizumab during standard trabeculectomy. This indicated that intravitreal bevacizumab might also be helpful in antiglaucomatous penetrating filtering surgery for clinical situations with anterior chamber neovascularization or persisting macular oedema [14]. Intravitreal bevacizumab (1 mg) was also successfully tested for NVG by another research team in combination with transscleral cyclophotocoagulation and panretinal photocoagulation [15]. Kahook applied 1 mg of bevacizumab subconjunctivally in a patient

with a failing bleb following trabeculectomy and noted that the bleb became more diffuse, with a decrease in superficial neovascularization [16]. The Bevacizumab Study Group reported a dramatic reduction of leakage from rubeotic vessels in iris rubeosis after intracameral injection of 1 mg of bevacizumab, with no relapse during the follow-up time of four weeks [17]. A combination of Ahmed glaucoma valve implantation with intravitreal bevacizumab injection was also found to be effective at times in controlling IOP in refractory neovascular glaucoma [18].

Grewal [19] published the results of a pilot study evaluating single subconjunctival injections of bevacizumab after trabeculectomy. In his small study cohort (12 eyes), trabeculectomy resulted in successful control of IOP in 92%, with an average IOP reduction of 52% after a mean follow-up of 182 days.

In our study, complete success of trabeculectomy was defined as a 30% decline in baseline IOP; this was observed in 78.1% of the bevacizumab patients after 12 months, a slightly lower rate than in the 5-FU group (86.7% respectively).

IOP results for bevacizumab in our study were slightly − but not significantly − better than for 5-FU. However, some studies [20,21] have found a similar difference between a 5-FU group and a placebo group. This may have relevance for our small study cohort as well, but will require further study, especially in light of the finding that medication usage after surgery was significantly higher in our bevacizumab group. On the other hand, the most recent randomized trial comparing intraoperative MMC to intraoperative 5-FU showed no significant differences between the two groups in reducing IOP 12 months after primary trabeculectomy [3].

It is generally accepted that there is a relationship between the level of intraocular pressure and visual field (VF) loss in patients with glaucoma [22]. The objective of trabeculectomy is not only to reduce IOP but also prevent a progression of glaucomatous VF loss. In our study, trabeculectomy with application of bevacizumab stopped visual field loss as effectively as trabeculectomy with 5-FU; for final confirmation, however, this result would require a more prolonged observation period.

As in other studies [23], visual acuity did not change significantly after trabeculectomy during the 12-month observation period . Other studies have reported an early deterioration of visual acuity due to complications after trabeculectomy [24,25]. However, half of the eyes in those studies had undergone cataract surgery within 3 years after glaucoma surgery, and there was a 78% increase in the risk of those eyes either developing a cataract or requiring cataract surgery [26].

Vascularisation in the bleb is an important prognosis factor after trabeculectomy. For this reason, the use of angiogenesis inhibitors as wound healing agents is recommended here. In our study, however, no statistical differences were found in either bleb vascularisation or the other remaining bleb morphologic features between bevacizumab and 5-FU patients.

A functioning endothelium is essential for corneal integrity and transparency. Specular microscopy has become a standard technique for assessing endothelial cell density and morphology [27]. Most glaucoma surgery can adversely affect the cornea. This often consists of mild endothelial loss, but occasionally corneal decompensation may also occur [28]. In the present study, the endothelial cells count difference was close to statistical significance and probably would become significant if larger study numbers had been achieved. The minor endothelial cytotoxicity would be a substantial advantage of using bevacizumab.

Mean corneal endothelial cell losses after glaucoma filtering surgery are reported to range from 0.2% to 14.9% [25]. The effect on the cornea depends on pre-existing corneal disease, the severity and chronicity of intraocular pressure elevation, and the prior history of intraocular procedures and complications [28]. Additionally, a toxic influence of 5-FU on endothelial cells when 5-FU is given in subconjunctival injections after filtrating procedures has been described [29,30]. Some studies have suggested that trabeculectomy can cause similar changes in the corneal endothelium when supplemented with 5-FU and MMC [31]. However, Mietz reported partial decompensation of the corneal endothelium in 2 patients who underwent routine trabeculectomy with MMC [32]. Additionally, subconjunctival MMC injection may cause limbal stem cell deficiency [28].

