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DESCRIPTION

FIELD OF THE INVENTION

[0001] The current invention relates to the improvement of trabeculectomy surgery. The improvement more specifically resides in an extended lifetime of the sclera-corneal drainage channel created by trabeculectomy surgery. The improvement is obtained by administration of an antibody, or antigen-binding fragment thereof, binding to placental growth factor (P1GF) and inhibiting P1GF activity.

BACKGROUND OF THE INVENTION

[0002] Glaucoma is a multifactorial, neurodegenerative disease and the second most important cause of irreversible blindness (Quigley, 1996, Br J Ophthalmol 80, 389-393). This disease is characterized by progressive retinal ganglion cell apoptosis, resulting in visual field loss. Current treatment of this disease is directed towards the reduction of intraocular pressure (IOP), which is the main risk factor for glaucoma (Collaborative Normal-Tension Glaucoma Study Group, 1998, Am J Opthalmol 126, 487-497).

[0003] Of all currently used treatments to lower IOP, glaucoma filtration surgery (trabeculectomy), or shortly filtration surgery, was shown to be the most effective (Burr et al., 2005, Cochrane Database Syst Rev 18(2):CD004399; Hitchings, 1998, Arch Ophthalmol 116, 241-242). A trabeculectomy creates a "controlled" leak of fluid (aqueous humor) from the eye, which percolates under the conjunctiva. During the operation a piece of trabecular meshwork in the drainage angle of the eye is removed, creating an opening. The opening is partially covered with a flap of tissue from the sclera and conjunctiva. A small conjunctival "bleb" (bubble) appears at the junction of the cornea and the sclera (limbus) where this surgically produced valve is made.

[0004] In 30% of the cases, however, the constructed channel closes due to excessive scar tissue formation, resulting in surgical failure (Addicks et al., 1983, Arch Ophthalmol 101, 795-798). The 4 important processes contributing to post-operative conjunctival scarring are: clot formation, inflammation, angiogenesis and fibrosis (Lee et al., 1995, J Ocul Pharmacol Ther 11, 227-232; Lama & Fechtner, 2003, Surv Ophthalmol 48, 314-346). Indeed, increased conjunctival infiltration of inflammatory cells and Tenon fibroblasts (Hitchings & Grierson, 1983, Trans Ophthalmol Soc UK 103, 84-88; Skuta & Parrish, 1987, Surv Ophthalmol 32, 149-170), and higher levels of bleb vascularisation (Jampel et al., 1988, Arch Ophthalmol 106, 89-94) are associated with surgical failure. These processes are mediated by various cytokines (e.g. IL-1 and INF-α2b) and growth factors (e.g. PDGF, FGF, TGF-β1 and VEGF (Lama & Fechtner, 2003; Gillies & Su, 1991, Aust NZ J Ophthalmol 19, 299-304)). The role of VEGF in these processes was initially unclear (Li et al. 2006, EVER 2006 Abstract e2251) but later elucidated

to some extent (Van Bergen et al. 2011, Exp. Eye Res. 93, 689-699), whereas the role of P1GF remains elusive (Li et al. 2006, EVER 2006 Abstract e2251; Van Bergen et al. 2011, Bull. Soc. Belge Ophthalmol. 317, 65-66), although P1GF was described as an ocular hypotensive peptide in WO 2010/097800. Peroperative anti-mitotics, such as mitomycin-C and 5-Fluorouracyl can improve surgical outcome (Quigley, 1996; Katz et al., 1995, Ophthalmol 102, 1263-1269). However, these antimetabolites carry a risk of vision-threatening complications such as scleral thinning and infections (Lama & Fechtner, 2003; Hitchings & Grierson, 1983; Skuta & Parrish, 1987; Jampel et al., 1988; Gillies & Su, 1991; Katz et al., 1995; Greenfield et al., 1998, Arch Ophthalmol 116, 443-447). Furthermore, blocking TGF-ß seemed promising in animal models (Cordeiro et al., 2003, Gene Ther 10, 59-71), but was not efficient in a clinical study (CAT-152 0102 Trabeculectomy Study Group, Kwah, Grehn, 2007, Ophthalmol 114, 1822-1830). The number of post-trabeculectomy interventions expressed as the incidence of post-surgery "bleb manipulations" was reported to be as high as 78% (King et al., 2007, Br J Ophthalmol 91, 873-877). Therefore, there is still a need for alternative strategies to prevent filtration failure and, thus, to reduce the incidence of bleb manipulations.

[0005] Microplasmin is a recombinant protein that dissolves blood clots by degrading fibrin. Recently, microplasmin has been shown to be efficient, well tolerated and safe for intra-ocular use (WO 2004/052228) and was approved by FDA in October 2012 for treating vitreomacular adhesion (JETREA®; non-proprietary name: ocriplasmin). Results of the phase III clinical trials leading to this approval were published by Stalmans et al. (2012, N Engl J Med 367, 606-615). Plasmin was previously shown to be able to induce PVD as well (e.g. US 5,304,118). The mechanism by which PVD is induced by plasmin or microplasmin is currently not fully understood. Unsupported by any or any conclusive experimental data, WO 2009/073457 and WO 2009/067407 propose subconjunctival plasmin injection for rescuing filtering blebs and the use of matrix metalloproteinase activating proteases for reducing IOP, respectively. WO 2011/023805 provides the evidence that anterior chamber injection of microplasmin was effective in prolonging bleb survival, i.e., the mode of administration of microplasmin in this indication is determining success.

[0006] Pegaptanib is a pegylated anti-VEGF aptamer (VEGF = vascular endothelial growth factor), a single strand of nucleic acid (50 kDa). It specifically binds the $VEGF_{165}$ isoform, thereby preventing the binding to the heparin binding domain.Van Bergen et al. 2011 (Exp Eye Res 93, 689-699) showed that single or repeated injection of pegaptanib after glaucoma filtration surgery (in a rabbit model) had marginal effect on bleb area and bleb survival. Bevacizumab is an antibody inhibiting all forms of VEGF-A. Li et al. 2009 (Invest Ophthalmol Vis Sci 50, 5217-5225) disclosed the effect of bevacizumab on glaucoma filtration surgery (in a rabbit model) which was, judging from the reported effect on bleb area, limited.

SUMMARY OF THE INVENTION

[0007] The invention relates to a neutralizing anti-PlGF (placental growth factor) antibody or a neutralizing anti-PlGF antibody fragment for use in treating filtration failure after

trabeculectomy surgery (or glaucoma filtration surgery) of an eye, or for use in preventing, reducing or retarding the occurrence of filtration failure after trabeculectomy surgery of an eye.

