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(54) Title: USE OF TRANSFORMING GROWTH FACTOR - BETA 1 (TGF-β1) INHIBITOR PEPTIDES FOR THE TREATMENT OF CORNEAL FIBROSIS AND/OR HAZE

(57) Abstract: The invention refers to the use of transforming growth factor - beta 1 (TGF-β1) inhibitor peptides or polynucleotides encoding said peptides for the prevention and/or treatment of corneal fibrosis and/or corneal haze.

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USE OF TRANSFORMING GROWTH FACTOR – BETA 1 (TGF-β1) INHIBITOR PEPTIDES FOR THE TREATMENT OF CORNEAL FIBROSIS AND/OR HAZE

FIELD OF THE INVENTION

The invention generally refers to products, compositions and methods for the prevention and/or treatment of corneal fibrosis and/or corneal haze. The methods of the present invention involve the use of transforming growth factor - beta 1 (TGF-β1) inhibitor peptides or polynucleotides encoding said peptides.

BACKGROUND OF THE INVENTION

The ocular surface, i.e., cornea, is covered with stratified epithelium and underlying connective tissue. It serves as the outercoat of the eye structure that blocks interfering by external stimuli of chemical components, microbial attack or mechanical trauma. The avascularity, transparency and the regular curvature of the cornea are all essential for proper light refraction and, therefore, vision.

Corneal haze is a clouding of the normally clear front surface of the eye and its development is thought to be secondary to side effects of the cornea's innate wound healing mechanisms. Once the tissues are injured, mesenchymal cells and infiltrated inflammatory cells, i.e., macrophages, secrete various growth factors/cytokines including transforming growth factor - TGF-β1 (TGF-β1), one of the most important fibrogenic growth factors. Although TGF-β1 is critical in the maintenance of the tissue integrity, it also promotes a fibrogenic reaction in the healing subepithelial tissues.

The main components involved in tissue repair of the cornea are quite similar to those of skin; stratified epithelium and a collagenous matrix containing mesenchymal cells (corneal fibroblasts) lying beneath it. An organized extracellular matrix structure of collagen fibers of types I, III, and V and proteoglycans among the fibers is essential to the maintenance of its transparency and the regular shape. Fibrosis in the cornea causes loss of transparency, tissue contraction and scar transformation, thus causing corneal haze.

The use of both topical anti-inflammatory steroids drugs such as dexamethasone, fluorometholone or prednisolone and antimetabolites such as mitomycin C in the

treatment of corneal haze is currently widespread. However these drugs can be associated with severe complications. The use of topical steroids may cause an elevation of intraocular pressure leading to optic nerve damage and visual field changes. Mitomycin C has been used because it can stop the proliferation or growth of certain types of cells such as those cells in the eye which produce scarring or haze; however, mitomycin C is very potent and potentially toxic, under certain circumstances. Some of the eye-related complications that have been reported following the use of mitomycin C include, but are not limited to, conjunctival injection (redness of the eye), permanent stem cell deficiency, corneal or scleral thinning or perforation requiring corneal transplantation, corneal decompensation, cataract, and retinal vascular occlusion.

TGF-β1 has emerged as a key regulator of haze and scarring in the cornea and other tissues. In fact, increased expression of TGF-β1 and TGF-β2 in adult tissue. Similarly, the addition of exogenous TGF-β1 to healing corneal wounds results in exaggerated scarring and fibrosis. Inhibition of TGF-β1 centered on the use of different strategies has proved successful *in vitro* and *in vivo*, such as by using specific neutralizing antibodies [see, e.g., Jester et al., Cornea 1997; 16(2):177-87; Moller-Pedersen et al., Current Eye Research 1998 Jul.; 17(7):736-47; Bühren et al., Invest. Ophthalmol. Vis. Sci. 2009 February; 50(2):634-43], antisense oligonucleotides of the gene encoding for TGF-β1 which block its expression [Cordeiro et al., Gene Therapy 2003; 10:59-71]. Although inhibition of TGF-β1 with anti-TGF-β1 antibodies accelerates corneal reepithelization while reducing cell repopulation of the stroma by keratocytes [Carrington et al. Invest. Ophthalmol Vis Sci 2006; 47:1886-1894], the use of antibodies affords total and specific blockage of this cytokine (TGF-β1), though certain side effects are facilitated by both the presence of exogenous immunoglobulins in blood and the effects derived from the systemic blockage of TGF-β1. In addition, immunoglobulin stability over time does not allow short-time control of the activity of this cytokine. Antisense oligonucleotides inhibit TGF-β1 production at the gene expression level, a fact that can generate important deregulation of all processes in which this cytokine participates.

Further TGF-β1 inhibitors have been proposed, such as mannose-6-phosphate, in order to prevent haze after refractive surgery [Angunawela & Marshall, J. Cataract. Refract. Surg. 2010; 36:121-126]. Nevertheless, mannose-6-phosphate has recently

failed phase II clinical trial in accelerating the healing of split thickness skin graft donor sites, no evidence of clinical antifibrotic efficacy has been provided.

US 6,399,107 discloses the use of inhibitors of glycosaminoglycan synthesis for the treatment of corneal haze. US 2004/0001821 discloses the use of plasminogen activators to prevent corneal and subepithelial haze after laser vision correction surgery but no clinical data in corneal haze improvement have been reported.

Nevertheless, although there are different strategies to prevent and/or treat corneal fibrosis and/or haze, there is a need in the art to develop new treatments which reduce the corneal fibrosis and/or haze minimizing or avoiding side effects.

It has now found that TGF-β1 inhibitor peptides can be used to prevent and/or treat corneal fibrosis and/or haze. The use of said peptides in the treatment of liver fibrosis has been previously disclosed (WO 2005/019244) as well as immunomodulating agent (WO 2007/048857).

SUMMARY OF THE INVENTION

The present invention is directed to products, compositions and methods for the prevention and/or treatment of corneal fibrosis and/or corneal haze. The compositions comprise at least one TGF-β1 inhibitor peptide or a polynucleotide encoding said peptide.

Thus, in an aspect the invention relates to a product selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of a), b), c), d), and/or e),

for use in the prevention and/or treatment of corneal fibrosis and/or corneal haze.

Alternatively, the invention relates to the use of a product selected from the

group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of a), b), c), d), and/or e),

in the manufacture of a pharmaceutical composition for the prevention and/or treatment of corneal fibrosis and/or corneal haze.

In another aspect, the invention relates to a method for the prevention and/or treatment of corneal fibrosis or corneal haze which comprises administering to a subject in need of treatment a therapeutically effective amount of a product selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of (a), (b), (c), (d), and/or (e).

In another aspect, the invention relates to an ophthalmic pharmaceutical composition comprising a therapeutically effective amount of a product selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as

defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of (a), (b), (c), (d), and/or (e); and

a pharmaceutically acceptable vehicle for said compound(s) wherein said pharmaceutically acceptable vehicle is a vehicle suitable for ophthalmic administration.

In a particular embodiment, said product is in the form of a composition, wherein said composition comprises one or more of said products a) to e). In another particular embodiment, said composition further comprises a pharmaceutically acceptable vehicle and/or a therapeutic compound.

In a particular embodiment, said TGF-β1 inhibitor peptide is selected from the group of peptides consisting of p144 (SEQ ID NO: 6), p17 (SEQ ID NO: 17) and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the evolution of the effect of TGF-β1 inhibitor peptides (p17 and p144) on corneal haze.

Figure 2 shows the evolution of the effect of peptides p17 and p144 on epithelial closure.

Figure 3 shows the evolution of corneal haze after the application of p17, wherein:

*** $p<0.001$; control vs. p17 in the fifth week highly significant;

††† $p<0.001$; the control at the fifth week vs. the control at day 0 highly significant; and

$p<0.001$; the one treated at the fifth week vs. the one treated at day 0 highly significant.

Figure 4 shows the evolution of the epithelial closure after the application of p17, wherein:

** $p<0.01$; control vs. p17 in the fifth week very significant;

†† $p<0.01$; the control at the fifth week vs. the control at day 0 very significant; and

p<0.001; the one treated at the fifth week vs. the one treated at day 0 highly significant.

Figure 5 shows the evolution of corneal haze after the application of p144, wherein:

*** p<0.001; control vs. p17 in the fifth week highly significant;

* p<0.05; control vs. p17 in the second and fourth week significant;

††† p<0.001; the control at the fifth week vs. the control at day 0 highly significant; and

p<0.001; the one treated at the fifth week vs. the one treated at day 0 highly significant.

Figure 6 shows the evolution of the epithelial closure after the application of p144, wherein:

*** p<0.001; control vs. p17 in the fifth week highly significant;

* p<0.05; control vs. p17 in the fourth week significant;

†† p<0.01; the control at the fifth week vs. the control at day 0 very significant; and

p<0.001; the one treated at the fifth week vs. the one treated at day 0 highly significant.

Figure 7 shows the results of a Western Blot against p-Smad 2, total Smad2 and β-actin (loading control) of p17 and p144.

Figure 8 shows the results of a Western Blot against Smad2/3 in rabbit corneal fibroblasts.

Figure 9 shows the results of a Western Blot against Smad7 in rabbit corneal fibroblasts treated with TGF-β with p17 and p144.

Figure 10 shows the results of the immunofluorescence studies for p-Smad2 on rabbit corneal fibroblasts without treatment with TGF-β1 and after treatment with 3 ng/ml and 5 ng/ml of TGF-β1.

Figure 11 shows the results of the immunofluorescence studies for p-Smad2 on rabbit corneal fibroblasts without treatment with TGF-β1 and p17 or p144 and after the treatment with different concentrations thereof.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have discovered that the administration of TGF-β1 inhibitor peptides to an experimental animal model of corneal fibrosis induced after corneal damage with a chemical (NaOH) surprisingly decreases corneal fibrosis and/or haze in said animals thus providing a new therapeutic window for the prevention and/or treatment of corneal fibrosis and/or haze.

Therefore, the invention relates to a TGF-β1 inhibitor peptide for the treatment of corneal fibrosis and/or haze, although the invention also contemplates the possibility of using more than one different TGF-β1 inhibitor peptides for the treatment of said diseases. Likewise, the person skilled in the art will understand that the TGF-β1 inhibitor peptide or peptides can be presented in multiple forms, such as for example, a peptide, a fusion protein, a polynucleotide, a vector, a cell, etc.

Thus, in a first inventive aspect, the invention relates to a product, hereinafter referred to as the product of the invention, selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of (a), (b), (c), (d), and/or (e),

for use in the prevention and/or treatment of corneal fibrosis and/or corneal haze.

Alternatively, the invention relates to the use of a product (the product of the invention) selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of (a), (b), (c), (d), and/or (e),

in the manufacture of a pharmaceutical composition for the prevention and/or treatment of corneal fibrosis and/or corneal haze.

a) TGF-β1 inhibitor peptide of the invention

According to the invention, in a particular embodiment, the product of the invention is a TGF-β1 inhibitor peptide, hereinafter referred to as the TGF-β1 of the invention, or a functionally equivalent derivative, variant, analog or fragment thereof.

In the present invention, the term "TGF-β1 inhibitor peptide" refers to a peptide having the ability to inhibit a biological activity of TGF-β1; the inhibition of the biological activity of TGF-β1 can be, for example, by interacting with the active form of TGF-β1 – in a particular embodiment, the TGF-β1 inhibitor peptide binds directly to TGF-β1 and inhibits a biological activity of said TGF-β1.

TGF-β1 is a glycoprotein belonging to a superfamily of structurally related regulatory proteins (cytokines) included within one of the three isoforms described in mammals (TGF-β1, 2 and 3). The most abundant isoform is TGF-β1, which consists of a 25 kDa homodimer composed of two subunits joined by a disulfide bond. The sequences of said growth factors are well known for several animal species. The amino acid sequence of human TGF-β1 has been disclosed by Derynck et al. [Derynck K et al., "Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells". Nature 316 (6030), 701-705 (1985)]. The GenBank accession No. for the human sequences correspond to: TGF-β1 and TGF-β1 precursor: NP _ 000651 (protein) and NM _ 000660 (cDNA), TGF-β2 and TGF-β2 precursor: NP _ 003229 (protein) and NM _ 003238 (cDNA) and TGF-β3 and TGF-β3 precursor: NP-003230 (protein) and NM _ 003239 (cDNA). A further amino acid sequence of TGF-β1 is shown under accession number GenBank: AAL27646.2. TGF-β1 is a molecule with a highly preserved sequence in evolutive terms. Although it was originally defined by its capacity to induce adhesion independent of proliferation and morphological changes in rat fibroblasts, subsequent investigations have shown that TGF-β1 is a general inhibitor of proliferation of a broad range of cell types. The

molecule is produced by a great variety of cell types and in different tissues during all phases of cell differentiation. It has a large series of biological effects, with the generation of potent and very often opposite effects in relation to development, physiology and immune response. Many diseases or pathological alterations are associated with excessive or deregulated expression of TGF-β1, e.g., fibrosis associated to organ or tissue function loss, or surgical or esthetic complications, for example liver fibrosis or pulmonary fibrosis.

