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(54) Title: EXTRACELLULAR VESICLES OR COMPOSITION THEREOF FOR USE AS A MEDICAMENT, SUCH AS FOR THE TREATMENT OF OCULAR SURFACE DISORDERS

(57) Abstract: The invention provides extracellular vesicles (EVs) or composition thereof for use as a medicament, in particular for use in the treatment or the prevention of an ocular surface blinding disorder (OSBD), wherein said EVs are produced by a genetically modified cell comprising one or more recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6) and/or recombinant type VII Collagen (rCOL7A1).



EXTRACELLULAR VESICLES OR COMPOSITION THEREOF FOR USE AS A MEDICAMENT, SUCH AS FOR THE TREATMENT OF OCULAR SURFACE DISORDERS

Technical field

- 5 This invention relates to Extracellular vesicles (EVs) produced by a genetically modified cell for use in the treatment or the prevention of an ocular surface blinding disorder (OSBD).

Background of the invention

10 The ocular surface is composed of the conjunctival and corneal epithelia. Despite exhibiting different phenotypes, these two tissues are in adjacent continuity. However, while the corneal epithelium is transparent, stratified and non-keratinized, relying on a transparent and avascular stroma, the conjunctival epithelium is semi-transparent and relies on an opaque vascularized substantia propria.

15 The limbus is a specific structure at the periphery of the cornea, creating a transition between the corneal and conjunctival epithelia. The limbus hosts the corneal stem cell niche, which is composed of various cell types, such as mesenchymal stromal cells (MSCs) within a specific extracellular matrix (ECM) and basement membrane that supports Limbal Epithelial Stem Cell (LESC) survival and self-renewal, proliferation and differentiation.

20 Both corneal cells and ECM properties are important to maintain corneal transparency. Loss of corneal cells or disorganized matrix may lead to a loss of transparency and decrease of vision.

Maintenance of corneal transparency after wounding relies on the capacity of corneal epithelial cells to regenerate from the limbal area and on interactions between epithelial and stromal cells and restoration of the epithelium basement membrane (EBM) in the wounded area.

25 Rapid epithelial healing is crucial to prevent stromal lysis and/or fibrosis that would hinder visual prognosis. Defective wound healing with chronic EBM disruption leads to abnormal epithelial-stromal interactions, which may lead to a massive induction of matrix proteolytic enzymes responsible for corneal lysis and irregular scarring.

30 In severe ocular surface blinding disorders (OSBD), corneal epithelial wound healing may be impaired because of inherited or acquired lesions associated with defective EBM healing

leading to fibrosis and loss of corneal transparency. Moreover, chronic or recurrent ulcerations result from the progressive destruction of the stem cell niche (limbus), responsible for a limbal stem cell deficiency (LSCD). LSCD can be the consequence of severe trauma (accidental ocular burns, iatrogenic lesions), chronic inflammation (severe dry eye, mucous membrane pemphigoid, toxic epidermal necrolysis) or congenital diseases (congenital aniridia, Recessive dystrophic epidermolysis bullosa). The corneal epithelium progressively undergoes a transdifferentiation toward a conjunctival or cutaneous phenotype, with keratinization and total corneal transparency loss.

Although allogenic corneal graft is successfully used to restore vision by replacing the opaque stroma, it cannot restore the limbal stem cell niche. Therefore, this treatment would fail in patients presenting LSCD. When the LSCD is unilateral, LESC can be transplanted from the fellow eye, prior to the corneal grafting. However, there is no available treatment for patients with bilateral LSCD (including genetic causes of LSCD).

More generally, there is a need of efficient methods for treating ocular surface blinding disorders (OSBD), such as conditions associated with corneal ulceration, conditions associated with conjunctivalization of the cornea, and/or conditions associated with fibrosis of the cornea.