[0008] The neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment as described above may be in a pharmaceutically acceptable formulation capable of being administered to an eye. In particular, said pharmaceutically acceptable formulation is compatible with administration into the anterior chamber of an eye or with in-bleb administration wherein the bleb is created by the trabeculectomy surgery

[0009] In any of the above, said treating of filtration failure after trabeculectomy surgery of an eye, or said preventing, reducing or retarding of the occurrence of filtration failure after trabeculectomy surgery of an eye with a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment in particular results from administering to said eye at least a single dose, or, alternatively, multiple doses, of an effective amount of said neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment. When multiple doses are administered to an eye, these may be administered with at least 6-hour time intervals. Said eye may be contacted further with one or more agents chosen from an agent for controlling the intra-ocular pressure, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine, an anesthetic, an agent to induce mydriasis and an agent to induce cycloplegia. Said further contacting may be occurring prior to, concurrent with, or after administering the neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment.

[0010] The neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment as described above may be in a pharmaceutically acceptable formulation further comprising one or more of an agent for controlling the intra-ocular pressure, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine, an anesthetic, an agent to induce mydriasis and an agent to induce cycloplegia.

[0011] In particular, the anti-mitotic agent in the above may be mitomycin C or 5-fluorouracyl.

FIGURE LEGENDS

[0012]

FIGURE 1. Fig. 1A shows result of measurement of the intra-ocular pressure (IOP) in two groups (group size n=10) that had undergone glaucoma filtration surgery (GFS). One group was treated with 1C8, an irrelevant mouse IgG antibody (4.8 mg/ml) and the other group was treated with DC101, an anti-murine VEGF-R2 antibody (6.2 mg/ml). IOPs were measured with a Tonolab (Technop®). Results are represented as mean ±SEM. IOP was not found to be significantly different in the 2 groups ($p>0.05$). Fig. 1B shows in a similar way the result of measurement of in two groups (group size n=10) that had undergone GFS. One group was treated with 1 C8, an irrelevant mouse IgG antibody (4.8 mg/ml) and the other group was

treated with 5D11D4, an anti-murine PlGF antibody (5.2 mg/ml). IOP was not found to be significantly different in the 2 groups (p>0.05).

FIGURE 2. Fig. 2A shows measurements of bleb area in two groups (group size n=10) that had undergone (GFS). One group was treated with 1C8, an irrelevant mouse IgG antibody (4.8 mg/ml) and the other group was treated with DC101, an anti-murine VEGF-R2 antibody (6.2 mg/ml). Results are represented as mean ±SEM. DC101 significantly improved bleb area as compared to 1C8 (p=0.05). Fig. 2B is similar to Fig. 2A except that both groups were larger in size (n=20) and were followed during a longer time period. Fig. 2C shows similar results as Fig. 2A and 2B except that the antibodies were injected repeatedly at days 0, 4 and 10 after surgery.

FIGURE 3. Fig. 3A shows bleb survival in two groups (group size n=10) that had undergone GFS. One group was treated with 1C8, an irrelevant mouse IgG antibody (4.8 mg/ml) and the other group was treated with DC101, an anti-murine VEGF-R2 antibody (6.2 mg/ml). Bleb survival was not found to be significantly different in the 2 groups (p=0.23). After longer follow-up of two larger groups (n=20; Fig. 3B), significantly increased bleb survival was observed in the DC101-treated group vs the 1C8-treated group (p=0.06). Repeated injection of DC101 (group size n=10) at days 0, 4 and 10 after surgery further increased the bleb survival compared to single injections (Fig. 3C).

FIGURE 4. Fig. 4A shows measurements of bleb area in two groups (group size n=10) that had undergone GFS. One group was treated with 1C8, an irrelevant mouse IgG antibody (4.8 mg/ml) and the other group was treated with 5D11D4, an anti-murine PlGF antibody (5.2 mg/ml). Results are represented as mean ±SEM. 5D11D4 significantly improved bleb area as compared to 1C8 (p=0.01). This effect extended till the end of a longer follow-up period of larger groups (n=20; Fig. 4B) and was markedly enhanced by repeat injections of 5D11D4 on days 0, 4 and 10 after surgery (group size n=10; Fig. 4C). The latter effect was significantly more pronounced compared to repeat injections of anti-murine VEGF-R2 antibody DC101 (Fig. 2C / Fig. 6 C)

FIGURE 5. Fig. 5A shows bleb survival in two groups (group size n=10) that had undergone GFS. One group was treated with 1C8, an irrelevant mouse IgG antibody (4.8 mg/ml) and the other group was treated with 5D11D4, an anti-murine PlGF antibody (5.2 mg/ml). Results are represented as mean ±SEM. 5D11D4 significantly improved bleb survival as compared to 1C8 (p=0.04). This effect extended till the end of a longer follow-up period of larger groups (n=20; Fig. 5B) which was significantly more pronounced compared to the anti-murine VEGF-R2 antibody DC101 (Fig. 3B), and was markedly enhanced by repeat injections of 5D11D4 on days 0, 4 and 10 after surgery (group size n=10; Fig. 5C).

FIGURE 6. Fig. 6A shows measurements of bleb area in two groups (group size n=10) that had undergone GFS. One group was treated with DC101, an anti-murine VEGF-R2 antibody (6.2 mg/ml) and the other group was treated with 5D11D4, an anti-murine PlGF antibody (5.2 mg/ml). Results are represented as mean ±SEM. 5D11D4 significantly improved bleb area as compared to 1C8 (Fig. 1). A trend towards an increased bleb area after 5D11D4 administration was observed compared to DC101 delivery (p=0.07). The latter was confirmed and

strengthened when observing two groups (group size n=20) for a longer time period (Fig. 6B). The stronger effect of 5D11D4 administration over DC101 administration was moreover clearly obviated when comparing the effect of multiple administrations (at days 0, 4 and 10 after surgery; group size n=10) of the antibodies as depicted in Fig. 6C.

DETAILED DESCRIPTION OF THE INVENTION

[0013] As known from clinical practice, each subject or patient undergoing trabeculectomy surgery is at significant risk to develop filtration failure. The present invention is based on the effect of administration of a neutralizing anti-PlGF antibody (antibody inhibiting the activity of placental growth factor, PlGF) on the clinical outcome of trabeculectomy surgery, said effect being positive and resulting in the prevention, reduction or retardation of the occurrence of filtration failure. The effects obtained with a neutralizing anti-PlGF antibody are moreover markedly and unexpectedly more pronounced than the effects obtained with an inhibitor of VEGF-R2 (vascular endothelial growth factor receptor 2, known to bind VEGF) or obtained with inhibitors of $VEGF_{165}$ (pegaptanib; Van Bergen et al. 2011, Exp Eye Res 93, 689-699) or of VEGF (bevacizumab; Li et al. 2009, Invest Ophthalmol Vis Sci 50, 5217-5225).