The TGF-β1 inhibitor peptide molecule is of peptidic nature, meaning that they comprise α-amino acids linked by an amide bond, i.e. the peptidic bond. The term "peptide" is not to be limited to short amino acid chains; it can include chains of more than 50 amino acids in length. As such, the term peptide as used herein encompasses as well polypeptides and proteins in general. Illustrative, non-limitative examples of TGF-β1 inhibitor peptides include those peptides disclosed in WO 00/31135 or US 7,528,226 (Tables 2-6) as well as in WO 2005/019244, incorporated herein by reference.

In a particular embodiment, the TGF-β1 inhibitor peptide is preferably a peptide having at least 70%, advantageously at least 80%, preferably at least 90%, more preferably at least 95% sequence identity to an amino acid sequence of any of the amino acid sequences shown under SEQ ID NO: 1-23 as depicted in Table 1 hereunder.

Table 1

TGF-β1 inhibitor peptides

Internal reference	SEQ ID NO:	Sequence
P11 (WO0031135, SEQ ID NO:1)	1	HANFCLGPCPYIWSL
P12	2	FCLGPCPYIWSLDT
P54	3	TSLDATMIWTMM
P106	4	SNPYSAFQVDIIVDI
P142	5	TSLMIWTMM
P144	6	TSLDASIIWAMMQN
P145	7	SNPYSAFQVDITID

P150	8	EAVLILQGPPYVSWL
P152	9	LDSLSFQLGLYLSPH
P29	10	HEPKGYHANFCLGPCPYIWSLDT
P11 (WO2005019244, SEQ ID NO:11)	11	WHKYFLRRPLSVRTR
P3	12	RFFTRFPWHYHASRL
P13	13	RKWFLQHRRMPVSVL
P14	14	SGRRHLHRHHIFSLP
P4	15	RLAHSHRHRSHVALT
P6	16	PPYHRFWRGHRHAVQ
P17	17	KRIWFIPRSSWYERA
P18	18	MPLSRYWWLFSHRPR
P17(1-14)	19	KRIWFIPRSSWYER
P17(1-12)	20	KRIWFIPRSSWY
P17(1-11)	21	KRIWFIPRSSW
P17(1-11)am	22	KRIWFIPRSSW; Trp-11; C-terminal amide
AcP17(1-11)am	23	KRIWFIPRSSW; Lys-1;- N-acetyl Trp-11; C-terminal amide

TGF-β1 inhibitor peptides of the invention may be obtained by conventional techniques; for example, they may be synthesized by chemical synthetic methods, including but not limited to, solid phase peptide synthesis (see, for example, WO 00/31135 or WO 2005/019244), and, if desired, purified with high performance liquid chromatography (HPLC), and analyzed by conventional techniques such as for example sequencing and mass spectrometry, amino acid analysis, nuclear magnetic resonance techniques, etc. Alternatively, said TGF-β1 inhibitor peptides of the invention can be obtained by means of the recombinant DNA technology from a variety of cell sources that synthesize said peptides including, for example, cells transfected with recombinant DNA molecules capable of directing the synthesis or secretion of the peptides. The nucleotide sequence encoding said peptide can easily be deduced from the amino acid sequence of the TGF-β1 inhibitor peptide. Further, said peptides may be also

commercially available, for instance P144 is supplied by Sigma-Genosys, Ltd. (Cambridge, UK).

As known in the art, "sequence identity" or "degree of identity" between two peptides or proteins is determined by comparing the amino acid sequence of a peptide or protein to the amino acid sequence of the other peptide or protein. As discussed herein, whether a particular peptide is at least about 70%, 80%, 90% or 95% identical to another peptide can be determined using methods and computer programs or software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group), the GCG package (GAP version 8, Genetics Computer Group, USA). BESTFIT uses the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics 2:482-489 (1981)), to find the best segment of homology between two sequences. The GCG package uses standard penalties for proteins: GAP weight 3.00, length weight 0.100, and the Matrix described in Gribskov and Brugess, *Nucl. Acids Res.* (1986) 14(16), 6745-6763. Alternatively, the degree of identity between two peptides or proteins factor can be determined by using the BLASTP algorithm [BLAST Manual, Altschul, S., *et al.,* NCBI NLM NIH Bethesda, Md. 20894, Altschul, S., *et al.*, J. Mol. Biol. 215: 403-410 (1990)].

The person skilled in the art will recognize that the present invention does not only relate to a specific TGF-β1 inhibitor peptide, i.e., to the specific amino acid sequence of a TGF-β1 inhibitor peptide, but also to functionally equivalent derivatives, variants, analogs and/or fragments thereof. Thus, a functionally equivalent derivative, variant, analog, or fragment of the amino acid sequence of a specific TGF-β1 inhibitor peptide is that sequence which preferably conserves at least one biological activity or function of said specific TGF-β1 inhibitor peptide which, in the context of the present invention, is the capacity to inhibit TGF-β1.

In a particular embodiment, the TGF-β1 inhibitor peptide is a peptide selected from the group of peptides whose sequence is shown in SEQ ID NO: 1-23, or a functionally equivalent salt, derivative, variant, analog, or fragment thereof (i..e., a derivative, variant, analog, or fragment of said TGF-β1 inhibitor peptide with capacity to inhibit TGF-β1). Therefore, in a particular embodiment, the TGF-β1 inhibitor peptide is a peptide whose amino acid sequence is shown in SEQ ID NO: 1, or in SEQ ID NO:

2, or in SEQ ID NO: 3, or in SEQ ID NO: 4, or in SEQ ID NO: 5, or in SEQ ID NO: 6, or in SEQ ID NO: 7, or in SEQ ID NO: 8, or in SEQ ID NO: 9, or in SEQ ID NO: 10, or in SEQ ID NO: 11, or in SEQ ID NO: 12, or in SEQ ID NO: 13, or in SEQ ID NO: 14, or in SEQ ID NO: 15, or in SEQ ID NO: 16, or in SEQ ID NO: 17, or in SEQ ID NO: 18, or in SEQ ID NO: 19, or in SEQ ID NO: 20, or in SEQ ID NO: 21, or in SEQ ID NO: 22, or in SEQ ID NO: 23, or a functionally equivalent derivative, variant, analog, or fragment thereof (i..e., a derivative, variant, analog, or fragment of said TGF-β1 inhibitor peptide with capacity to inhibit TGF-β1).

In another particular embodiment, the TGF-β1 inhibitor peptide is selected from a peptide having at least 70%, advantageously at least 80%, preferably at least 90%, more preferably at least 95% sequence identity to the amino acid sequences of SEQ ID NO: 1-23, or a functionally equivalent derivative, variant, analog, or fragment thereof.

Therefore, in a particular embodiment, the TGF-β1 inhibitor peptide is a peptide whose amino acid sequence has at least 70%, advantageously at least 80%, preferably at least 90 %, sequence identity to the amino acid sequences shown in SEQ ID NO: 1, or in SEQ ID NO: 2, or in SEQ ID NO: 3, or in SEQ ID NO: 4, or in SEQ ID NO: 5, or in SEQ ID NO: 6, or in SEQ ID NO: 7, or in SEQ ID NO: 8, or in SEQ ID NO: 9, or in SEQ ID NO: 10, or in SEQ ID NO: 11, or in SEQ ID NO: 12, or in SEQ ID NO: 13, or in SEQ ID NO: 14, or in SEQ ID NO: 15, or in SEQ ID NO: 16, or in SEQ ID NO: 17, or in SEQ ID NO: 18, or in SEQ ID NO: 19, or in SEQ ID NO: 20, or in SEQ ID NO: 21, or in SEQ ID NO: 22, or in SEQ ID NO: 23, or a functionally equivalent derivative, variant, analog, or fragment thereof.

In a particular embodiment of the invention, the TGF-β1 inhibitor peptide is selected from peptide p144 (SEQ ID NO: 6), peptide p17 (SEQ ID NO: 17), their combinations, or functionally equivalent derivatives, variants, analogs, or fragments thereof; preferably, said TGF-β1 inhibitor peptide is selected from peptide p144 (SEQ ID NO: 6), peptide p17 (SEQ ID NO: 17), and a combination thereof.

Thus, within the scope of this invention are the functionally equivalent derivatives of the TGF-β1 inhibitor peptide of the invention. The term "derivative" as used herein covers derivatives which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically

acceptable, i.e., they do not destroy the biological activity of the peptides as described above (i.e., the ability to inhibit the biological activity of TGF-β1) and do not confer toxic properties on compositions containing it. Derivatives may have chemical moieties, such as carbohydrate or phosphate residues, provided such a derivative retains the biological activity of the TGF-β1 inhibitor peptide and remains pharmaceutically acceptable. For example, derivatives may include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives or free amino groups of the amino acid residues formed with acyl moieties (e.g., alkanoyl or carbocyclic aroyl groups), or O-acyl derivatives of free hydroxyl group (e.g., that of seryl or threonyl residues) formed with acyl moieties. Such derivatives may also include for example, polyethylene glycol side-chains, which may mask antigenic sites and extend the residence of the molecule in body fluids.

A functionally equivalent variant of the TGF-β1 inhibitor peptide of the invention is also within the scope of the invention. As used herein, the term functionally equivalent "variant" refers to any peptide the amino acid sequence of which can be obtained by insertion, substitution or elimination of one or more amino acids from the original sequence of a TGF-β1inhibitor peptide [the guideline referring to which amino acid changes are likely to be phenotypically silent can be found in Bowie, J. U., *et al.*, (Science 1990, vol. 247:1306-1310)] and which at least partially conserves the capacity to inhibit the biological activity of TGF-β1.

The ability of a peptide to inhibit TGF-β1 may be determined by any suitable conventional bioassay for measuring TGF-β1 activity, for example by the assays described by Meager [Meager A. Journal of Immunological Methods (1991) 141:1-14]. Amongst these methods, the Mv-1-Lu cell growth inhibition assay is particularly suitable. Mv-1-Lu cell line is a cell line derived from mink lung epithelium, whose proliferation is inhibited by TGF-β1. A description of said Mv-1-Lu cell growth inhibition assay is provided in WO2005/019244. Typically, the TGF-β1 inhibitor peptide of the present invention has an inhibitory activity of 10% or higher, advantageously of 15% or higher, preferably of 20% or higher, in the Mv-1-Lu cell growth inhibition assay. More preferably the TGF-β1 inhibitor peptide of the present invention has an inhibitory activity of at least 25%, at least 30%, at least 35%, at least

40%, at least 45% or at least 50% in the Mv-1-Lu cell growth inhibition assay. Further, the capacity of a peptide to *in vivo* inhibit the biological activity of TGF-β1 can be evaluated and, if desired, quantified by testing in an animal model of acute liver damage induced for example by the administration of carbon tetrachloride (CCl_4). A description of said assays for determining the ability of a peptide to *in vivo* inhibit TGF-β1 is also provided in WO2005/019244.

Variations in the peptide primary structure, as well as variations in higher levels of structural organization, e.g. variation in the type of covalent bonds linking the amino acid residues or addition of groups to the terminal residues of the peptide, are within the scope of the invention.

In a particular embodiment, a functionally equivalent variant can be a variant in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code. Thus, a variant may include conservative or non-conservative alterations in the amino acid sequence that result in silent changes that preserve the functionality of the molecule including, for example, deletions, additions, and substitutions. Such altered molecules may be desirable where they provide certain advantages in their use. As used herein, conservative substitutions involves the substitution of one or more amino acids within the sequence of the corresponding peptide with another amino acid having similar polarity and hydrophobicity/hydrophilicity characteristics resulting in a functionally equivalent molecule. Such conservative substitutions include but are not limited to substitutions within the following groups of amino acids: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; phenylalanine, tyrosine; and methionine, norleucine. The skilled person in the art will understand that mutations in the nucleotide sequence encoding a TGF-β1 inhibitor peptide which give rise to conservative amino acid substitutions in positions that are non-critical for the functionality of the peptide are evolutionarily neutral mutations which do not affect its global structure or its functionality.