Methods of treatment using autologous epithelial cells from other origin have been developed, such as cultivated oral mucosal epithelial transplantation (COMET). Despite an attractive concept, COMET rarely achieves significant and long-lasting improvement in vision. Moreover, COMET could induce corneal stromal neovascularization that is the major risk factor of corneal graft rejection if subsequently performed [1] [2] [3].

Promising cell therapies using MSCs are under way but present several drawbacks that include the need of repeated injections or surgeries, and the impossibility to control cell survival and migration.

Therapies using extracellular vesicles (EVs) derived from MSCs are also disclosed for treating corneal scarring. EVs derived from MSCs share the bioactive properties of their parental cells and therefore represent flexible, ready to use, off-the-shelf alternatives to stem cell therapy for tissue regeneration, immune modulation, or drug delivery, without the risk of allogenic reaction and with limited side effects.

WO2019/169380 discloses that exosomes derived from corneal MSCs, adipose derived MSCs, umbilical cord MSCs, or bone marrow derived MSCs have the ability to reduce the scar of a mouse model of corneal wounding. One clinical trial using Exosomes from umbilical cord MSCs to treat ocular surface graft versus host disease is underway (NCT04213248).

- 5 The present invention provides improved therapies to the use of extracellular vesicles (EVs) for the treatment of OSBD.

Summary of the invention

The present invention provides an advanced therapy medicinal product for patients with OSBD, combining advantages of EVs therapy and gene therapy using EVs produced by
10 genetically modified cells, especially from mesenchymal stromal cells.

In a first aspect, the invention provides extracellular vesicles (EVs) or composition thereof for use as a medicament, wherein said EVs are produced by a genetically modified cell comprising one or more recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6) and/or recombinant type VII Collagen (rCOL7A1), and
15 wherein said EVs contain (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs.

In a second aspect, the invention provides extracellular vesicles (EVs) or composition thereof for use in the treatment or the prevention of an ocular surface blinding disorder (OSBD), wherein said EVs are produced by a genetically modified cell comprising one or more
20 recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6) and/or recombinant type VII Collagen (rCOL7A1); and wherein said EVs contain (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs.

25 Detailed description of the invention

Definitions

Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood

meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

5 The term "extracellular vesicles" or "EVs" means lipid bound vesicles secreted by cells into the extracellular space. EVs have a diameter from near the size of the smallest physically possible unilamellar liposome (around 20-30 nanometers) to as large as 10 microns or more, although the vast majority of EVs are smaller than 200 nm. EVs are secreted by a number of different cell types for communicating with other cells via the proteins and nucleic acids they carry. Depending on their cellular origin, EVs carry a uniquely distinct profile of proteins,
10 lipids and polynucleotides, which can trigger signaling pathways in other cells and/or transfer EVs products into other cells by EVs fusion with cellular plasma membranes or by endocytosis. EVs can mediate intercellular communication by transferring membrane and cytosolic proteins, lipids, and nucleic acids between cells. These transferred molecules are functional in the recipient cells. The three main subtypes of EVs are microvesicles (MV),
15 exosomes, and apoptotic bodies, which are differentiated based upon their biogenesis, release pathways, size, content, and function.

"PAX6", for "paired box 6", also known as AN, AN1, AN2, FVH1, MGDA, WAGR, ASGD5 or D11S812E, is a protein that in humans is encoded by the *PAX6* gene. PAX6 is a transcription factor acting as a key regulator of eye development. PAX6 deficiency is associated to ocular
20 defects, such as aniridia. Both corneal epithelial and corneal stromal stem cells express high levels of PAX6 in their limbal stem cell niche [4][5].

"COL7A1", for "Collagen Type VII Alpha 1 Chain", also known as EBDCT, EBD1, EBR1 or NDNC8 is a protein that in humans is encoded by the *COL7A1* gene. It functions as an anchoring fibril between the external epithelia and the underlying stroma. Mutations in this
25 gene are associated with all forms of dystrophic epidermolysis bullosa. In the absence of mutations, however, an autoimmune response against type VII collagen can result in an acquired form of this disease called epidermolysis bullosa acquisita.