[0014] Therefore, the invention relates to a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment for use in treating filtration failure after trabeculectomy surgery of an eye, , or for use in preventing, reducing or retarding the occurrence of filtration failure after trabeculectomy surgery of an eye; all evidently as compared to trabeculectomy surgery performed without using a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment. Alternatively, the invention relates to the use of a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment for the manufacture of a medicament for treating filtration failure after trabeculectomy surgery of an eye, or for/of preventing, reducing or retarding the occurrence of filtration failure after trabeculectomy surgery of an eye; all evidently as compared to trabeculectomy surgery performed without using a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment. The terms "glaucoma filtration surgery", "filtration surgery" and "trabeculectomy surgery" are used herein interchangeably.

[0015] The "trabecular meshwork (TM)" is a mesh-like structure inside the eye at the iris-scleral junction of the anterior chamber angle. The TM filters the aqueous fluid and controls its flow into the canal of Schlemm prior to its leaving the anterior chamber. Increased resistance in the TM leads to reduced aqueous fluid outflow and thus increased intra-ocular pressure (IOP).

[0016] When left untreated, this elevated IOP leads to glaucomatous damage to the optic nerve and retinal nerve fibers, and leads to loss of vision. This vision loss can be prevented or halted by administering medication, an "agent for controlling the intra-ocular pressure", which controls the intra-ocular pressure. Such medicaments include adrenergic blocking agents (beta blockers or sympatholytic drugs such as betaxolol, carteolol, levobunolol, metipanolol and

timolol), adrenergic stimulating agents (sympathomimetic drugs such as aproclonidine, epinephrine, hydroxyamphetamine, phenylephrine, naphazoline and tetrahydrozaline), carbonic anhydrase inhibitors (such as systemic acetozolamide, and topical brinzolamide and dorzolamide), miotics (cholinergic stimulating agents, parasympathomimetic drugs such as carbachol and pilocarpine), osmotic agents (such as glycerin and mannitol), prostaglandin and prostaglandin analogues (prostamides, bimatoprost, unoprostone isopropyl, travoprost, latanoprost, natural prostaglandin, prostaglandin F2α, and FP prostanoid receptor agonists). When such medicaments are not efficient (or not anymore), then filtration surgery is a viable treatment.

[0017] "Trabeculectomy", "trabeculectomy surgery" or "filtration surgery", or "glaucoma filtration surgery", is defined as a surgical procedure on the eye wherein part of the trabecular meshwork is removed whereby a filtration site (a sclera-corneal drainage channel) is created that increases the outflow of aqueous fluid from the eye; this type of filtering procedure is commonly used in the treatment of glaucoma, and more specifically to reduce the IOP in an eye subject to/suffering from glaucoma.

[0018] "Filtration failure" is a condition reversing the clinically desired effect of trabeculectomy surgery, i.e., reversing the desired drop in IOP. The initial post-operative time is crucial in the sense that eye-healing activities are highest in this period. This period of high eye-healing capacity is dependent upon the species and spans about 2 weeks for rabbits and up to 1- to 2-months in humans. Upon contacting a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment with an eye according to the current invention, the frequency of occurrence of filtration failure over a given period of time is lowered. The neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment used according to the current invention thus results in the prevention, reduction or retarding of the occurrence of filtration failure, or in an improvement, enhancement or increase of the success rate of trabeculectomy surgery (compared to trabeculectomy surgery without administering or using a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment).

[0019] The neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment, or a medicament comprising it, may be in a pharmaceutically acceptable formulation (or composition or solution) capable of being administered to an eye. In particular, said formulation (or composition or solution) is capable of being administered into the anterior chamber of the eye or compatible with administration into the anterior chamber of the eye. Alternatively, said formulation (or composition or solution) is capable of being administered into the surgically created bleb (i.e., in-bleb administration) or compatible with administration into bleb. Said administration may e.g. be by injection of the formulation (or composition or solution) or medicament comprising a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment. Although not required, there may be an additional advantage in said formulation being a slow-release formulation such as a gel-like formulation.

[0020] The treatment of filtration failure after trabeculectomy surgery (or glaucoma filtration surgery) of an eye, or the prevention, reduction or retardation of the occurrence of filtration

failure after trabeculectomy surgery of an eye may result from introduction into the eye of an effective amount of at least a single dose of a neutralizing anti-PlGF antibody (or neutralizing anti-PlGF antibody fragment) or of a formulation (or composition or solution) or medicament comprising it. In particular, said administration is into the anterior chamber of an eye, such as by injection. Alternatively, multiple doses of an effective amount of said neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment (or of a formulation (or composition or solution) or medicament comprising it) may be administered, such as to increase efficacy. When multiple doses are administered to an eye, these may be administered with at least 6-hour time intervals, with about 12-hour time intervals, with about 18-hr time intervals, with about 1-day time intervals, with about 2-day time intervals, with about 3-day time intervals, with about 4-day time intervals, with about 7-day time intervals, with about 2-week time intervals with about 1-month time intervals, with about 2-month time intervals or with about 3-month time intervals. When multiple doses are administered to an eye with time intervals, the time interval between two subsequent doses may change during the treatment depending on the evolution of the clinical result. For example, time intervals between subsequent doses may be short immediately after the trabeculectomy surgery and may increase with increasing time after the trabeculectomy surgery.

[0021] In any of the above, said anti-PlGF antibody may be any type of antibody or any fragment of any thereof that is capable of binding to PlGF and of inhibiting the activity of PlGF. In particular, said anti-PlGF antibody or fragment thereof may be neutralizing the activity of PlGF, thus may be a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment. Such antibodies include all types of antibodies known in the art, such as human or humanized antibodies, cameloid antibodies, nanobodies, domain antibodies, mono- or plural-specific antibodies, etc., and any fragment of any thereof. Examples of neutralizing anti-PlGF antibodies are described in WO 01/85796 and WO 2006/099698. In particular, an anti-PlGF antibody for use as described herein is effective in inhibiting the activity of placental growth factor as present in the subject undergoing trabeculectomy. In particular, said subject is a mammal, more in particular a human.