Intravitreal bevacizumab has been reported to cause no changes in the viability of retinal neurons or the expression of neurofilaments, a marker of neuronal differentiation [33]. *In vitro* studies have concluded that bevacizumab is not toxic to corneal cells of human origin, including corneal endothelial cells, at doses usually used for treatment of corneal neovascularization (i.e. 20-fold higher than those used for intravitreal application [34]). A pilot study evaluating experimental treatment with bevacizumab eye drops in corneal neovascularization induced by alkali burn showed that bevacizumab eye drops can sufficiently penetrate the corneal stroma and anterior chamber. When administered soon after alkali burn, it appeared to reduce corneal damage significantly [35].

The potential side effects of bevacizumab after subconjunctival application require further investigation. In our patients no differences were found in complications after trabeculectomy with bevacizumab from those typical for trabeculectomy with adjunctive antimetabolites, nor did the number of complications differ significantly.

Histologic studies have shown that maximum proliferation of subconjunctival fibroblasts, an important factor in bleb failure, occurs on the third to fifth postoperative day [36,37]. Since it is known that bevacizumab's half-life is 3-4 days, multiple injections of antiangiogenic agents are to be recommended. However, the pharmacokinetics of bevacizumab after subconjunctival injections require further study.

The use of bevacizumab in glaucoma is currently used off- label, and several issues need to be addressed in this regard, such as the duration of action and the profile of toxicity to the corneal endothelium, lens, and trabecular meshwork. However, it is worthwhile pointing out that neither 5FU or MMC has a licence for use in many countries.

Since the pharmacokinetics of bevacizumab after subconjunctival injection had not been widely studied when our study began, we decided to apply the dose typically injected intravitreally. Recent studies with animal models have successfully tested similar doses [38]. In our study, the dosage schedule was consistent with that applied in a phase III study on CAT-152 [5]. Multiple dosage of bevacizumab is expected to be more effective as bevacizumab specifically neutralizes VEGF. Our experience indicates that an additional injection 14 days after surgery might be beneficial [39]. However, experimentation with parameters such as dosages, routes of application (e.g. topical), depot preparation and/or combined therapy in future might improve the effect of bevacizumab-augmented trabeculectomy [19].

In conclusion, the twelve-month IOP results showed no significant differences between the 2 groups of patients after bevacizumab or 5-FU to augment trabeculectomy. However, to obtain successful IOP control more patients in bevacizumab group needed medical therapy. On the other hand, adjunctive bevacizumab caused minor decrease in corneal endothelial cell count. Safety of bevacizumab and 5-FU for augmentation of primary trabeculectomy indicated no significant differences between the two. However, to confirm the results a larger randomisd clinical trial has been planned.

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TABLES

Table 1: Demographic data of patients scheduled for trabeculectomy

Baseline Characteristics	**Type of modification**		**p***
	5-FU n=30	Bevacizumab n=32	
Age (mean ± SD)	71.1 ± 7.4	70.6 ± 5.8	0.56
Sex			0.29
Female (%)	19 (63.3)	16 (50)	
Male (%)	11 (36.7)	16 (50)	
Type of glaucoma			0.71
POAG	21 (70.0)	21 (65.5)	
PEXG	9 (30.0)	11 (34.4)	
IOP before surg. (mean ± SD)	28.9 ± 9.5	29.3 ± 9.4	0.96
VA (logMAR) (mean ± SD)	0. 6± 0.2	0.6 ± 0.2	0.74
VF parameters			
MD (dB)	-19.6 ± 7.9	-19.3 ± 8.2	0.96
PSD (dB)	7.9 ± 3.3	8.4 ± 2.6	0.59
Endothelial cell density	2013.9 ± 296.1	2108.4 ± 351.3	0.13

*- χ^2 test or U Mann-Whitney test

Abbreviations: 5-FU 0 =-Fluorouracil; POAG = primary open angle glaucoma; PEXG = pseudoexfoliative glaucoma; IOP = intraocular pressure; VA= visual acuity; VF = visual field; MD = mean deviation; PSD = pattern standard deviation.