A variant may be conveniently prepared by direct chemical synthesis of the variant peptide, using methods well known in the art. Alternatively, variants of a defined TGF-β1 inhibitor peptide can be prepared by mutations in the corresponding

DNA sequence. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence. Obviously, the mutations that will be made in the DNA encoding the variant must not alter the reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. At the gene level, said variants ordinarily are prepared by site-directed mutagenesis of nucleotides in the DNA encoding the defined TGF-β1 inhibitor peptide molecule, thereby producing a DNA encoding the variant, and thereafter expressing the DNA in a recombinant cell culture. The variants typically exhibit the same qualitative biological activity as the non-variant peptide.

Also within the scope of this invention are functionally equivalent analogs of the TGF-β1 inhibitor peptide of the invention. An "analog" of a TGF-β1 inhibitor peptide above defined, according to the present invention, refers to a non-natural molecule, which is substantially similar to either, the entire molecules or to an active fragment thereof. Such analog will exhibit the same activity as the corresponding TGF-β1 inhibitor peptide.

A functionally equivalent fragment of a TGF-β1 inhibitor peptide of the invention is also within the scope of this invention. A "fragment", according to the present invention, can be that which comprises the amino acid sequence of a TGF-β1 inhibitor peptide, but lack a continuous series of residues (i.e., a continuous region, part or portion) including the amino end, or a continuous series of residues including the carboxyl end or, as in double truncation mutants, a deletion of two continuous series of residues, one including the amino end and the other one including the carboxyl end. Once again, these truncation mutants conserve at least one biological activity of the complete TGF-β1 inhibitor peptide, namely, the ability to inhibit TGF-β1. The fragments of the amino acid sequence of a TGF-β1 inhibitor peptide can be used to produce the corresponding full-length peptide by means of peptide synthesis; therefore, the fragments can be used as intermediate products to produce the full-length peptides. Fragments may readily be prepared by removing amino acids from either end of the molecule and testing the resultant for its properties as a TGF-β1 inhibitor peptide. Proteases for removing one amino acid at a time from either the N-terminal or the C-terminal of a polypeptide are known in the art.

In a particular embodiment, said fragment is a fragment comprising 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acid residues of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, or SEQ ID NO: 23, wherein said fragment maintains the capacity to inhibit TGF-β1.

The skilled person in the art will understand that also within the scope of this invention are the functionally equivalent salts of the TGF-β1 inhibitor peptide of the invention. The term "salt" herein refers to both salts of carboxyl groups and acid addition salts of amino groups of the TGF-β1 inhibitor peptides. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric, or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine, lysine, piperidine, procaine, and the like. Acid addition salts include, for example, salts with mineral acids, such as, for example, hydrochloric acid, bromic acid, sulfuric acid, phosphoric acid, etc., and salts with organic acids, such as, for example, acetic acid, citric acid, lactic acid, tartaric acid, fumaric acid, oxalic acid, methanesulfonic acid, benzenesulfonic acid, maleic acid, etc. Of course, any such salt must retain the biological activity of the TGF-β1 inhibitor peptide. For therapeutic use, salts of TGF-β1 inhibitor peptides are those wherein the counter-ion is pharmaceutically acceptable. The nature of the pharmaceutically acceptable salt is not a critical consideration, provided it is pharmaceutically acceptable. Non-pharmaceutically acceptable salts are also encompassed in the ambit of the present invention since they can be used in the production of pharmaceutically acceptable end products.

b) Fusion protein comprising the TGF-β1 inhibitor peptide of the invention

According to the invention, in another particular embodiment, the product of the invention is a fusion protein, hereinafter referred to as the fusion protein of the invention, comprising a TGF-β1 inhibitor peptide of the invention. Effectively, in an embodiment, the TGF-β1 inhibitor peptide of the present invention can be bound to another molecule, for example, another peptide conferring to the TGF-β1 inhibitor peptide

a property or advantage with respect to the native TGF-β1 inhibitor peptide, to give rise to a fusion protein.

In the present invention, the term "fusion protein" refers to a polypeptide having at least two portions covalently bound together in which each of the portions is derived from different proteins, one of the portions being a TGF-β1 inhibitor peptide of the invention, as previously defined. As the skilled person in the art will understand, the fusion protein described herein has the capacity to inhibit the biological activity of TGF-β1.

Illustrative, non-limitative, examples of peptides which can be fused to the TGF-β1 inhibitor peptide are, for example, molecules facilitating the extraction and purification of the fusion protein, such as polyhistidine tags (His-tags) (for example H6 and H10, etc.) or other tags for use in IMAC systems, for example, Ni^{2+} affinity columns, etc., GST fusions, MBP fusions, streptavidin tags, the BSP biotinylation target sequence of the BIRA bacterial enzyme and tag epitopes directed by antibodies (for example c-myc tags, FLAG tags, among others). As it will be observed by a person skilled in the art, said tag peptide can be used for purifying, inspecting, selecting and/or viewing the fusion protein of the invention. Furthermore, fluorescent, radioactive or enzymatic moieties, in addition to the molecule enhancing the stability in assay conditions such as the binding of DNA or transcriptional activation domains of the GAL4 gene and the like, are also suitable tags.

The TGF-β1 inhibitor peptide of the invention can be connected to other peptide by means of well known processes including bifunctional linkers, formation of a fusion polypeptide and formation of biotin/streptavidin or biotin/avidin complexes by means of the binding of either biotin or streptavidin/avidin to the complementary molecule. Depending on the nature of the reactive groups in the TGF-β1 inhibitor peptide and in the other compounds, a linker can be formed, simultaneously or sequentially allowing the reactive groups to react with one another. For example, the agent selected as a target can be prepared with a sulfhydryl group in, for example, the carboxyl end, which is then coupled to a derivatization agent to form a carrier molecule. The carrier molecule then binds by means of its sulfhydryl group to the peptide. In addition, the bond can be formed by allowing the reactive groups of the TGF-β1 inhibitor peptide and the molecule or molecules which will form the fusion protein to from a preferably covalent bond using coupling chemistries known for the persons skilled in the art. A number of processes accepted in the art can be used to form a covalent bond [see, for example, March, J.,

Advanced Organic Chemistry, 4th ed., New York, N.Y., Wiley and Sons, 1985, pp. 326-1120]. The person skilled in the art knows many other possible linkers.

In addition, the peptides forming part of the fusion protein of the invention can be directly connected by means of a single peptide bond or by means of a peptide linker containing one or more amino acid residues. Generally, the portions and the linker will be in the same reading frame with one another and are produced using recombinant techniques. Illustrative, non-limitative, examples of possible flexible linkers/linker sequences include SGGTSGSTSGTGST (SEQ ID NO: 24), AGSSTGSSTGPGSTT (SEQ ID NO: 25), GGSGGAP (SEQ ID NO: 26) [Muller, K. M., Arndt, K. M. and Alber, T., Meth. Enzymology, 2000, 328:261-281], GGGVEGGG (SEQ ID NO: 27) or GSGGS (SEQ ID NO: 28). Preferred examples of connector or linker peptides include those which have been used to connect peptides or proteins without substantially damaging the function of the connected peptides or proteins or at least without substantially damaging the function of one of the connected peptides or proteins.

The linker is preferably of a peptide nature. The linker peptide preferably comprises at least 2 amino acids, such as at least 3 amino acids, for example at least 5 amino acids, such as at least 10 amino acids, for example at least 15 amino acids, such as at least 20 amino acids, for example at least 30 amino acids, such as at least 40 amino acids, for example at least 50 amino acids, such as at least 60 amino acids, for example at least 70 amino acids, such as at least 80 amino acids, such as at least 90 amino acids, such as approximately 100 amino acids.

If desired, it is possible to include a proteolytic cleavage site between the portions (peptides - partners) of the fusion protein to allow the separation of the partners (peptides) of the fusion proteins. In the event that the fusion protein has reduced activity, the linker between said partners (peptides) can be selected to be sufficiently labile (e.g., for enzymatic cleavage by means of an enzyme present in the target tissue) such that it is easily cleaved, the molecules thus being released.

c) Polynucleotide encoding a TGF-β1 inhibitor peptide of the invention or a fusion protein of the invention

In another embodiment, the product of the invention comprises a polynucleotide, hereinafter referred to as the polynucleotide of the invention, encoding one or more

different TGF-β1 inhibitor peptides of the invention, and/or a polynucleotide encoding a fusion protein of the invention. The polynucleotide of the invention can contain expression regulatory sequences preceding the nucleotide sequence encoding the TGF-β1 inhibitor peptide or the fusion protein, said expression regulatory sequences being operatively bound to said nucleotide sequence encoding the TGF-β1 inhibitor peptide or the fusion protein, i.e., said TGF-β1 inhibitor peptide or fusion protein is within the correct reading frame for its expression under the control of said regulatory sequences.

The regulatory sequences useful for the present invention can be nuclear promoter sequences or, alternatively, enhancer sequences and/or regulatory sequences which increase the expression of the nucleotide sequence. The promoter can be constitutive or inducible. If the constant expression of the polynucleotide is desired, then a constitutive promoter is used. Examples of well known constitutive promoters include the derivatives of the genomes of eukaryotic viruses such as such as the polyomavirus, adenovirus, SV40, CMV, avian sarcoma virus, hepatitis B virus, the metallothionein gene promoter, the herpes simplex virus thymidine kinase gene promoter, LTR regions of retroviruses, the immunoglobulin gene promoter, the actin gene promoter, the EF-1 alpha gene promoter as well as inducible promoters in which the expression of the peptide or protein depends on the addition of a molecule or on an exogenous signal, such as the tetracycline system, the NFkappaB/UV light system, the Cre/Lox system and the heat shock gene promoter. Several other examples of constitutive promoters are well known in the art and can be used to implement the invention.

If the controlled expression of the nucleotide sequence encoding the TGF-β1 inhibitor peptide or the fusion protein is desired, then an inducible promoter must be used. In a non-induced state, the inducible promoter can be "silent". "Silent" is understood to mean that in the absence of an inducer, little or no expression of the polynucleotide of the invention is detected; however, in the presence of an inducer the expression of the polynucleotide of the invention occurs. The expression level can frequently be controlled by varying the concentration of the inducer. By controlling the expression, for example, varying the concentration of the inducer such that an inducible promoter is more strongly or more weakly stimulated, the concentration of the transcribed product of the polynucleotide of the invention can be affected thus controlling the amount of peptide or fusion protein synthesized. It is therefore possible to vary the concentration of the

therapeutic product. Examples of well known inducible promoters are: an androgen- or estrogen-sensitive promoter, a doxycycline-sensitive promoter, a metallothionein promoter or an ecdisone-responsive promoter. Other various examples are well known in the art and can be used to implement the invention. In addition to constitutive and inducible promoters (which normally work in a large variety of cell or tissue types), tissue-specific promoters can be used to achieve the specific expression of the polynucleotide sequence in specific cells or tissues. Well known examples of ocular tissue-specific promoters include the cornea-specific promoters of keratin 12 and keratocan genes, the retina-specific promoters of IRBP (interstitial retinol-binding protein) (also known as retinol-binding protein 3 (RBP3)) and Rhodopsin kinase (Rk or GRK1) genes, etc.

d) Vector comprising a polynucleotide of the invention

In another embodiment, the product of the invention comprises a vector, hereinafter referred to as the vector of the invention, comprising a polynucleotide of the invention as it has been defined above, i.e., a polynucleotide which encodes one or more different TGF-β1 inhibitor peptides or functionally equivalent derivatives, variants, analogs or fragments thereof, or a polynucleotide which encodes a fusion protein comprising said TGF-β1 inhibitor peptide. In a particular embodiment, said polynucleotide of the invention is preceded by expression regulatory sequences.

Vectors suitable for housing said polynucleotide of the invention include a plasmid or a vector which, when it is introduced in the host cell, is integrated or is not integrated in the genome of said cell. Said vector of the invention can be obtained by means of conventional processes known by persons skilled in the art and which can be found in, for example, Sambrook *et al.*, Cold Spring Harbor Laboratory Press, third edition, 2001.