[0022] The invention further covers a neutralizing anti-PlGF antibody (or any neutralizing anti-PlGF antibody fragment) as described above for use in treating filtration failure after trabeculectomy surgery of an eye, or for use in preventing, reducing or retarding the occurrence of filtration failure after trabeculectomy surgery of an eye, wherein the neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibodyfragment is in a pharmaceutically acceptable formulation (or composition or solution) that may further comprise one or more of an agent for controlling the intra-ocular pressure, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine, an anesthetic, an agent to induce mydriasis and an agent to induce cycloplegia. Alternatively, when said further agent is, or said further agents are, not included in the pharmaceutically acceptable formulation (or composition or solution) containing said neutralizing anti-PlGF antibody (or any neutralizing anti-PlGF antibody fragment), said eye may be contacted further with one or more agents chosen from an agent for controlling the intra-ocular pressure, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an anti-angiogenic agent, an

anti-mitotic agent, an antihistamine, an anesthetic, an agent to induce mydriasis and an agent to induce cycloplegia. Such further contacting may be prior to, concurrent with, or after the administration of a neutralizing anti-PlGF antibody or any neutralizing anti-PlGF antibody fragment (or of a formulation, composition, solution, or medicament comprising it).

[0023] The invention further covers the use of a neutralizing anti-PlGF antibody (or any neutralizing anti-PlGF antibody fragment) as described above for the manufacture of a medicament for treating filtration failure after trabeculectomy surgery of an eye, or for preventing, reducing or retarding the occurrence of filtration failure after trabeculectomy surgery of an eye, wherein the neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment is in a pharmaceutically acceptable composition that may further comprise one or more of an agent for controlling the intra-ocular pressure, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine, an anesthetic, an agent to induce mydriasis and an agent to induce cycloplegia. Alternatively, when said further agent is, or said further agents are, not included in the pharmaceutically acceptable formulation (or composition or solution) or medicament containing said neutralizing anti-PlGF antibody (or any neutralizing anti-PlGF antibody fragment), said eye may be contacted further with one or more agents chosen from an agent for controlling the intra-ocular pressure, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine, an anesthetic, an agent to induce mydriasis and an agent to induce cycloplegia. Such further contacting may be prior to, concurrent with, or after the administration of a neutralizing anti-PlGF antibody or any neutralizing anti-PlGF antibody fragment (or of a formulation, composition, solution, or medicament comprising it).

[0024] "Contacting" means any mode of administration that results in interaction between an agent or composition such as a medicament and an object (such as conjunctiva or subconjunctival tissue) with which said agent or composition is contacted. The interaction between the agent or composition and the object can occur starting immediately or nearly immediately with the administration of the agent or composition, can occur over an extended time period (starting immediately or nearly immediately with the administration of the agent or composition), or can be delayed relative to the time of administration of the agent or composition. More specifically the "contacting" results in delivering an effective amount of the agent, composition or medicament to the object.

[0025] The term "effective amount" refers to the dosing regimen of the agent, composition or medicament according to the invention, in particular of the active ingredient of the medicament according to the invention, i.e., a neutralizing anti-PlGF antibody or a neutralizing anti-PlGF antibody fragment. The effective amount will generally depend on and will need adjustment to the mode of contacting or administration. The effective amount of the agent, composition or medicament, more particular its active ingredient, is the amount required to obtain the desired clinical outcome or therapeutic or prophylactic effect without causing significant or unnecessary toxic effects. To obtain or maintain the effective amount, the agent, composition or medicament may be administered as a single dose or in multiple doses. The effective amount may further

vary depending on the severity of the condition that needs to be treated or the expected severity of the condition that needs to be prevented or treated; this may depend on the overall health and physical condition of the patient and usually the treating doctor's or physician's assessment will be required to establish what is the effective amount. The effective amount may further be obtained by a combination of different types of contacting or administration. In the context of the present invention the effective amount may more particularly be obtained by either one or more of administration of topical eye drops, administration by injection into the anterior chamber of an eye or administration by subconjunctival injection. A typical dose of a single administration of the agent, composition or medicament of the invention may comprise 10 µg to 20 mg of the active compound, or alternatively may comprise 10 µg/kg body weight to 20 mg/kg body weight of the active compound. Administration of the medicament of the invention by means of injection typically is kept to a minimum, i.e., the frequency of repeat injections is kept to a minimum. As the first weeks or months post-trabeculectomy (species dependent as described higher) are crucial in the sense that eye-healing activities are highest in this period, the duration of treatment with an agent, composition or medicament according to the present invention should be adjusted to this period.

[0026] In general, the formulation (or composition or solution) or medicament of the invention comprising a neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment according to the invention may, depending on its ultimate use and mode of administration, comprise one or more further active ingredients such as an agent controlling the intra-ocular pressure (see higher), an anticoagulant, a thrombolytic agent, an anti-inflammatory agent, an antiviral agent, an antibacterial agent, an antifungal agent, an anti-angiogenic agent, an anti-mitotic agent, an antihistamine or anesthetic.

[0027] "Anticoagulants" include hirudins, heparins, coumarins, low-molecular weight heparin, thrombin inhibitors, platelet inhibitors, platelet aggregation inhibitors, coagulation factor inhibitors, anti-fibrin antibodies and factor VIII-inhibitors (such as those described in WO 01/04269 and WO 2005/016455).

[0028] "Thrombolytic agents" include urokinase, streptokinase, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) and staphylokinase or any variant or derivative of any thereof such as APSAC (anisoylated plasminogen streptokinase activator complex), alteplase, reteplase, tenecteplase, and scuPA (single chain uPA), plasmin or any truncated variant thereof such as midiplasmin, miniplasmin, deltaplasmin and microplasmin.

[0029] "Anti-inflammatory agents" include steroids (e.g. prednisolone, methylprednisolone, cortisone, hydrocortisone, prednisone, triamcinolone, dexamethasone) and non-steroidal anti-inflammatory agents (NSAIDs; e.g. acetaminophren, ibuprofen, aspirin).

[0030] "Antiviral agents" include trifluridine, vidarabine, acyclovir, valacyclovir, famciclovir, and doxuridine.

[0031] "Antibacterial agents" or antibiotics include ampicillin, penicillin, tetracycline,

oxytetracycline, framycetin, gatifloxacin, gentamicin, tobramycin, bacitracin, neomycin and polymyxin.

[0032] "Anti-mycotic/fungistatic/antifungal agents" include fluconazole, amphotericin, clotrimazole, econazole, itraconazole, miconazole, 5-fluorocytosine, ketoconazole and natamycin.