Table 2: Changes in intraocular pressure in operated patients

	pre	**1 day**	**7 day**	**1 month**	**3 month**	**6month**	**12 month**
5-FU	28.9±9.5	9.8±3.5	9.1±3.1	11.7±4.8	12.7±4.7	13.4±4.6	13.6±4.4
Bevacizumab	29.3±9.4	10.2±3.3	9.3±3.1	12.0±6.1	14.0±6.5	13.7±5.2	14.7±4.7
p*	p=0.96	p=0.62	p=0.80	p=0.99	p=0.39	p=0.85	p=0.35

IOP= mean value (mmHg)±SD

*p = adequacy of parameters in 5-FU and bevacizumab groups; U Mann-Whitney test

Abbreviations; 5-FU = 5-Fluorouracil;

Table 3: Comparison of parameters between the 2 study groups

	5-FU before surgery	**Bevacizumab before surgery**	**p***	**5-FU after surgery**	**Bevacizumab after surgery**	**p***
VA (logMAR)	0.6±0.2	0.6±0.2	0.74	0.6±0.2	0.6±0.2	0.64
MD (dB)	-19.6±7.9	-19.3 ±8.2	0.96	-19.4 ±8.5	-19.1 ±8.5	0.97
PSD (dB)	7.9 ±3.3	8.4 ±2.6	0.59	8.0 ±3.0	8.1 ±2.8	0.89
Endothelial cell density (cell/mm^2)	2013.9± 296.0	2108.4± 351.3	0.13	1908.8± 313.4	2026.8± 326.1	0.08

*p = adequacy of parameters in 5-FU and bevacizumab groups; U Mann-Whitney test
Abbreviations; 5-FU = 5-Fluorouracil; VA = visual acuity; MD = mean deviation; PSD = pattern standard deviation.

Table 4: Assessment of bleb morphology 12 months after trabeculectomy

		Group		Statistical analysis (χ^2 test)
		5-FU **n=30**	Bevacizumab **n=32**	
A bleb	1	2 (6.7%)	0 (0%)	χ^2=3.99 p=0.41
	2	12 (40.0%)	9 (28.1%)	
	3	7 (23.3%)	11 (34.4%)	
	4	7 (23.3%)	8 (25.0%)	
	5	2 (6.7%)	4 (12.5%)	
B bleb	1	2 (6.7%)	1 (3.1%)	χ^2=2.68 p=0.61
	2	11 (36.7%)	8 (25.0%)	
	3	8 (26.6%)	13 (40.6%)	
	4	7 (23.3%)	6 (18.8%)	
	5	2 (6.7%)	4 (12.5%)	
H bleb	1	3 (10.0%)	5 (15.6%)	χ^2=1.98 p=0.37
	2	18 (60.0%)	22 (68.8%)	
	3	9 (30.0%)	5 (15.6%)	
V bleb	1	19 (63.4%)	19 (59.4%)	χ^2=0.12 p=0.94
	2	10 (33.3%)	12 (37.5%)	
	3	1 (3.3%)	1 (3.1%)	

5-FU = 5-Fluorouracil

Table 5: Complications after trabeculectomy in the two study groups

	5-FU n=30	**Bevacizumab n=32**	**p***
Tenon's cyst	2 (6.7%)	3 (9.4%)	**χ^2=0.75 p=0.86**
Conjunctival leak	2 (6.7%)	2 (6.3%)	
Shallow AC	1 (3.3%)	1 (3.1%)	
Significant cataract	1 (3.3%)	0	

* χ^2 test

5-FU = 5-Fluorouracil

AC = anterior chamber

FIGURE

Figure 1: Kaplan Meier survival curve for time (months) to percentage of surgical success defined as 30% decline in initial IOP (complete and qualified success).