However, within the scope of the invention, the vector of the invention is preferably a viral or non-viral vector suitable for use in therapy. Illustrative, non-limitative, examples of said vectors include viral vectors based on retroviruses, adenoviruses, alphaviruses, etc., or in the case of non-viral vectors the vectors can be DNA-liposome, DNA-polymer, DNA-polymer-liposome complexes, polymer nanoparticles, lipid nanoparticles, dendrimers, etc. [see "Nonviral Vectors for Gene Therapy", edited by Huang, Hung and Wagner, Academic Press (1999)]. Said viral and non-viral vectors comprising a polynucleotide of the invention can be directly administered to the human or

animal body by means of conventional processes. Viral vectors can be, for example, an alphavirus, such as Semliki Forest virus, Sindbis virus or Venezuelan equine encephalitis virus (EEV); a high-capacity adenovirus; a conditionally replicative adenovirus; an adenoassociated virus; etc.

e) Cell comprising a vector of the invention

The vectors of the invention can alternatively be used to transform, transfect or infect cells, preferably mammalian cells including human cells, for example, *ex vivo*, and, subsequently implant them into the human or animal body to obtain the desired therapeutic effect. For their administration to the subject, said cells will be formulated in a suitable medium which does not adversely affect their viability. Likewise, said vector can contain, among others, multiple cloning sites, expression regulatory sequences, replication origins suitable for the host cell in which the vector is to be introduced, selection markers, etc.

Therefore, the person skilled in the art will understand that, in an embodiment, the product of the invention comprises a cell, hereinafter referred to as the cell of the invention, comprising a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof, a fusion protein of the invention, a polynucleotide of the invention, or a vector of the invention, as defined, respectively, in sections a), b) and c) of the first inventive aspect of the invention. The characteristics of said TGF-β1 inhibitor peptide, fusion protein, polynucleotide and vector have been explained above.

Cells suitable for housing a TGF-β1 inhibitor peptide of the invention, fusion protein of the invention, a polynucleotide of the invention, or a vector of the invention, can be a eukaryotic or prokaryotic cell. In the present invention, virtually any host cell which can be transformed by a polynucleotide of the invention or which can be transformed, transfected or infected by a vector of the invention, for example animal cells (e.g., mammalian cells, bird cells, insect cells, etc.), plant cells, yeasts, etc., can be used.

Any eukaryotic cell which has been genetically modified to express a TGF-β1 inhibitor peptide of the invention, a fusion protein of the invention, a polynucleotide of the invention and/or the vector of the invention can be used in the present invention, but mouse, rat, primate and human cells are the preferred cells. Thus, cells suitable for carrying out the invention are cardiomyocytes, endothelial cells, epithelial cells, lymphocytes (B and T cells), mastocytes, eosinophils, vascular intima cells, primary

cultures of cells isolated from different organs, preferably of cells isolated from islets of Langerhans, hepatocytes, leukocytes, including mononuclear leukocytes, embryonic, mesenchymal, umbilical cord or adult (skin, lung, kidney and liver) stem cells, osteoclasts, chondrocytes and other connective tissue cells. Cells of established lines such as Jurkat T cells, NIH-3T3 cells, CHO cells, Cos cells, VERO cells, BHK cells, HeLa cells, COS cells, MDCK cells, 293 cells, 3T3 cells, C2C12 myoblasts and W138 cells are also suitable. The cells of the invention can be obtained by means of conventional processes known by persons skilled in the art [Sambrook *et al.*, 2001, mentioned above].

f) Combinations

In another embodiment, the invention contemplates the possibility of using a combination of two or more of said products a) to e). The particulars of said products a) to e) have been previously mentioned.

Compositions of the invention

In another embodiment, the product of the invention is in the form of a composition, wherein said composition comprises one or more of said products a) to e). Thus, in a particular embodiment, the invention provides a composition comprising a combination of one or more of a TGF-β1 inhibitor peptide of the invention, a fusion protein of the invention, a polynucleotide of the invention, a vector of the invention and/or a cell of the invention as defined, respectively, in sections a), b), c), d) and e) of the first inventive aspect of the invention. Thus, the composition provided by the instant invention also contemplates the possibility of comprising:

- at least two different TGF-β1 inhibitor peptides, alone or in combination with fusion proteins, polynucleotides and vectors such as those defined, respectively, in sections b), c) and d) of the first inventive aspect of the invention;
- at least two polynucleotides, each of them encoding a different TGF-β1 inhibitor peptide, alone or in combination with TGF-β1 inhibitor peptides or vectors such as those defined, respectively, in sections a) and d) of the first inventive aspect of the invention;

- at least two vectors, each of them comprising one or several polynucleotides such as those defined in section c) of the first inventive aspect of the invention which encode different TGF-β1 inhibitor peptides, alone or in combination with TGF-β1 inhibitor peptides such as those defined in section a) of the first inventive aspect of the invention;
- at least two cells comprising different TGF-β1 inhibitor peptides, fusion proteins, polyucleotides or vectors, such as those defined, respectively, in sections a)-d) of the first inventive aspect of the invention; etc.

As it has been mentioned at the beginning of the present description, the administration of TGF-β1 inhibitor peptides to a corneal fibrosis or haze model rat surprisingly causes a decrease in the corneal fibrosis and/or haze, thus providing a new therapeutic window for the treatment of corneal fibrosis and/or corneal haze. The person skilled in the art will understand that, for a TGF-β1 inhibitor peptide of the invention, or any other product of the invention, to be able to be used for therapeutic purposes, it must be suitably formulated for its administration.

Therefore, in a particular embodiment, the composition provided by the instant invention is a pharmaceutical composition and further comprises a pharmaceutically acceptable vehicle and, optionally, if desired, another therapeutic compound aimed at the treatment of corneal fibrosis and/or corneal haze.

Thus, the invention also provides a pharmaceutical composition comprising a therapeutically effective amount of a product or composition according to the present invention.

In the present invention, a "therapeutically effective amount" is understood as the amount of TGF-β1 inhibitor peptide of the invention which is sufficient for the latter to be able to delay, reduce or eliminate the symptoms associated with corneal fibrosis and/or haze or the severity thereof. As used herein, the term "pharmaceutically acceptable vehicle" refers to any formulation which is safe and provides an effective delivery of an effective amount of at least one product of the invention to the target tissue.

Depending on the nature of the products of the invention or the components of the composition provided by the invention, i.e., peptides, fusion proteins, polynucleotides, vectors or cells as defined above, the amount thereof in the pharmaceutical composition can vary. For example, if the composition comprises a viral vector, the amount of vector in

the composition provided by the instant invention can vary between 10^5 and 10^{13} viral particles per dose depending on the viral vector used. On the other hand, if the vector is a non-viral vector, for example, a plasmid, the amount of the vector in the composition provided by the invention can vary between 100 ng and 5 mg per dose.

In a particular embodiment, the pharmaceutical composition of the invention comprises two or more components; in this embodiment, said components can be formulated for their separate, simultaneous or successive use.

The products of the invention may be contained in various types of pharmaceutical compositions in accordance with formulation techniques known to those skilled in the art. The route of administration (e.g., topical or intraocular) and the dosage regimen will be determined by skilled clinicians, based on factors such as the exact nature of the condition being treated, the severity of the condition, the age and general physical condition of the patient, the particular product of the invention used, the pharmacokinetic properties of the individual and so on.

The products of the invention, or the composition of the invention can be administered by means of different processes, for example topically, intraocularly, etc., and can be administered locally or systemically or directly to the target site. A review of the different processes for administering active ingredients, the excipients to be used and the processes for preparing them can be found in Tratado de Farmacia Galénica, C. Faulí i Trillo, Luzán 5, S.A. de Editions, 1993 and in Remington's Pharmaceutical Sciences (A.R. Gennaro, Ed.), 20^{th} edition, Williams & Wilkins PA, USA. (2000).

In a particular embodiment, in the context of the present invention, the products of the invention, or the composition of the invention is administered to the eye(s), so that the TGF-β1 inhibitor peptide can cause the inhibition of TGF-β1 in the eye(s), although it can also be administered at other level.

The method of administration of the product of the invention or the pharmaceutical composition of the invention will depend on the disease to be treated and other factors such as the duration of therapy and whether the products of the invention or the pharmaceutical composition will be administered prophylactically or during acute phases, e.g., after surgery. The products of the invention and the pharmaceutical composition may be used as an adjunct to ophthalmic surgery, such as by vitreal or subconjunctival injection following ophthalmic surgery. The compounds

may be used for acute treatment of temporary conditions, or may be administered chronically, especially in the case of degenerative disease. The compounds may also be used prophylactically, especially prior to ocular surgery or non-invasive ophthalmic procedures, or other types of surgery.

Therefore, in an embodiment, the pharmaceutical composition provided by the instant invention is an ophthalmic pharmaceutical composition, i.e., a pharmaceutical composition comprising a therapeutically effective amount of a product or composition according to the present invention and a pharmaceutically acceptable vehicle for said compound(s) wherein said pharmaceutically acceptable vehicle is a vehicle suitable for ophthalmic administration. As used herein, the term "ophthalmic pharmaceutical composition" denotes a composition intended for application in the eye or intended for treating a device to be placed in contact with the eye such as a contact lens. Thus, in a particular embodiment, the invention relates to an ophthalmic pharmaceutical composition comprising a therapeutically effective amount of a product selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of (a), (b), (c), (d), and/or (e); and

a pharmaceutically acceptable vehicle for said compound(s) wherein said pharmaceutically acceptable vehicle is a vehicle suitable for ophthalmic administration.

In a particular embodiment, the pharmaceutical composition will be formulated and administered for topical application. Topical formulations are generally aqueous in nature, buffered to a physiological acceptable pH and typically preserved for multi-dispensing. Thus, in a particular embodiment, the invention also provides a topical ophthalmic pharmaceutical composition comprising a therapeutically effective amount of a product or composition according to the present invention and a pharmaceutically

acceptable vehicle for said compound(s) wherein said pharmaceutically acceptable vehicle is a vehicle suitable for topical ophthalmic administration. As it is well-known by the skilled person in the art, various types of vehicles may be utilized. The vehicles will generally be aqueous in nature. Aqueous solutions are generally preferred, based on ease of formulation, as well as patients' ability to easily administer such compositions by means of instilling one to two drops of the solutions in the affected eyes. However, the products of the invention may also be readily incorporated into other types of compositions, such as suspensions, viscous or semi-viscous gels or other types of solid or semi-solid compositions. Suspensions may be preferred for products of the invention which are relatively insoluble in water. The ophthalmic compositions of the present invention may also include various other ingredients, such as buffers, preservatives, co-solvents and viscosity building agents.

An appropriate buffer system (e.g., sodium bicarbonate, sodium phosphate, sodium acetate, sodium citrate, sodium ascorbate or sodium borate) may be added to prevent pH drift under storage conditions.

Ophthalmic products are typically packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. Suitable preservatives include, for example: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, polyquaternium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0 percent by weight, based on the total weight of the composition (wt. %).

Some of the products of the invention may have limited solubility in water and therefore may require a surfactant or other appropriate co-solvent in the composition. Such co-solvents include, for example: polyethoxylated castor oils, Polysorbate 20, 60 and 80; Pluronic® F-68, F-84 and P-103 (BASF Corp., Parsippany N.J., USA); cyclodextrins; or other agents known to those skilled in the art. Such co-solvents are typically employed at a level of from 0.01 to 2 wt. %.

Viscosity greater than that of simple aqueous solutions may be desirable to increase ocular absorption of the active compound (products of the invention), to decrease variability in dispensing the formulations, to decrease physical separation of components of a suspension or emulsion of formulation and/or otherwise to improve the

ophthalmic formulation. Such viscosity building agents include, for example, polyvinyl alcohol, polyvinyl pyrrolidone, methyl cellulose, hydroxypropyl methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose or other agents known to those skilled in the art. Such agents are typically employed at a level of from 0.01 to 2 wt. %.

In another particular embodiment, the products of the invention will be formulated for intraocular use. Thus, in a particular embodiment, the invention also provides an intraocular ophthalmic pharmaceutical composition comprising a therapeutically effective amount of a product or composition according to the present invention and a pharmaceutically acceptable vehicle for said compound(s) wherein said pharmaceutically acceptable vehicle is a vehicle suitable for intraocular ophthalmic administration. As it is well-known by the skilled person in the art, various types of vehicles may be utilized. Formulations for intraocular use generally will comprise a surgical irrigating solution such as a fluorinated hydrocarbon in BSS Plus® Sterile Irrigating Solution or BSS Plus® Sterile Irrigating Solution alone, as described below. The use of physiologically balanced irrigating solutions as pharmaceutical vehicles for the products of the invention is preferred when said products are administered intraocularly. As utilized herein, the term "physiologically balanced irrigating solution" means a solution which is adapted to maintain the physical structure and function of tissues during invasive or noninvasive medical procedures. This type of solution will typically contain electrolytes, such as sodium, potassium, calcium, magnesium and/or chloride; an energy source, such as dextrose; and a buffer to maintain the pH of the solution at or near physiological levels. Various solutions of this type are known (e.g., Lactated Ringers Solution). BSS® Sterile Irrigating Solution and BSS Plus® Sterile Intraocular Irrigating Solution (Alcon Laboratories, Inc., Fort Worth, Tex., USA) are examples of physiologically balanced intraocular irrigating solutions. The latter type of solution is described in U.S. Pat. No. 4,550,022, the entire contents of which are hereby incorporated in the present specification by reference.