[0033] "Anti-angiogenic agents" include antibodies (or fragments thereof) such as anti-VEGF (vascular endothelial growth factor) or anti-PlGF (placental growth factor) antibodies and agents such as macugen (pegaptanib sodium), trypthophanyl-tRNA synthetase (TrpRS), anecortave acetate, combrestatin A4 prodrug, AdPEDF (adenovector capable of expressing pigment epithelium-derived factor), VEGF-trap, inhibitor of VEGF receptor-2, inhibitors of VEGF, PlGF or TGF-β, Sirolimus (rapamycin) and endostatin.

[0034] "Anti-mitotic agents" include mitomycin C and 5-fluorouracyl.

[0035] "Antihistamine" includes ketitofen fumarate and pheniramine maleate.

[0036] "Anesthetics" include benzocaine, butamben, dibucaine, lidocaine, oxybuprocaine, pramoxine, proparacaine, proxymetacaine, tetracaine and amethocaine.

[0037] Other adjunct agents or drugs that can be used in conjunction with the anti-PlGF antibody or fragment thereof according to the invention include scopoloamine, atropine or tropicamide, to induce mydriasis (pupillary dilation) and/or cycloplegia (paralysis of the eye focusing muscle).

[0038] In addition to the neutralizing anti-PlGF antibody or neutralizing anti-PlGF antibody fragment, each of the above listed agents as well as antihistamine and anesthetics is to be considered as an "active ingredient".

[0039] A "pharmaceutically acceptable formulation" is, in the context of the current invention more particular an "ophthalmologically acceptable formulation". A formulation in general is a composition comprising a carrier, diluent or adjunvant compatible with the one or more active ingredients to be formulated, the whole formulation being compatible with the intended use in the intended tissue or organ, etc. Examples of pharmaceutically acceptable formulations as well as methods for making them can be found, e.g., in Remington's Pharmaceutical Sciences (e.g. 20th Edition; Lippincott, Williams & Wilkins, 2000) or in any Pharmacopeia handbook (e.g. US-, European- or International Pharmacopeia).

[0040] "Lubricants" include propylene glycerol, glycerin, carboxymethylcellulose, hydroxypropylmethylcellulose, soy lecithin, polyvinyl alcohol, white petrolatum, mineral oil, povidone, carbopol 980, polysorbate 80, dextran 70.

EXAMPLES

[0041] The Examples included hereafter demonstrate the invention.

[0042] PURPOSE. Excessive postoperative wound healing with subsequent inflammation and scarring frequently leads to surgical failure of glaucoma filtration surgery (GFS). The hypothesis was checked that placental growth factor (PlGF) plays a role in scar formation after GFS, and that it may be a target for improvement of the outcome of this surgery.

[0043] METHODS. Aqueous humor and plasma samples of glaucoma and control patients (n=10) were collected and PlGF levels were determined by ELISA. The effect of the anti-murine PlGF-antibody (5D11D4) was investigated in a mouse model of GFS in C75B1/6 mice. In the single-injection setting, 5D11D4 (1µl; 5.2mg/ml; antibody described in detail in WO 01/85796) or 1C8, an irrelevant mouse IgG antibody against human tissue plasminogen activator (1µl; 4.8 mg/ml; antibody available at ThromboGenics), were injected in the anterior chamber (n=10 eyes or n=20 eyes for both groups) immediately after surgery ("day 0"). An anti-murine VEGF-R2 antibody (DC101) was used as a positive control (1µl; 6.2 mg/ml; n=10). Mice were killed on post-operative day 8. In the multiple- or repeated-injection setting, the antibodies were administered as above, albeit it on days 0, 4 and 10 after surgery; groups of n=10 eyes were assessed; and mice were killed on post-operative day 13. Treatment outcome was studied by clinical investigation of intra-ocular pressure (IOP), bleb area and bleb survival every other day.

[0044] RESULTS. PlGF levels in aqueous humor were found to be significantly upregulated in glaucoma compared to control patients (17 ± 2 pg/ml versus 12 ± 0.75 pg/ml, p=0.03). No significant differences were found in plasma concentrations of PlGF. In the mouse model of GFS, single administration of the anti-PlGF antibody (5D11D4) significantly improved surgical outcome by increasing bleb survival (p=0.04) and bleb area (p=0.01) with 29% compared to negative control (1C8). A single administration of anti-VEGF-R2 (DC101) also significantly improved bleb area with 7% as compared to 1C8 (p=0.05), but had no effect on bleb survival (p=0.23). A trend towards an increased bleb area after 5D11D4 administration was observed compared to DC101 delivery (p=0.07). IOP was not found to be significantly different in any of the groups (p>0.05). Results of single administration of the antibodies are depicted in Figure 1 (IOP); Figures 2A, 2B, 4A, 4B, 6A and 6B (all bleb area); and Figures 3A, 3B, 5A and 5B (bleb survival).

[0045] Multiple- or repeated administrations of the antibodies (at days 0, 4 and 10 after surgery) led to a more pronounced improvement of surgical outcome with both 5D11D4 and DC101 separately compared to 1C8, and with a more pronounced positive outcome with 5D11D4 compared to DC101. Furthermore, the trend towards an increased bleb area after 5D11D4 administration compared to DC101 upon single administration (p=0.07) was converted into a significant difference. The latter further proves that an anti-PlGF antibody (5D11D4) is more efficient in improving surgical outcome of GFS than an anti-VEGF-R2 antibody (DC101) (p=0.005). Results of repeated administrations of the antibodies are depicted in Figures 2C, 4C

and 6C (all bleb area); and Figures 3C and 5C (bleb survival).

[0046] CONCLUSIONS. Local production of PlGF in the eye may indicate an important role for this growth factor in wound healing after GFS. Indeed, targeting PlGF with an inhibitory monoclonal antibody is efficacious in improving GFS outcome, even more efficacious than inhibition of VEGF-R2 as described herein, and more efficacious than inhibition of VEGF-165 (Van Bergen et al. 2011, Exp Eye Res 93, 689-699) or VEGF-A (Li et al. 2009, Invest Ophthalmol Vis Sci 50, 5217-5225). This effect is seen with single administration of an anti-PlGF antibody and is significantly enhanced upon multiple administrations of an anti-PlGF antibody. These results render PlGF an validated target for ocular wound healing and point to the therapeutic benefits of PlGF-inhibition in this setting.