The term "ocular surface blinding disorder" or "OSBD" means an ocular surface disorder that may lead to blindness. For example, OSBD is known as "severe ocular surface cicatrizing
30 disorder" or "OSCD" for conjunctive disorders. Therefore, in the present invention, OSBD may be replaced by OSCD for conjunctive disorders.

The term "medium" or "culture medium", as used herein, refers to any substance or preparation used for the cultivation of living cells, including the components of the environment surrounding the cells. The medium can be any medium adequate for culturing cells. In a particular embodiment, the medium does not comprise any type of sera, including fetal bovine serum, bovine serum (BS), calf serum (CS), fetal calf serum (PCS), newborn calf serum (NCS), goat serum (GS), horse serum (HS), porcine serum, sheep serum, rabbit serum, rat serum (RS), platelet lysate, such as human platelet lysate.

The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. or European Pharmacopeia or other generally recognized pharmacopeia for use in animals and Humans.

The terms "pharmaceutically acceptable excipient", or "pharmaceutically acceptable carrier, or "pharmaceutically acceptable diluent", or "pharmaceutically acceptable vehicle" used interchangeably herein, refer to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any conventional type. A pharmaceutically acceptable carrier is essentially non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. Suitable carriers include, but are not limited to water, dextrose, glycerol, saline, ethanol, and combinations thereof. The carrier can contain additional agents such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the formulation.

The term "pharmaceutical composition", as used herein, means a composition comprising pharmaceutically acceptable carrier. For example, a carrier can be a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. In the context of the invention, "pharmaceutical composition" refers to a composition comprising a therapeutically effective amount of EVs and at least one pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises EVs, such as isolated EVs, in combination with a non-naturally occurring pharmaceutically acceptable carrier.

The term "Recombinant" is used consistently with its usage in the art to refer to or denoting an organism, cell, protein or genetic material formed or produced by recombination. For example, a recombinant nucleotide sequence (or transgene) is created by a process that involves the human intervention and/or is generated from a nucleic acid that was created by human intervention (e.g., by one or more cycles of replication, amplification, transcription, etc.). For example, a recombinant protein is one that is coded by a recombinant nucleotide

sequence. For example, a recombinant cell is a genetically modified cell comprising one or more recombinant nucleic acid sequence(s) (or transgene) expressing one or more recombinant protein(s).

The term "subject", "patient" or "individual", as used herein, refers to a human or non-human mammal (such as a rodent (mouse, rat), a feline, a canine, or a primate). Preferably, the subject is a human, man or woman.

First and second medical use

In a first aspect, the invention provides extracellular vesicles (EVs) or composition thereof for use as a medicament,

wherein said EVs are produced by a genetically modified cell comprising one or more recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6) and/or recombinant type VII Collagen (rCOL7A1), and

wherein said EVs contain (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs.

In a second aspect, the invention provides extracellular vesicles (EVs) or composition thereof for use in the treatment or the prevention of an ocular surface blinding disorder (OSBD),

wherein said EVs are produced by a genetically modified cell comprising one or more recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6) and/or recombinant type VII Collagen (rCOL7A1); and

wherein said EVs contain (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs.

In some embodiments, EVs are produced by a genetically modified cell comprising a recombinant nucleic acid sequence expressing rPAX6 isoform A. The amino acid sequence of rPAX6 isoform A is SEQ ID NO: 1. According to these embodiments, the genetically modified cell comprises one or more recombinant nucleic acid sequence(s) expressing rPAX6 isoform A, such as one or more recombinant nucleic acid sequence(s) of SEQ ID NO: 2.