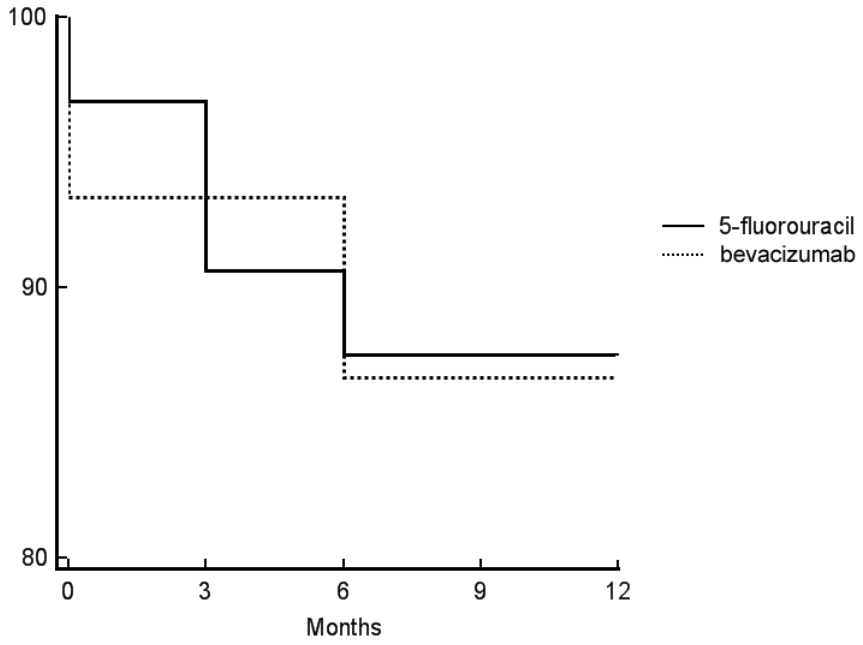
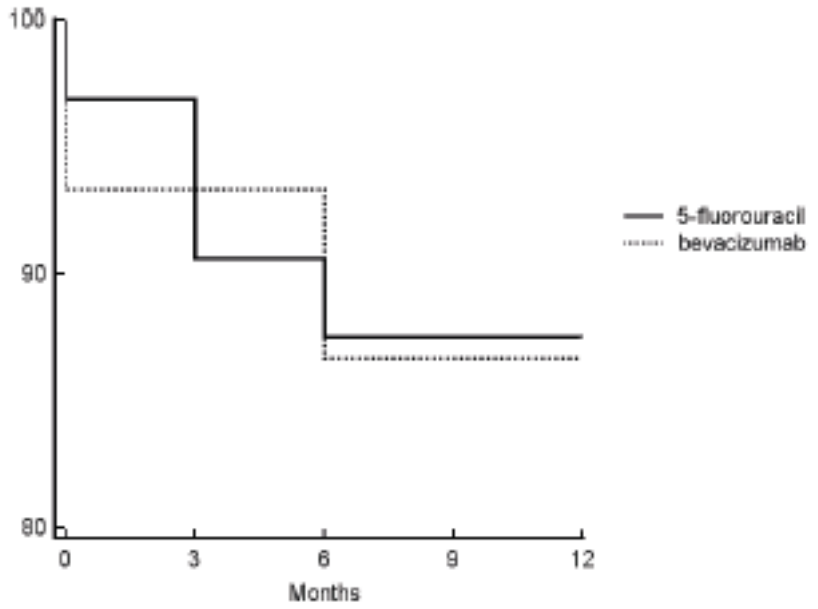


Figure 1: Kaplan Meier survival curve for time (months) to percentage of s
defined as 30% decline in initial IOP (complete and qualified success).



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