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- Remington's Pharmaceutical SciencesLippincott, Williams & Wilkins20000000 [0039]

Patentkrav

1. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse til behandling af manglende filtreringsevne efter trabekulektomi-operation i et øje eller til anvendelse til forebyggelse, reducering eller forsinkelse af forekomsten af manglende filtreringsevne efter trabekulektomi-operation i et øje.

2. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 1, som er i en farmaceutisk acceptabel formulering, der kan administreres til et øje.

3. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 2, som er i en farmaceutisk acceptabel formulering, der kan administreres i forkammeret i et øje.

4. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 2, som er i en farmaceutisk acceptabel formulering, der kan administreres i blisten, der dannes ved trabekulektomi-operationen.

5. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 1, hvor behandlingen af manglende filtreringsevne efter trabekulektomi-operation i et øje eller forebyggelsen, reduceringen eller forsinkelsen af forekomsten af manglende filtreringsevne efter trabekulektomi-operation i et øje opnås ved administrering til øjet af mindst en enkelt dosis af en effektiv mængde af det neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment.

6. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge et hvilket som helst af kravene 1 til 5, som er i en farmaceutisk

acceptabel formulering, der yderligere omfatter et eller flere af et middel til at kontrollere det intraokulære tryk, et antiinflammatorisk middel, et antiviralt middel, et antibakterielt middel, et antiangiogent middel, et antimitotikum, et antihistamin, et anæstetikum, et middel til inducering af pupildilatation og et middel til inducering af cycloplegi.

7. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 5, hvor øjet bringes yderligere i kontakt med et eller flere midler udvalgt blandt et middel til at kontrollere det intraokulære tryk, et antiinflammatorisk middel, et antiviralt middel, et antibakterielt middel, et antiangiogent middel, et antimitotikum, et antihistamin, et anæstetikum, et middel til inducering af pupildilatation og et middel til inducering af cycloplegi.

8. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 7, hvor den yderligere kontakt forekommer før, samtidig med eller efter administrering af det neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment.

9. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 1, hvor behandlingen af manglende filtreringsevne efter trabekulektomi-operation i et øje eller forebyggelsen, reduceringen eller forsinkelsen af forekomsten af manglende filtreringsevne efter trabekulektomi-operation i et øje opnås ved administrering til øjet af flere doser af en effektiv mængde af det neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment.

10. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 9, hvor de flere doser administreres med mindst 6 timers intervaller.

11. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 9 eller 10, som er i en farmaceutisk acceptabel formulering, der yderligere omfatter et eller flere af et middel til at kontrollere det intraokulære tryk, et antiinflammatorisk middel, et antiviralt middel, et antibakterielt middel, et antiangiogent middel, et antimitotikum, et antihistamin, et anæstetikum, et middel til inducering af pupildilatation og et middel til inducering af cycloplegi.

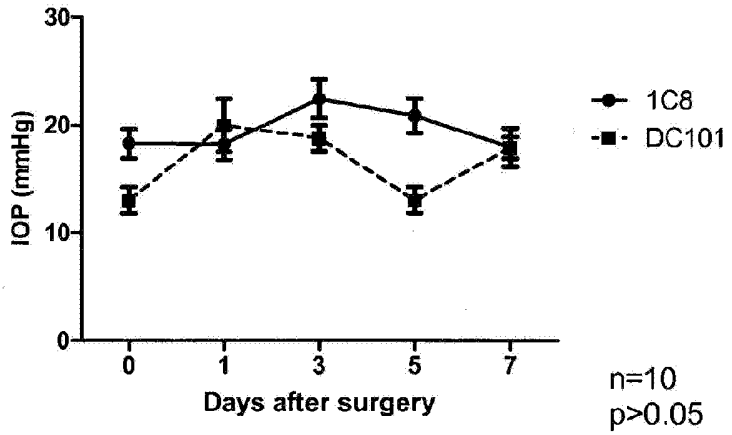
12. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 9 eller 10, hvor øjet bringes yderligere i kontakt med et eller flere midler udvalgt blandt et middel til at kontrollere det intraokulære tryk, et antiinflammatorisk middel, et antiviralt middel, et antibakterielt middel, et antiangiogent middel, et antimitotikum, et antihistamin, et anæstetikum, et middel til inducering af pupildilatation og et middel til inducering af cycloplegi.

13. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge krav 12, hvor den yderligere kontakt forekommer før, samtidig med eller efter administrering af det neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment.

14. Neutraliserende anti-PlGF-antistof eller neutraliserende anti-PlGF-antistoffragment til anvendelse ifølge et hvilket som helst af kravene 6 til 8 og 11 til 13, hvor antimitotikummet er mitomycin C eller 5-fluoruracyl.

DRAWINGS

A



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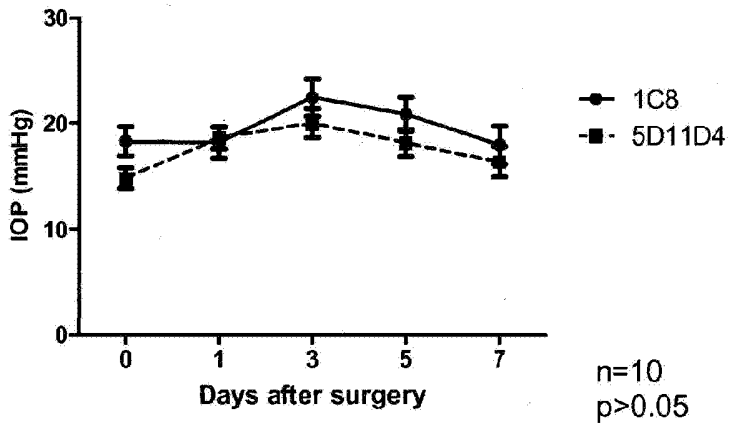