In some embodiments, EVs are produced by a genetically modified cell comprising a recombinant nucleic acid sequence expressing rPAX6 isoform B. The amino acid sequence of rPAX6 isoform B is SEQ ID NO: 3. According to these embodiments, the genetically modified

cell comprises one or more recombinant nucleic acid sequence(s) expressing rPAX6 isoform B, such as one or more recombinant nucleic acid sequence(s) of SEQ ID NO: 4.

In some embodiments, EVs are produced by a genetically modified cell comprising a recombinant nucleic acid sequence expressing rCOL7A1 having the amino acid sequence SEQ
5 ID NO: 5. According to these embodiments, the genetically modified cell comprises one or more recombinant nucleic acid sequence(s) expressing rCOL7A1 of SEQ ID NO: 5, such as one or more recombinant nucleic acid sequence(s) of SEQ ID NO: 6.

In some embodiments, the OSBD is a condition associated with corneal ulceration, a condition associated with conjunctivalization of the cornea and/or a condition associated with
10 fibrosis of the cornea.

In some embodiments, the condition is limbal stem cell deficiency (LSCD).

In some embodiments, the LSCD is the consequence of:

- a lesion of the cornea, such as burn of the cornea (e.g. chemical burn), physical lesions of the cornea (e.g. corneal limbal scarring);
- 15 - a chronic inflammation of the cornea, such as severe dry eye (e.g. due to Sjögren's Syndrome), mucous membrane pemphigoid, toxic epidermal necrolysis; or
- a congenital diseases of the cornea, such as congenital aniridia, aniridia-associated keratopathy (AAK) or recessive dystrophic epidermolysis bullosa (RDEB).

In some other embodiments, the condition is neurotrophic keratitis, recurrent corneal erosion
20 syndrome or mild dystrophic epidermolysis bullosa.

In some other embodiments, the condition is an ocular GVHD (Graft Versus Host Disease).

In some embodiments, the EVs or composition thereof as disclosed herein have the property to restore the structure of the corneal epithelial cells, to restore the interactions between epithelial and stromal cells and/or to restore the epithelium basement membrane. The EVs or
25 composition thereof as disclosed herein may also promote rapid epithelial healing, favor EBM maturation and/or strengthens epithelial adhesion to prevent recurrences.

In some embodiments, the EVs or composition thereof as disclosed herein also prevent opacification of the cornea and/or improve corneal healing.

In some embodiments, the EVs or composition thereof as disclosed herein also promote corneal tissue regeneration and/or corneal tissue healing.

EVs or composition thereof as disclosed herein may also have one or more of the following properties, but not limited to:

- 5 - Slowing and/or preventing degeneration of epithelial stem cells,
- reduction of the scar of corneal wounding,
- Corneal wound repair,
- Treatment and prevention of corneal scarring,
- Preservation of cornea transparency,
- 10 - Prevention and treatment of cornea ulceration and erosion,
- Prevention and delay of LSCD,
- Improvement of epithelium attachment to basement membrane.
- Limitation of associated pain,
- Prevention of conjunctive fibrosis, including symblepharon and fornix foreshortening,
- 15 and/or
- Prevention of ocular surface conjunctivalization and keratinization.

This represents a groundbreaking therapeutic innovation for patients with OSBD.

In some embodiments, the EVs are produced by a genetically modified cell, such as a genetically modified mesenchymal stromal cell, comprising one or more recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6), and said EVs contain rPAX6 proteins and/or rPAX6 mRNAs. In these embodiments, the EVs or composition thereof for use according to the invention are particularly useful in the treatment of corneal damages due to Sjögren's syndrome, congenital aniridia, aniridia-associated keratopathy (AAK), Ocular GVHD, Stevens Johnson Lyell syndrome and all other disorders associated with severe dry eyes.

In some embodiments, the EVs are produced by a genetically modified cell, such as a genetically modified mesenchymal stromal cell, comprising one or more recombinant nucleic acid sequence(s) expressing recombinant type VII Collagen (rCOL7A1), and said EVs contain rCOL7A1 proteins and/or rCOL7A1 mRNAs. In these embodiments, the EVs or composition thereof for use according to the invention are particularly useful in the treatment of RDEB, recurrent corneal erosions syndromes, neurotrophic keratitis.