In a particular embodiment, the TGF-β1 inhibitor peptide is administered in the form of a topical pharmaceutical composition suitable for ophthalmic application, such as eyedrops, ointments, creams, etc. In a particular embodiment, said topical pharmaceutical composition comprises hyaluronic acid or a pharmaceutically

acceptable salt thereof, such as sodium hyaluronate, suitable for ophthalmic administration.

The doses utilized for any of the above-described purposes of topical, periocular or intraocular administration will generally be from about 0.01 to about 100 milligrams per kilogram of body weight (mg/kg), administered one to several, e.g., four, six, eight or even more, times per day.

Alternatively, the pharmaceutical composition of the invention can be formulated in a solid pharmaceutical dosage form (e.g., tablets, capsules , etc.), liquid pharmaceutical dosage form (e.g., solutions, suspensions, etc.) or semisolid pharmaceutical dosage form (e.g., gels, etc.). The compositions comprising said vehicles can be formulated by means of conventional processes known in the state of the art.

In a particular embodiment, the pharmaceutical composition of the invention further comprises, in addition to the product of the invention, another therapeutic compound aimed at the treatment of corneal fibrosis and/or corneal haze. Virtually any therapeutic compound aimed at the treatment of corneal fibrosis and/or corneal haze can be used. Illustrative, non-limitative, examples of therapeutic agents used in corneal fibrosis and/or haze include topical anti-inflammatory steroids drugs such as dexamethasone, fluorometholone, prednisolone, etc., antimetabolites such as mitomycin C, etc., and the like.

The products of the invention as well as the composition provided by the invention can be in the form of a kit. In the present invention, a "kit" is understood as a product containing the different active ingredients or products of the invention as defined in sections a)-e) of the first inventive aspect of the invention and/or the additional therapeutic compounds forming the packaged composition such that the transport, storage and simultaneous or successive administration thereof is allowed. Therefore, the kits of the invention can contain one or more suspensions, syringes, and the like which contain the active ingredients of the invention and which can be prepared in a single dose or as multiple doses. The kit can additionally contain a vehicle suitable for resuspending the products of the invention such as aqueous media such as saline solution, Ringer's solution, dextrose and sodium chloride, water-soluble media such as alcohol, polyethylene glycol, propylethylene glycol and water-insoluble vehicles if necessary. Another component which may be present in the kit is a package which allows maintaining the compositions of

the invention within determined limits. Materials suitable for preparing such packages include glass, plastic (polyethylene, polypropylene, polycarbonate and the like), bottles, vials, paper, sachets and the like.

The kit of the invention can additionally contain instructions for the simultaneous, successive or separate administration of the different pharmaceutical formulations present in the kit. Therefore, the kit of the invention can further comprise instructions for the simultaneous, successive or separate administration of the different components. Said instructions can be in the form of printed material or in the form of an electronic support which can store the instructions such that they can be read by a subject, such as electronic storage media (magnetic disks, tapes and the like), optical media (CD-ROM, DVD) and the like. The media can additionally or alternatively contain Internet webpages providing said instructions.

As it has been indicated above, the findings described in the present invention are useful for the treatment of corneal fibrosis and/or corneal haze. Fibrosis in the cornea (corneal fibrosis) causes loss of transparency, tissue contraction and scar transformation, thus causing corneal haze. Example 1 shows that two TGF-β1 inhibitor peptides [p144 (SEQ ID NO: 6) and p17 (SEQ ID NO: 17)] are capable of completely or partially reducing corneal fibrosis and haze induced by chemical attack in an animal model.

In the present invention, "corneal fibrosis" is understood as that disease which is characterized by an opacification, or cloudiness, of the normally clear and transparent part of the central or peripheral area of the coat of the eyeball (cornea), secondary to inflammation, infection or irritant exposure by a foreign body or chemical. This corneal disorder includes any corneal tissue alteration built up of scar tissue (collagen excessive deposition or/and collagen altered orientation) in response to injury or chronic disease. This gives the cornea a white-gray color in the damaged areas and the usual degree of transparency becomes to different grade of opacity.

Similarly, as used herein, the term "corneal haze" means corneal subepithelial haze, and it refers to disturbances in corneal transparency that makes vision hazy or cloudy. Haze is caused by the presence of unstructured collagen fibers excreted by activated keratocytes and affected extracellular matrix. When present, haze appears weeks to several months after laser eye surgery, usually during the first month, its

intensity being highest during the postoperative 3-6 months. Haze can last up to a year before resolving. In a particular embodiment of the invention, corneal haze or cloudiness following laser refractive surgery occurs as a result of the disrupted structure of the anterior stromal lamellae. Corneal haze is not apparent until a few weeks to a few months postoperatively. Its duration can be from weeks to months, with some occurrences lasting over a year and in some cases irreversible. Its severity can be mild to strong. There is a wide variability in the reported prevalence of haze.

In another aspect, the invention relates to a method for the prevention and/or treatment of corneal fibrosis and/or corneal haze which comprises administering to a subject in need of treatment a therapeutically effective amount of a product selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d),

f) a combination of one or more of (a), (b), (c), (d), and/or (e).

In a particular embodiment, said product is in the form of a composition, wherein said composition comprises one or more of said products a) to e). In another particular embodiment, said composition further comprises a pharmaceutically acceptable vehicle and/or a therapeutic compound aimed at the treatment of corneal fibrosis and/or corneal haze.

In a particular embodiment, the above method can be used for preventing or reducing postoperative corneal subepithelial haze following laser surgery by applying to the surface of the affected eye, a therapeutically effective amount of one or more products of the invention. The method of the present invention is especially designed to prevent haze following photorefractive keratectomy (PRK) and laser *in situ* keratomileusis (LASIK), however the method prevents or reduces haze following any eye injury that is likely to cause haze including any photoablation of the cornea during

ophthalmic surgery, eye surface infections (bacterias, virus, any other microbiological agent, parasites), mechanical abrasion, chemical injury, etc.

In a particular embodiment, the TGF-β1 inhibitor peptide is a peptide selected from the group of peptides whose sequence is shown in SEQ ID NO: 1-23, or a functionally equivalent derivative, variant, analog, or fragment thereof (i.e., a derivative, variant, analog, or fragment of said TGF-β1 inhibitor peptide with capacity to inhibit TGF-β1). Thus, in a particular embodiment, the TGF-β1 inhibitor peptide is a peptide whose amino acid sequence is shown in SEQ ID NO: 1, or in SEQ ID NO: 2, or in SEQ ID NO: 3, or in SEQ ID NO: 4, or in SEQ ID NO: 5, or in SEQ ID NO: 6, or in SEQ ID NO: 7, or in SEQ ID NO: 8, or in SEQ ID NO: 9, or in SEQ ID NO: 10, or in SEQ ID NO: 11, or in SEQ ID NO: 12, or in SEQ ID NO: 13, or in SEQ ID NO: 14, or in SEQ ID NO: 15, or in SEQ ID NO: 16, or in SEQ ID NO: 17, or in SEQ ID NO: 18, or in SEQ ID NO: 19, or in SEQ ID NO: 20, or in SEQ ID NO: 21, or in SEQ ID NO: 22, or in SEQ ID NO: 23, or a functionally equivalent derivative, variant, analog, or fragment thereof, such as a fragment comprising 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acid residues of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, or SEQ ID NO: 23, or their pharmaceutically acceptable salts, wherein said fragment maintains the capacity to inhibit TGF-β1.

In another particular embodiment, the TGF-β1 inhibitor peptide is a peptide selected from the group of peptides p144 (SEQ ID NO: 6), p17 (SEQ ID NO: 17) or a fragment comprising 9, 10, 11, 12, 13, or 14 consecutive amino acid residues of SEQ ID NO: 6 or SEQ ID NO: 17, or their pharmaceutically acceptable salts, wherein said fragment maintains the capacity to inhibit TGF-β1.

In a preferred embodiment said TGF-β1 inhibitor peptide is selected from the group of peptides consisting of p144 (SEQ ID NO: 6), p17 (SEQ ID NO: 17) and combinations thereof.

The following examples illustrate the invention and must not be considered as limiting the scope thereof.

EXAMPLE 1

Treatment of corneal fibrosis and haze

TGF-β1 is one of the main fibrosis induction mediators. Fibrosis in cornea causes loss of transparency, tissue contraction and scar transformation, thus causing corneal haze. This example was focused on studying corneal fibrosis in experimental animals and its complete or partial decrease after the topical administration of TGF-β1 inhibitor peptides: p144 (SEQ ID NO: 6) and p17 (SEQ ID NO: 17).

1.1 Materials and Methods

1.1.1 Corneal Damage Induction

Animal fibrosis model

In this study, domestic hens (*Gallus gallus domesticus*) aged between 12 and 16 months were initially used as experimental animals but due to the difficulty of the model, especially in relation to obtaining and handling the animals, it was decided to use Long-Evans rats. Furthermore, it was bibliographically verified that these animals are used for *in vivo* models for studying corneal fibrosis because they allow to follow the evolution of the damage with more precision since they are pigmented rats and, like humans, have a Bowman's membrane.

Experimentation groups

Male Long-Evans rats aged 10 weeks were used, divided into 5 groups:

- Group 1: 12 rats, 6 treated with dexamethasone and 6 treated with mitomycin C.
- Group 2: 12 rats, 6 treated with p17 of the first formulation (see section 1.1.2.b) and 6 treated with p144 of the first formulation (see section 1.1.2.b).

- Group 3: 12 rats, 6 treated with p17 of the second formulation (see section 1.1.2.b) and 6 treated with p144 of the second formulation (see section 1.1.2.b).
- Group 4: 12 rats, 6 treated with p17 of the third formulation (see section 1.1.2.b) and 6 treated with p144 of the third formulation (see section 1.1.2.b).
- Group 5: 12 rats, 6 treated with p17 of the fourth formulation (see section 1.1.2.b) and 6 treated with p144 of the fourth formulation (see section 1.1.2.b).
- Group 6: 12 rats, 6 treated with p17 of the fifth formulation (see section 1.1.2.b) and 6 treated with p144 of the fifth formulation (see section 1.1.2.b).

To perform the damage in the cornea, the animals were anesthetized with isoflurane and a 5 mm Whatman paper disc impregnated in 1 N NaOH for 30 seconds was applied to them in the central part of the eye. After the damage, they were washed with abundant physiological saline serum and an antibiotic ointment was applied to them to prevent possible infections in the affected area.

After conducting several *in vivo* studies with the previously described groups of animals, it could be concluded that the chemical damage occurring after the application of NaOH for 30 seconds caused an excessive lesion for the study which was to be conducted, therefore, it was decided to conduct new experiments reducing the time to 15 seconds.

1.1.2 Treatment

Firstly, an experiment was conducted to corroborate the clinical efficacy of the drugs usually used in the treatment of corneal fibrosis: dexamethasone and mitomycin C. In said first experiment, it was observed that the obtained results did indeed correspond to those described in the scientific literature. For this reason and from that time onwards, the following studies were conducted with groups of animals treated exclusively with p17 and p144. It was thus possible to work with a larger number of animals, thus obtaining more significant results.

a) Summary of the posology:

First Week

Treatment	Time of Treatment
Peptides (1 drop)	8:30
Antibiotic ointment	13:00
Peptides (1 drop)	18:00

Second-Sixth Week

Treatment	Time of Treatment
Peptides (1 drop)	8:30
Peptides (1 drop)	18:00

It was randomly established that the left eye of each animal would act as a control and that the right eye would be the treated eye, such that the same animals acts as a control in addition to being treated. It must be pointed out that all the animals received antibiotic treatment in both eyes daily only during the first week of the study for the purposes of avoiding microbial keratitis.

b) Formulation of the eye drops:

One of the objectives of this study was to achieve the most suitable formulation to achieve clinical results similar to those obtained with dexamethasone and mitomycin C. To that end, the different formulations listed below were tested:

First formulation

- 2% Carbopol (1 g in 50 ml) heated at 60ºC and sonicated in MQ water filtered with a 0.45 μm filter

- p144 and p17 at a final concentration of 1 mg/ml
- 10 mg were weighed and sonicated in 5 ml of 2% carbopol
- at 4ºC over the weekend until the use thereof on Tuesday

Note: The formulations were maintained at 4ºC throughout the study.