FIGURE 1

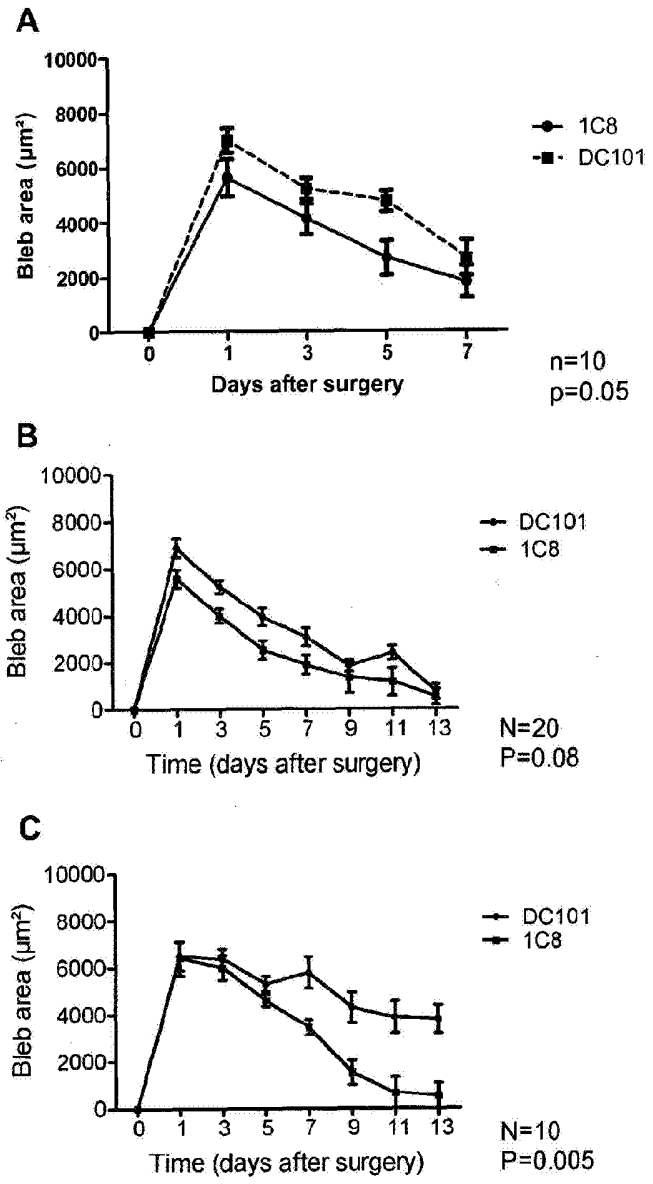
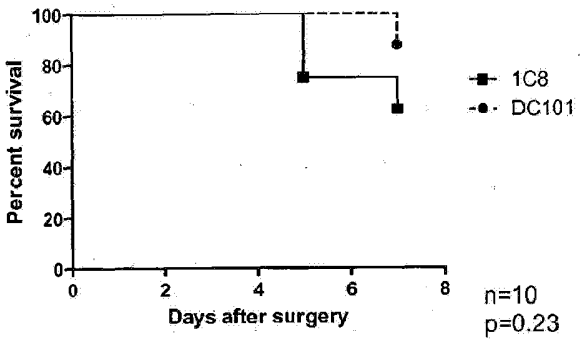
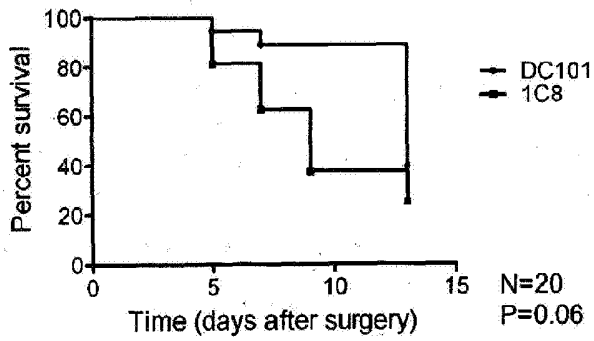

A
Bleb area (µm²)
10000
8000
6000
4000
2000
0
0
1
3
5
7
Days after surgery
1C8
DC101
n=10
p=0.05
B
Bleb area (µm²)
10000
8000
6000
4000
2000
0
0
1
3
5
7
9
11
13
Time (days after surgery)
DC101
1C8
N=20
P=0.08
C
Bleb area (µm²)
10000
8000
6000
4000
2000
0
0
1
3
5
7
9
11
13
Time (days after surgery)
DC101
1C8
N=10
P=0.005

FIGURE 2

A


100
80
60
40
20
0
Percent survival
0
2
4
6
8
Days after surgery
1C8
DC101
n=10
p=0.23

B


100
80
60
40
20
0
Percent survival
0
5
10
15
Time (days after surgery)
DC101
1C8
N=20
P=0.06

C

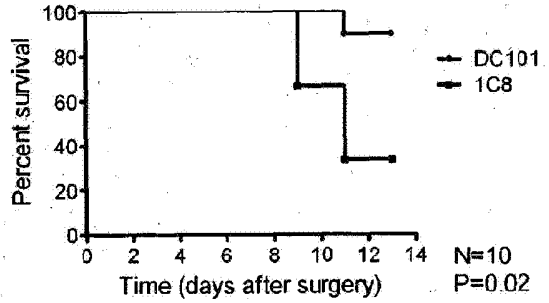

100
80
60
40
20
0
Percent survival
0
2
4
6
8
10
12
14
Time (days after surgery)
DC101
1C8
N=10
P=0.02