In some embodiments, the EVs are produced by a genetically modified cell, such as a genetically modified mesenchymal stromal cell, comprising one or more recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6) and recombinant type VII Collagen (rCOL7A1), and said EVs contain (i) rPAX6 proteins and/or rPAX6 mRNAs; and (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs. In this embodiment, the EVs or composition thereof for use according to the invention are particularly useful in the treatment of to Sjögren's syndrome, congenital aniridia, aniridia-associated keratopathy (AAK), Ocular GVHD, Stevens Johnson Lyell syndrome, RDEB, recurrent corneal erosions syndroms, neurotrophic keratitis, and all other disorders associated with sever dry eyes.

10 The EVs or composition thereof as disclosed herein are delivery vehicles (or cargos) to deliver Col7A1 and/or PAX6 proteins/mRNA, into cells, tissue, or organs, preferably into the corneal tissue cells. The EVs or composition thereof as disclosed herein are internalized into cells of the tissue or organs, thereby delivering (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs into cells of the tissue or organs, preferably into the corneal tissue cells. Therefore, in some embodiments, the EVs are used to treat cells, tissue, or organs damages, such as corneal damages, in a patient by administering to said patient a therapeutically effective amount of said EVs or composition thereof.

20 In some embodiments, EVs contain a therapeutically effective amount of (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs.

25 According to the invention, the amount of (i) PAX6 proteins and/or PAX6 mRNAs (including recombinant and naturally produced by the cell); and/or (ii) COL7A1 proteins and/or COL7A1 mRNAs (including recombinant and naturally produced by the cell) in the EVs produced by the genetically modified cell as disclosed in the present invention is higher compared to the amount of (i) PAX6 proteins and/or PAX6 mRNAs (i.e. naturally produced by the cell); and/or (ii) COL7A1 proteins and/or COL7A1 mRNAs (i.e. naturally produced by the cell) in the EVs produced by a non-genetically modified cell.

In some embodiments, the genetically modified cell is an induced pluripotent stem cell (iPS).

30 In some embodiments, the genetically modified cell is a mesenchymal stromal cell (MSC), such as a bone marrow-derived MSC (BMMSC), a dental pulp MSC, an adipose tissue MSC, a cornea limbal MSC, a placental MSC, or an umbilical cord MSC (UCMSC), preferably a

UCMSC. UCMSC are particularly advantageous due to the innate pro-healing, anti-fibrotic and anti-inflammatory properties of EVs derived from a UCMSC.

The EVs are produced by a genetically modified cell, such as a mesenchymal stromal cell (e.g. a MSC). Where the cell, such as a MSC, is in cell culture, the EVs may be secreted into the cell culture medium. The EVs may be formed by inward budding of the endosomal membrane. The EVs may comprise one or more polynucleotides or proteins present in the cell that produces the EVs, such as a protein characteristic or specific to the MSC. EVs as disclosed herein contain (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs.

5 In some embodiments, the protein and/or mRNA loading of rPAX6 and/or rCOL7A1 into EVs is increased by modulating shear forces, such as implementing "Turbulence technologies" [6]. Other methods can also force the packaging of rPAX6 and/or rCOL7A1 protein/mRNA into EVs, such as electroporation of exogenous rPAX6 and/or rCOL7A1 protein/mRNA [7] or sonication of exogenous rPAX6 and/or rCOL7A1 protein/mRNA. Cellular-nanoporation was also described to encapsulate selected mRNA in EVs [8].