Second formulation

- The carbopol solution was prepared as follows: 40 ml at 1.5% carbopol-2001 sonicated and centrifuged 5 min 2000 rpm (bubbles) + 400 µl of triethanolamine (99%) and 1.2 ml 1N HCl (stirred and centrifuged)
- p144: 1 ml of carbonate + 10 mg of p144. Sonicate. 9 ml of carbopol solution were added and sonication
- p144 control: the same but without p144
- p17: 10 ml of the carbopol solution + 10 mg of p17. Sonicate
- p17 control: carbopol solution (i.e., without p17)

Note: The carbonate raises the pH of the carbopol solution, increasing the viscosity. All the solutions were gel in water. The formulations were maintained at 4ºC throughout the study.

Third formulation

- The carbopol solution was prepared as follows: 40 ml at 1.5% carbopol-2001 sonicated and centrifuged 5 min 2000 rpm (bubbles)
- p144: 1 ml of carbonate + 10 mg of p14. Sonicate. 9 ml of carbopol solution were added and sonication
- p144 control: the same but without p144
- p17: 10 ml carbopol solution + 10 mg of p17. Sonicate
- p17 control: carbopol solution (i.e., without p17)

Note: Adjustment of viscosity by adding triethanolamine (10-20 µl) and HCl (32%) (2-10 µL) until obtaining a suitable viscosity. The formulations were maintained at 4ºC throughout the study.

Fourth formulation

Starting from 23 mg /ml Healon 5™ (AMO) sodium hyaluronate in phosphate buffered saline (PBS), a dilution was performed until obtaining a final concentration of 1 mg/ml of hyaluronate. A 0.6 ml syringe of 23 mg/ml hyaluronate (HLNC) until 14 ml of PBS and carbonate:

- p144: 14 mg were dissolved in 7 ml of carbonate with 1 mg/ml of HLNC. Sonicate
- p17: 4 mg were dissolved in 7 ml of PBS with 1 mg/ml of HLNC

Note: The formulations were maintained at 4ºC throughout the study.

Fifth formulation

Starting from 0.15% sodium hyaluronate (Hyabak 10 mL, Thea) without preservatives:

- p144: 20 mg were dissolved in 5 ml of eye drops, 40 µl of saturated concentrated NaOH were incorporated, stirring and adding in portions of 5 µl. It was sonicated until solubilization. To adjust the pH, 500 µl of 1M HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) were added, such that the pH changed from 10.5 to 7
- p144 control: 40 µl of saturated NaOH and 500 µl of 1 M HEPES (final concentration of HEPES 50 mM)
- p17: 20 mg were dissolved in 5 ml of the eye drops, it was sonicated until complete solubilization and it was incorporated to the vial with a 10 ml syringe and a blue needle to perforate the filter of the contained (final concentration 2 mg/ml)
- p17 control: non-manipulated eye drops

Note: The formulations were maintained at 4ºC throughout the study.

After all the *in vivo* studies conducted on the different groups (section 1.1.1) it was determined that the most suitable formulation to attempt reversing the damage generated with 1 N NaOH was the fifth formulation. Therefore, all the results shown in relation to *in vivo* will refer to this type of treatment.

1.1.3 Weekly Follow-up of the Corneal Lesions

a) Semi-quantitative analysis by means of Corneal Biomicroscopy

The administration of the treatment was initiated twenty-four hours after the damage. After that time a follow-up of the damage was performed, which consisted of taking photographs at days 0, 7, 14, 21, 28 and 35. To take the photographs the animals were anesthetized with isoflurane. Photographs were taken of both the control eye (left) and of the treated eye (right) with and without fluorescein. The photographs with fluorescein allowed to observe the evolution of the epithelial closure of the generated lesion, whereas the photographs without fluorescein show the evolution of the haze of the lesioned area. The photographs without fluorescein were taken first. These photographs were taken with the white color filter of the camera. Then, the photographs with fluorescein were taken such that a drop of commercial fluorescein (Colircusí Fluorescein) was added on the eye for 60 seconds. After that time, the area was washed with physiological saline and the eyes were photographed in darkness and using the blue filter of the camera.

b) Quantitative analysis of Corneal Haze

This analysis allowed to obtain numerical area values from the study conducted in section 1.1.3.a). To that end, the ImageJ program was used in which both the total area of the eye of each animal and the damaged area were measured. In order to be able to carry out comparable measurements, the damaged area was divided by the total area. Thus, the error due to differences in the size of the eyes between different animals or the differences in relation to the perspective with which the photograph is taken was minimized. All the data obtained were referred to the initial value obtained with the photographs at day 0, the initial damage in all the animals was thus considered to be 100%.

1.1.4 Sacrifice of the animals and enucleation of the eyes

The animals were sacrificed by neck dislocation after having been anesthetized with isoflurane. After the sacrifice, the enucleation was performed and the eyes were placed for 24 hours in Davidson's solution for the purpose of fixing them. After this time, the eyes of the animals were passed from Davidson's solution to 4% formol, 24

hours later the eyes were passed to 70% ethanol, where they can remain up to one month.

1.1.5 Inclusion of the tissue in paraffin

The fixed tissues, which were in 70% ethanol, were dehydrated so that they could be included in paraffin. Once included, the samples can be cut to 3-5 μm in the microtome and subsequently the corresponding histological and immunohistochemical analysis can be conducted.

1.1.6 Histological analysis of the sections

Initially, the decision was made to study the samples at immunohistochemical level by means of using fibrosis markers such as α-SMA or fibronectin, quantify the amount of collagen after the staining with Sirius Red and quantify the number of inflammatory cells. However, it was not possible to carry out most of these techniques due to problems with the choice of the suitable antibody because there are not many specific antibodies against rats. Furthermore, by consulting other research centers (IOVA, Valladolid (Spain)) it was seen that it was convenient to quantify the efficacy of the peptides in the inhibition of corneal fibrosis in a more refined manner by means of molecular signaling studies.

Therefore, Western Blot and immunofluorescence studies were conducted to determine, from cells extracted from the *in vivo* studies, the signaling pathway used by the peptides administered topically. It should be emphasized that said studies were conducted on fibroblasts from rabbit cornea and not from rat cornea. This decision was made due to the difficulty entailed in isolating fibroblasts from rodent eyes.

1.1.7 Statistical Study of the Data

The comparison of the obtained results was performed by means of the Student's t-test for related samples using the SPSS 15.0 software.

1.2. Results

1.2.1 *In vivo* results

The results obtained in the different groups of animals are shown below. A clear decrease of the haze (Figure 1) and of the epithelial closure (Figure 2) in the eyes treated with peptide, both p17 and p144, with respect to the control, can be seen.

After analyzing the images with ImageJ program a series of area data were obtained from which the graphs shown in Figures 3, 4, 5 and 6 were made. Said graphs show the mean ± the standard deviation of all the animals (n=12) of each group at days 0, 7, 14, 21, 28 and 35.

1.2.2 Biochemical results

a) Western Blot Studies

Since the scientific literature repeatedly describes the TGF-β1 signaling pathways, the decision was made to go into depth in such pathways by means of the techniques described in the methodology section. Said pathways involve the study of the Smad protein family, specifically the study of the isoforms Smad2, 2/3 and 7.

To that end, protein extracts obtained from the primary culture of rabbit fibroblasts were treated with TGF-β1 at concentrations of 0, 3 and 5 ng/ml and, 50 and 200 μg/ml of peptide p17 and p144.

Expression of p-Smad2

Decrease of the activation of p-Smad2 after the application of the inhibitory peptide p17 (Figure 7) was not observed, whereas with p144 a slight inhibition of the expression of p-Smad2 after the treatment with 50 μg/ml was observed. The inhibition is greater with the treatment of 200 μg/ml reaching the values of the controls (Figure 7).

Expression of Smad 2/3

The expression of Smad 2/3 in the primary culture was not affected with the treatment of TGF-β1 or with the inhibitory peptides p17 and 144 (Figure 8). Figure 8 shows the inhibitory peptide p17 in the first place and the action of the p144 in the second place.

Expression of Smad 7

The expression of Smad 7 in the primary culture of rabbit fibroblasts was not affected with the treatment of TGF-β1 or with the inhibitory peptides (Figure 9).

b) Immunofluorescence studies

Treatment with TGF-β1

The application of 3 and 5 ng of TGF-β1 induced the translocation of p-Smad2 to the nucleus (stained green) [Figure 10].

Treatment with TGF-β1 inhibitor peptides (p17 and p144)

Treatment with p17 did not inhibit the translocation of p-Smad2 to the nucleus but it was inhibited by treatment with p144, which indicates that this peptide inhibits the TGF-β1 signaling pathway in the primary culture of rabbit corneal fibroblasts (Figure 11).

1.3 Conclusions

Treatment of the chemical lesions induced after corneal damage with 1N NaOH in the different experimentation groups decreases significantly after the treatment with p17 and p144, which undoubtedly has an extraordinary clinical importance.

Additionally, the results obtained show that:

- the most suitable formulation for the treatment of the lesions made with 1N NaOH was the fifth formulation (comprising sodium hyaluronate);
- treatment with p17 or with p144 during the six-week assay period significantly reversed the initial damage generated with NaOH in relation to haze and epithelial closure;
- TGF-β1 activates the phosphorylation of Smad2 in the primary culture of rabbit corneal fibroblasts but does not modify the levels of Smad2/3 and Smad7. The latter are not modified after treatment with p17 or p144;

- treatment with p17 does not have any effect on the signaling cascade of TGF-β1 in the primary culture of rabbit corneal fibroblasts, which shows a discrepancy with the results obtained in the *in vivo* studies; a non-binding explanation leads to thinking that the signaling pathway used by p17, which shows great effectiveness in the animal models studied, is not mediated by the proteins studied in this work but by other different ones from the Smad2, Smad2/3 and Smad7 proteins; and
- treatments with p144, at concentrations of 50 μg/ml and 200 μg/ml block the TGF-β1 transduction signal, inhibiting the phosphorylation of Smad2 and therefore its subsequent translocation to the nucleus; these findings are perfectly correlated with the results found in the *in vivo* studies and suggest that the intracellular effects are mediated by the Smad2 pathway.

CLAIMS

1. A product selected from the group consisting of:
   a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,
   b) a fusion protein comprising a peptide as defined in a),
   c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),
   d) a vector comprising a polynucleotide as defined in c),
   e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and
   f) a combination of one or more of a), b), c), d), and/or e),

   for use in the prevention and/or treatment of corneal fibrosis and/or corneal haze.

2. Product according to claim 1, in the form of a composition comprising one or more of said products a) to e).

3. Product according to claim 1 or 2, wherein said TGF-β1 inhibitor peptide is a peptide having at least 70% sequence identity to an amino acid sequence of any of the amino acid sequences shown under SEQ ID NO: 1-23.

4. Product according to claim 1 or 2, wherein said TGF-β1 inhibitor peptide is a peptide selected from the group of peptides whose amino acid sequence is shown in SEQ ID NO: 1, or in SEQ ID NO: 2, or in SEQ ID NO: 3, or in SEQ ID NO: 4, or in SEQ ID NO: 5, or in SEQ ID NO: 6, or in SEQ ID NO: 7, or in SEQ ID NO: 8, or in SEQ ID NO: 9, or in SEQ ID NO: 10, or in SEQ ID NO: 11, or in SEQ ID NO: 12, or in SEQ ID NO: 13, or in SEQ ID NO: 14, or in SEQ ID NO: 15, or in SEQ ID NO: 16, or in SEQ ID NO: 17, or in SEQ ID NO: 18, or in SEQ ID NO: 19, or in SEQ ID NO: 20, or in SEQ ID NO: 21, or in SEQ ID NO: 22, or in SEQ ID NO: 23, or a functionally equivalent derivative, variant, analog, or fragment thereof, or a salt thereof.

5. Product according to claim 4, wherein said fragment comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 consecutive amino acid residues of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, or SEQ ID NO: 23, and maintains the capacity to inhibit TGF-β1.

6. Product according to claim 1 or 2, wherein said TGF-β1 inhibitor peptide is a peptide selected from the group of peptides p144 (SEQ ID NO: 6), p17 (SEQ ID NO: 17) or a fragment comprising 9, 10, 11, 12, 13, or 14 consecutive amino acid residues of SEQ ID NO: 6 or SEQ ID NO: 17, or their pharmaceutically acceptable salts, wherein said fragment maintains the capacity to inhibit TGF-β1.