FIGURE 3

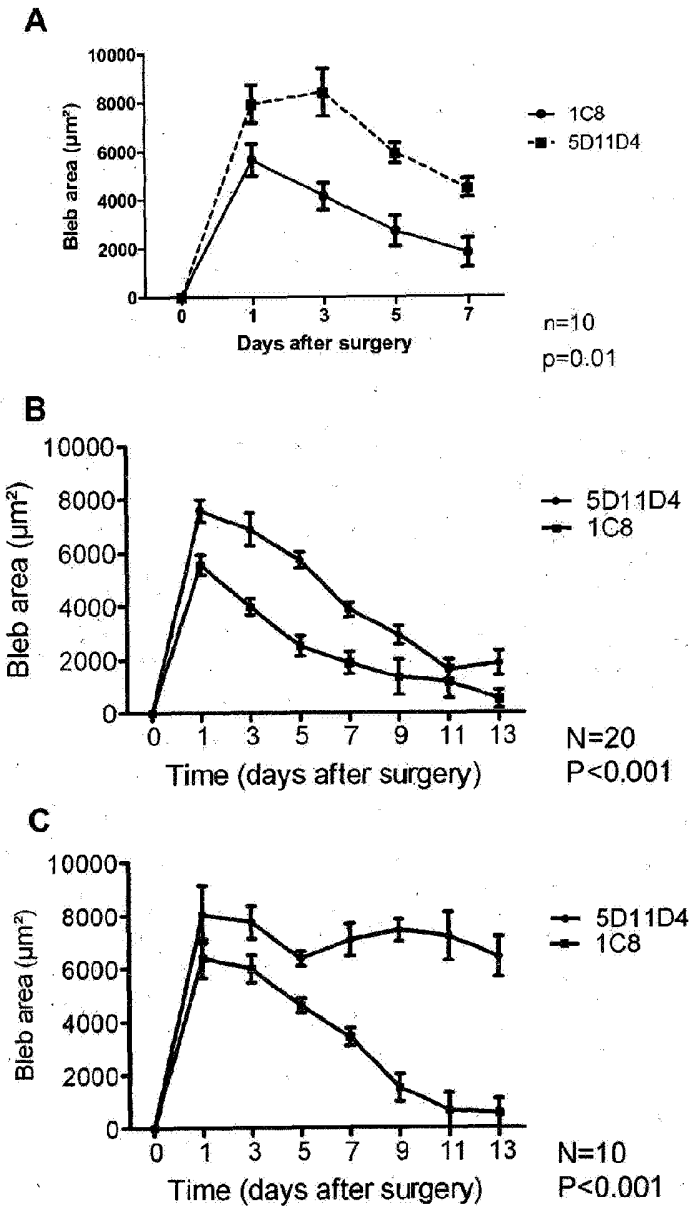

A
Bleb area (µm²)
10000
8000
6000
4000
2000
0
0
1
3
5
7
Days after surgery
1C8
5D11D4
n=10
p=0.01
B
Bleb area (µm²)
10000
8000
6000
4000
2000
0
0
1
3
5
7
9
11
13
Time (days after surgery)
5D11D4
1C8
N=20
P<0.001
C
Bleb area (µm²)
10000
8000
6000
4000
2000
0
0
1
3
5
7
9
11
13
Time (days after surgery)
5D11D4
1C8
N=10
P<0.001

FIGURE 4

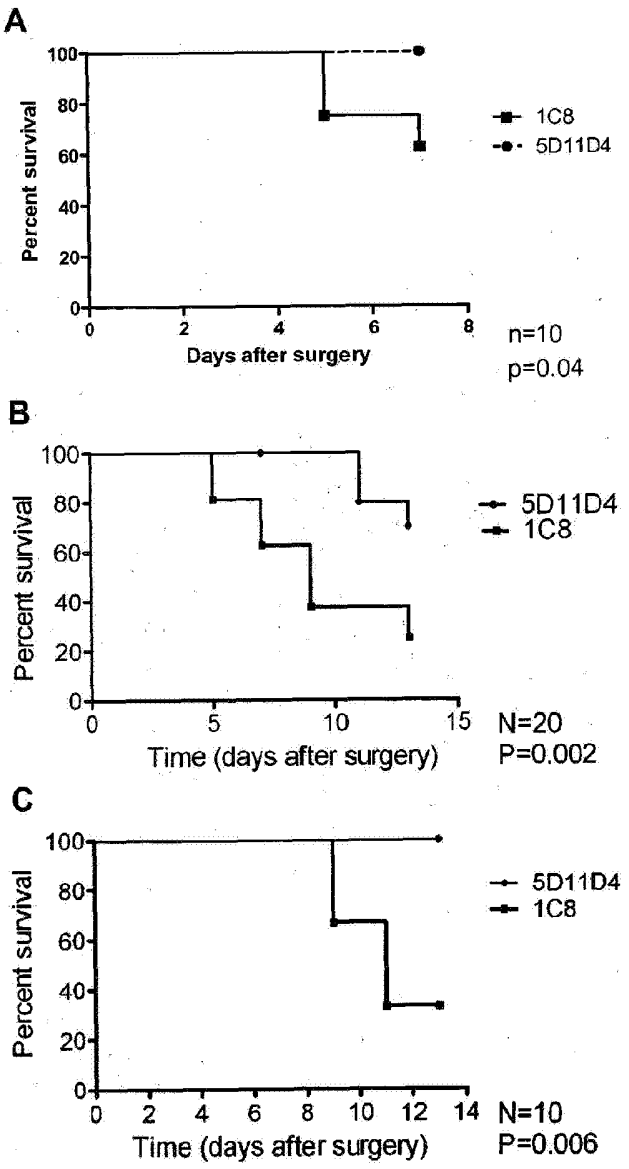
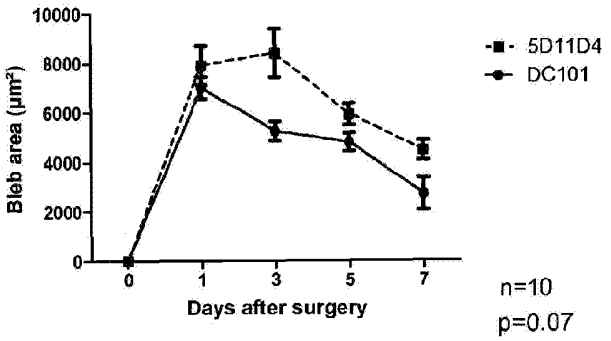

A
Percent survival
Days after surgery
1C8
5D11D4
n=10
p=0.04
B
Percent survival
Time (days after surgery)
5D11D4
1C8
N=20
P=0.002
C
Percent survival
Time (days after surgery)
5D11D4
1C8
N=10
P=0.006

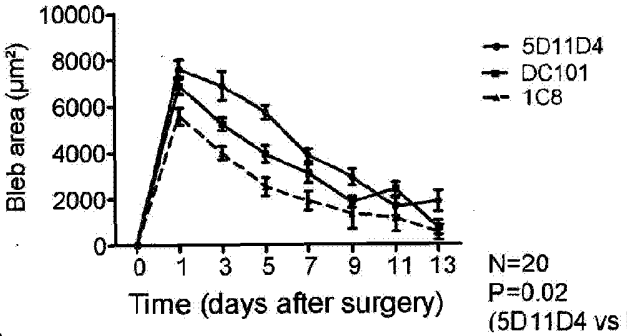
FIGURE 5

A



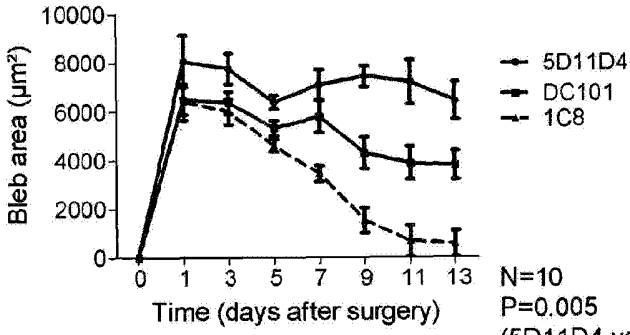
n=10
p=0.07

B



N=20
P=0.02
(5D11D4 vs DC101)

C



N=10
P=0.005
(5D11D4 vs DC101)

FIGURE 6