15 The recombinant cell that produces the EVs is modified to contain the so-called one or more recombinant nucleic acid sequence(s) expressing rPAX6 and/or Col7A1 rCOL7A1 placed under control of proper regulatory sequences for its expression, such as a promoter, an enhancer, etc. Natural or synthetic 5'-UTR and/or 3'-UTR can be added to the recombinant cDNA of COL7A1 and/or PAX6 to potentiate an enrichment of mRNA of rCOL7A1 and/or rPAX6 loading in EV.

25 Methods for introducing a recombinant nucleotide sequence into cells are known in the art and including, as non-limiting examples, stable transduction methods wherein the recombinant nucleotide sequence is integrated into the genome of the cell (e.g. recombinant viral vector-mediated methods, liposomes, microinjection, electroporation, particle bombardment, polyethyleneimine). Transduction methods, such as electroporation and polyethyleneimine, are preferably performed with a sleeping beauty transposon system. The recombinant nucleotide sequence may be included in a vector, more particularly a plasmid or a viral vector, in view of being expressed into cells. In a preferred embodiment, the method for introducing a recombinant nucleotide sequence encoding rPAX6 and/or rCOL7A1 into cells is a recombinant viral vector-mediated methods (e.g. lentivirus), electroporation or polyethyleneimine.

The cell that produces EVs may be immortalized. Therefore, in some embodiments, the EVs are produced by an immortalized cell line. Cells can be immortalized using conventional techniques, such as using a lentiviral construction overexpressing the oncogene cMYC, SV40 antigen or the telomerase reverse transcriptase (TERT). The immortalization of cells offers
5 the possibility- for constant supply of EVs having defined properties, less variability in the EVs production, elimination of variation of donors and a safe technology for treating subjects. The immortalization of the cells offers the possibility for constant supply of EVs, less variability in the EVs production, elimination of variation of donors and a safe technology for treating patients.

10 The EVs may be isolated, for example from a culture medium of cells producing the EVs, based on any property of the EVs. For example, the EVs may be isolated based on molecular weight, size, shape, composition or biological activity. Any appropriate techniques can be used to isolate the EVs, such as centrifugation, filtration, chromatography, size exclusion and/or affinity, preferably tangential flow filtration.

15 In some embodiments, the EVs or composition thereof is administered in the eye and/or by subconjunctival injection.

Composition comprising the EVs can be formulated as a pharmaceutical composition in a variety of forms adapted to the chosen route of administration, for example, in an ocular infusion, such as eye drops.

20 The pharmaceutical composition may be administered in a variety of ways, for example intraocular administration. The composition can be prepared in water, optionally mixed with a nontoxic surfactant. The pharmaceutical composition can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. The pharmaceutical composition can also be prepared in biologic mixtures, such as Warthon Jelly. Under ordinary
25 conditions of storage and use, the pharmaceutical composition can contain a preservative to prevent the growth of microorganisms.

The pharmaceutical composition suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the EVs which are adapted for the extemporaneous preparation of sterile infusible composition. In all cases, the ultimate
30 dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol,

liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof.

Useful liquid carriers include water, alcohols or glycols or water/alcohol/glycol blends, in which the EVs can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as additional antimicrobial agents can be added to optimize the properties for a given use.

Effective dosages and routes of administration of EVs or composition thereof of the invention are conventional. The exact amount (effective dose) of the EVs will vary from subject to subject, depending on, for example, the species, age, weight and general or clinical condition of the subject, the severity or mechanism of any disorder being treated, the particular agent or vehicle used, the method and scheduling of administration, and the like. A therapeutically effective dose can be determined empirically, by conventional procedures known to those of skill in the art. For example, an effective dose can be estimated initially either in cell culture assays or in suitable animal models. The animal model may also be used to determine the appropriate concentration ranges and routes of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutic dose can also be selected by analogy to dosages for comparable therapeutic agents.

The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (e.g. the subject, the disease, the disease state involved, and whether the treatment is prophylactic). Treatment may involve daily or multi-daily doses EVs or composition thereof over a period of a few days to months.