7. Product according to claim 1 or 2, wherein said TGF-β1 inhibitor peptide is selected from the group of peptides consisting of p144 (SEQ ID NO: 6), p17 (SEQ ID NO: 17) and combinations thereof.

8. Use of a product selected from the group consisting of:
   a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,
   b) a fusion protein comprising a peptide as defined in a),
   c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),
   d) a vector comprising a polynucleotide as defined in c),
   e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and
   f) a combination of one or more of a), b), c), d), and/or e),

   for use in the manufacture of a pharmaceutical composition for the prevention and/or treatment of corneal fibrosis and/or corneal haze.

9. A method for the prevention and/or treatment of corneal fibrosis or corneal haze which comprises administering to a subject in need of treatment a therapeutically effective amount of a product selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of (a), (b), (c), (d), and/or (e).

10. An ophthalmic pharmaceutical composition comprising a therapeutically effective amount of a product selected from the group consisting of:

a) a TGF-β1 inhibitor peptide, or a functionally equivalent derivative, variant, analog or fragment thereof,

b) a fusion protein comprising a peptide as defined in a),

c) a polynucleotide encoding a peptide as defined in a) or a fusion protein as defined in b),

d) a vector comprising a polynucleotide as defined in c),

e) a cell comprising a peptide as defined in a), a fusion protein as defined in b), a polynucleotide as defined in c) and/or a vector as defined in d), and

f) a combination of one or more of (a), (b), (c), (d), and/or (e); and

a pharmaceutically acceptable vehicle for said compound(s) wherein said pharmaceutically acceptable vehicle is a vehicle suitable for ophthalmic administration.

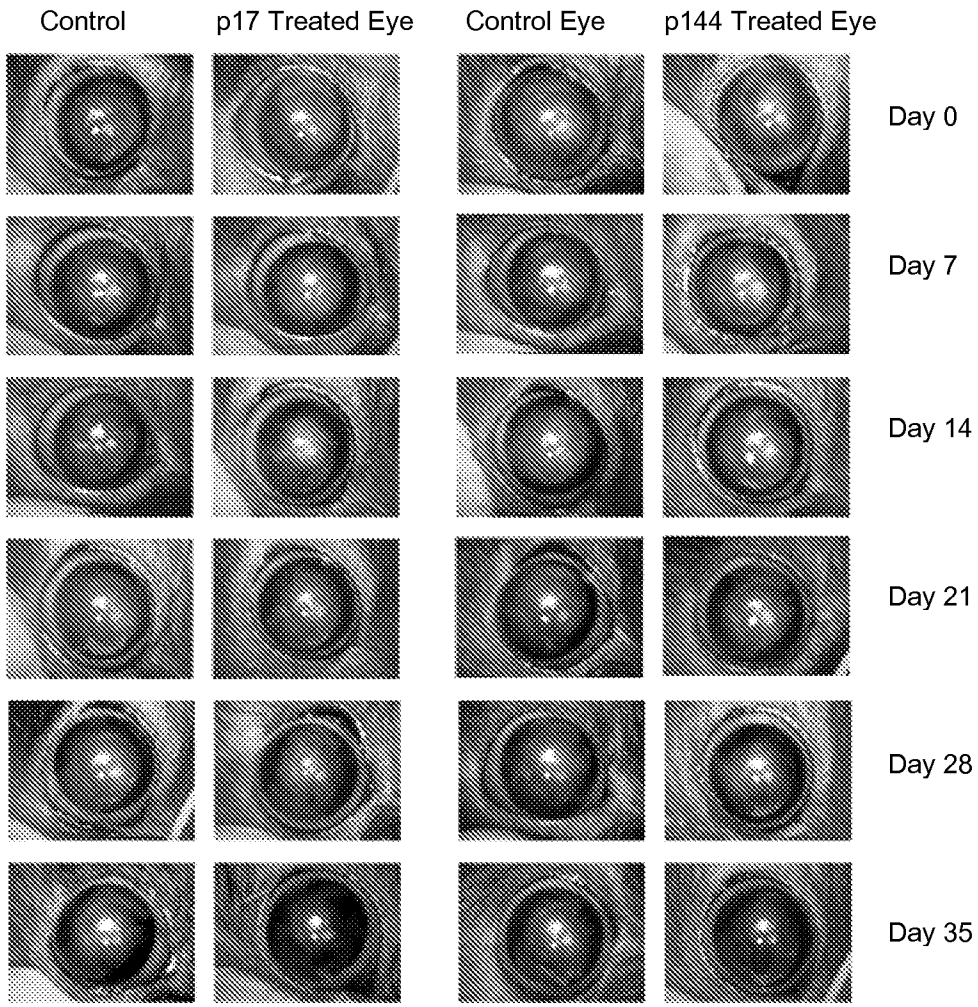
Control
p17 Treated Eye
Control Eye
p144 Treated Eye
Day 0
Day 7
Day 14
Day 21
Day 28
Day 35

Figure 1

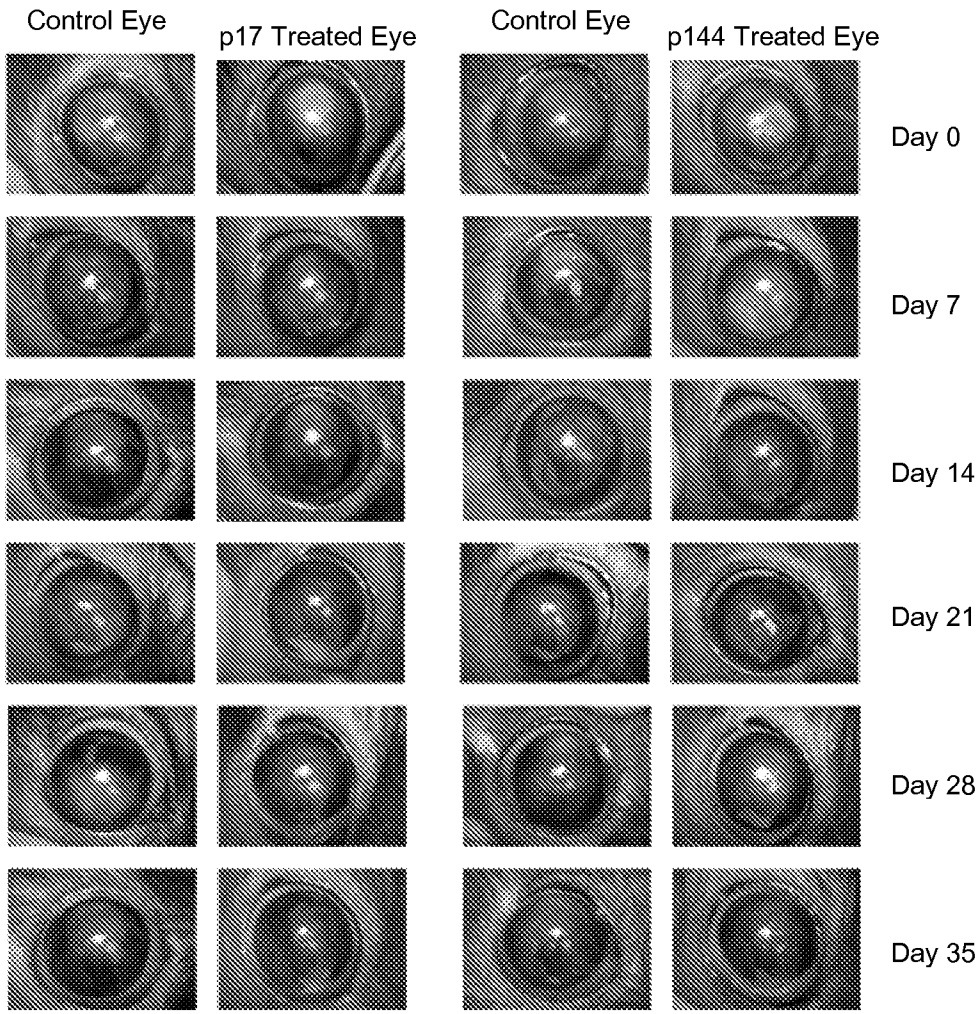
Control Eye
p17 Treated Eye
Control Eye
p144 Treated Eye
Day 0
Day 7
Day 14
Day 21
Day 28
Day 35

Figure 2

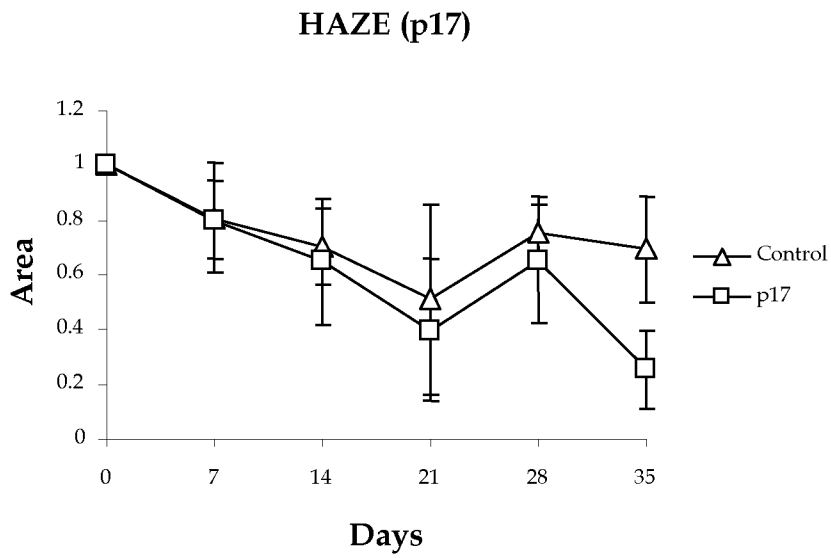

Figure 3

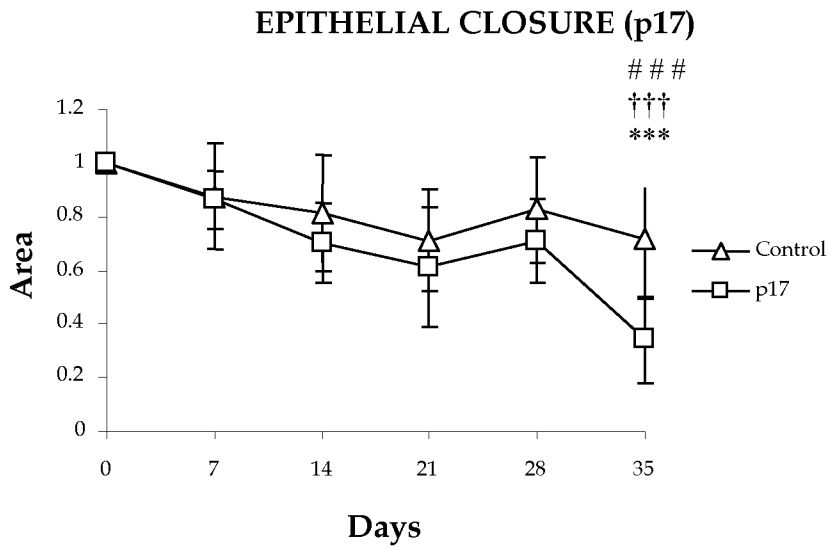

Figure 4

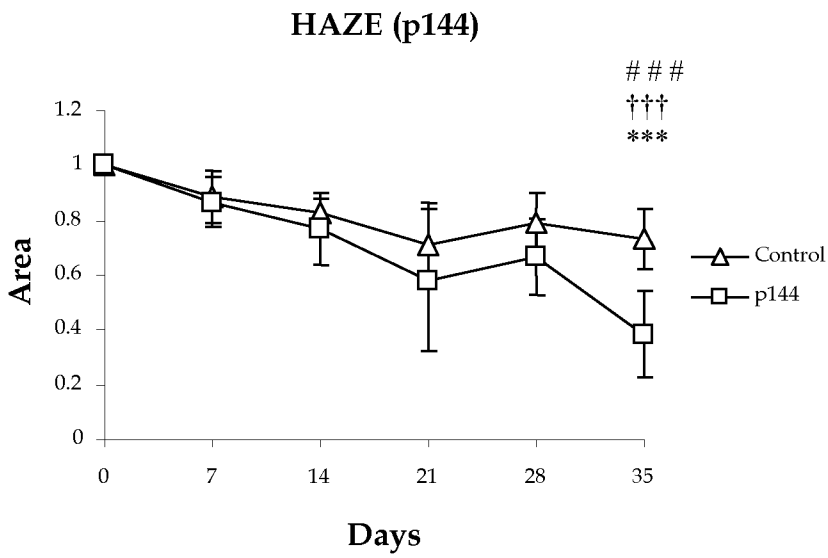

Figure 5

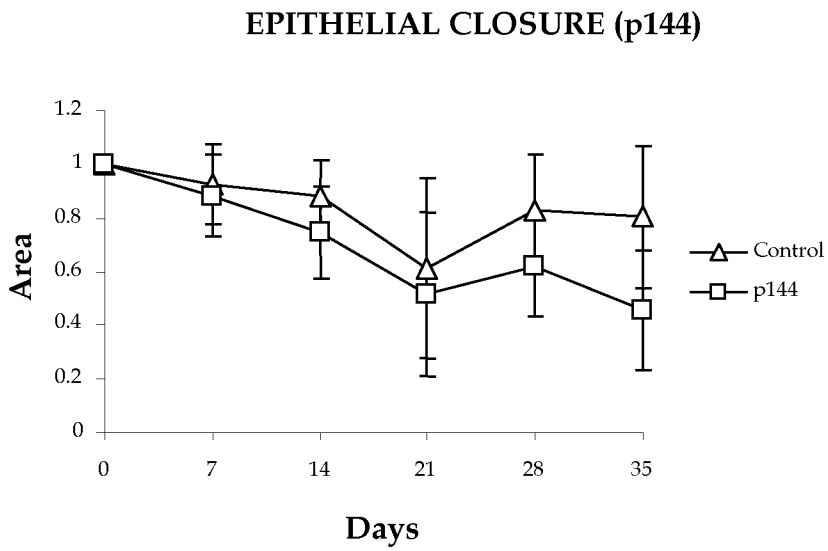

Figure 6

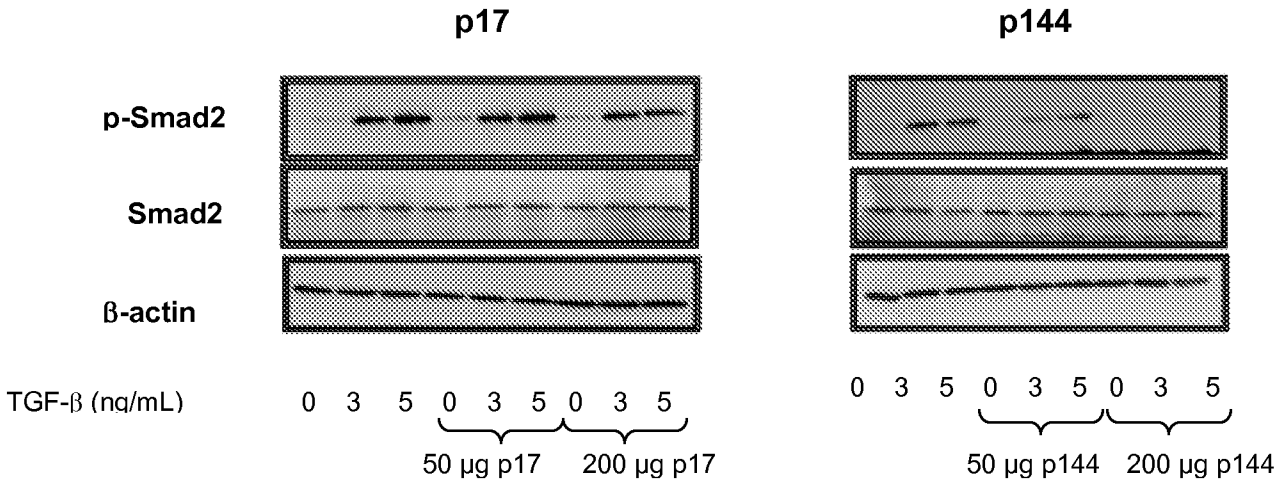

Figure 7

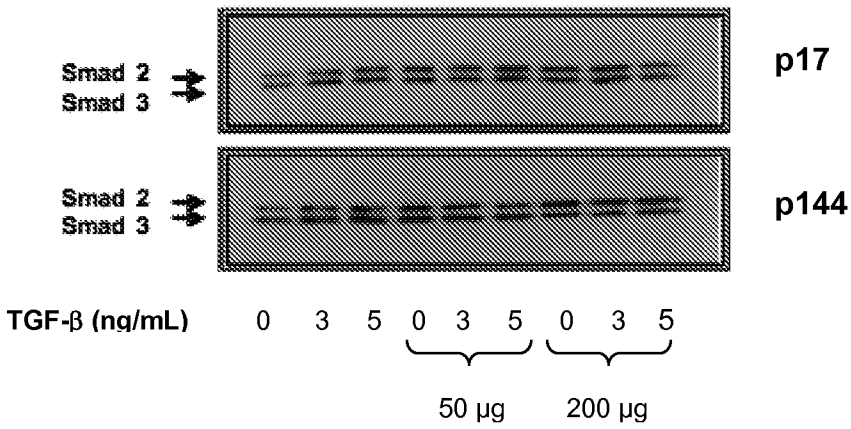

Figure 8

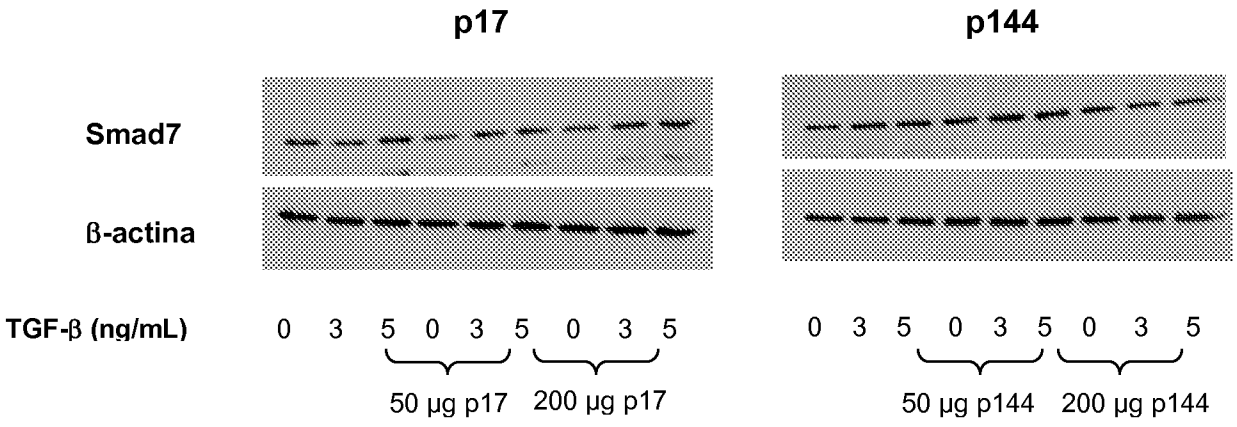

Figure 9

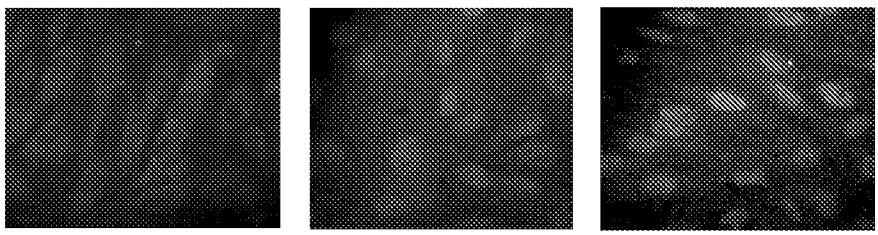

Figure10

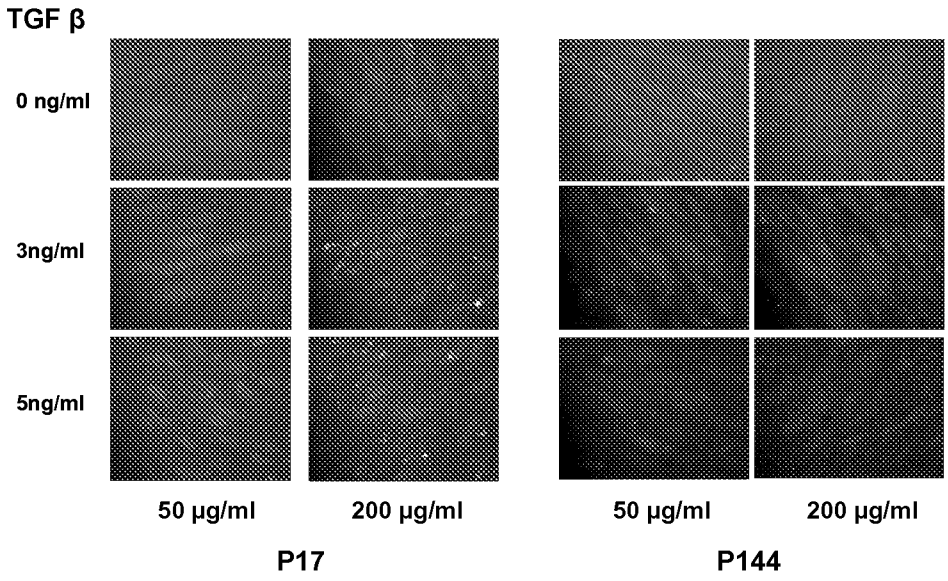
TGF β
0 ng/ml
3ng/ml
5ng/ml
50 µg/ml
200 µg/ml
50 µg/ml
200 µg/ml
P17
P144

Figure 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/052543

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/18 A61P27/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 669 367 A1 (PROYECTO BIOMEDICINA CIMA SL [ES]) 14 June 2006 (2006-06-14) paragraphs [0042] - [0048]; claim 11; table 4; sequences 11,17	1-10
X	WO 98/24466 A1 (ALCON LAB INC [US]; NIXON JON C [US]; STEELY HENRY T JR [US]; KUNKLE H) 11 June 1998 (1998-06-11) page 7, lines 14-20; claims 1-3,7	1,2,8-10
X	EP 1 132 403 A1 (INST CIENTIFICO TECNOL NAVARRA [ES] PROYECTO BIOMEDICINA CIMA SL [ES]) 12 September 2001 (2001-09-12)	10
Y	sequences 1-10	1-9
X	EP 1 974 740 A1 (PROYECTO BIOMEDICINA CIMA SL [ES]) 1 October 2008 (2008-10-01)	10
Y	claim 1; sequences 1-5	1-9

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 March 2011	05/04/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bochelen, Damien

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/052543

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1669367 A1	14-06-2006	AT 479702 T	15-09-2010
		AU 2004266856 A1	03-03-2005
		AU 2010212466 A1	09-09-2010
		BR PI0413215 A	19-06-2007
		CA 2535807 A1	03-03-2005
		CN 1839149 A	27-09-2006
		DK 1669367 T3	08-11-2010
		ES 2351865 T3	11-02-2011
		ES 2304069 A1	01-09-2008
		WO 2005019244 A1	03-03-2005
		HR 20100637 T1	31-12-2010
		JP 2007525204 T	06-09-2007
		PT 1669367 E	07-12-2010
		RU 2333917 C2	20-09-2008
		SI 1669367 T1	30-11-2010
		US 2007142275 A1	21-06-2007
WO 9824466 A1	11-06-1998	AU 5593398 A	29-06-1998
EP 1132403 A1	12-09-2001	AT 322505 T	15-04-2006
		AU 767498 B2	13-11-2003
		BR 9915604 A	07-08-2001
		CA 2352537 A1	02-06-2000
		CN 1328569 A	26-12-2001
		DE 69930763 T2	30-11-2006
		DK 1132403 T3	14-08-2006
		ES 2146552 A1	01-08-2000
		WO 0031135 A1	02-06-2000
		ES 2262349 T3	16-11-2006
		JP 3836677 B2	25-10-2006
		JP 2002530431 T	17-09-2002
		JP 4002287 B2	31-10-2007
		JP 2006241166 A	14-09-2006
		JP 4068654 B2	26-03-2008
		JP 2007186519 A	26-07-2007
		JP 4237807 B2	11-03-2009
		JP 2008056685 A	13-03-2008
		JP 4272255 B2	03-06-2009
		JP 2009040796 A	26-02-2009
		PT 1132403 E	31-07-2006
		RU 2232771 C2	20-07-2004
		SI 1132403 T1	31-08-2006
		US 2007014767 A1	18-01-2007
		US 7057013 B1	06-06-2006
EP 1974740 A1	01-10-2008	AU 2005337776 A1	03-05-2007
EP 1974740 A1		CA 2627298 A1	03-05-2007
		CN 101340927 A	07-01-2009
		WO 2007048857 A1	03-05-2007
		JP 2009512727 T	26-03-2009
		US 2009263410 A1	22-10-2009