In a particular preferred embodiment, the pharmaceutical composition of the invention is formulated as an ophthalmic formulation for administration to the eye and for this reason have formulation elements specifically selected for this purpose. For example, the most widely used ophthalmic buffer solutions are boric acid vehicle and Sorensen's modified phosphate buffer. The boric acid vehicle is a 1.9% solution of boric acid in purified water or preferably sterile water. It is isotonic with tears. It has a pH of approximately 5 and is useful when extemporaneously compounding ophthalmic solutions of drugs that are most stable at acid pH. Optionally, the buffer solution is convenient to use as a tonicity adjustor. In circumstances when an ophthalmic solution without a buffer is desired, any compatible salt

or non-electrolyte that is approved for ophthalmic products may be used. Sodium chloride, sodium nitrate, sodium sulfate, and dextrose are common neutral tonicity adjustors.

The use of preservatives is common ophthalmic solutions in order to prevent the growth of, or to destroy, microorganisms accidentally introduced when the container is opened during
5 use. Benzyl alcohol, thimerosal and the parabens are the preservatives commonly found in ophthalmic solutions.

In addition, certain active ingredient(s) may be susceptible to oxidative degradation. If oxidation is a problem, an antioxidant can be included. Sodium metabisulfite and Sodium bisulfite are acceptable for this purpose in drug preparations.

10 Finally, an increase in the viscosity of ophthalmic products will result in a longer residence time in the eye, providing a longer time for drug absorption and effect. Numerous viscosity enhancing materials can be used, among winch methylcellulose is a common example.

In some embodiments, the EVs or composition thereof as disclosed herein are delivered by direct injection into the corneal stroma. In other embodiments, the EVs or a composition
15 thereof are delivered by coating an EVs containing hydrogel in the inner surface of a contact lens and contacting an eye with the contact lens. Such embodiments can employ a number of reagents and/or method steps that are performed in treatment of OSBD.

Description of the figures

Figure 1: rPAX6 expression in umbilical cord mesenchymal stromal cells (UC-MSC). A) UC-MSC are transduced (UC-MSC_PAX6) or not (UC-MSC_∅) by a lentiviral vector allowing stable PAX6 expression. 3 days post transduction UC-MSC were immunostained for PAX6 (orange) and nucleic acids are stained using DAPI (blue), ~65% of UC-MSC were stained for PAX6. B) *PAX6* mRNA relative expression quantified by RT-qPCR between UC-MSC_∅ and UC-MSC_PAX6, 15 days post lentiviral transduction and puromycin selection. C) UC-MSC were electroporated (UC-MSC_PAX6) or not (UC-MSC_∅) by a pFAR4 plasmid allowing stable PAX6 expression thanks to sleeping beauty transposon system. 7 days post transduction UC-MSC were immunostained for PAX6 (orange) and nucleic acids are stained using DAPI (blue), ~15% of UC-MSC were stained for PAX6. D) *PAX6* mRNA expression in EVs from control UC-MSC (WT, black bars) and UC-MSC transduced by PAX6 lentivirus (PAX6, dash bar) relative to 3 independent housekeeping genes *GAPDH*, *ACTB* and *PPIA*. E) Quantification of luciferase activity in skin epithelial cells carrying, or not carrying (WT cells), the PAX6 transcriptional responsive element, driving the expression of a luciferase. Cells were treated for 24h with EVs from unmodified UC-MSC (EV WT) or EVs from UC-MSC transduced with PAX6 lentivirus (EV PAX6) at MOI $20 \cdot 10^3$ (MOI20K) or MOI $80 \cdot 10^3$ (MOI80K) or not treated (∅). Luciferase activity is increased when cells are treated with EV PAX6. In a model of skin epithelial cell expressing a low level of PAX6, the treatment with EVs PAX6 was sufficient to increase PAX6 transcriptional activity.

Figure 2: A) representative example of Nano tracking analysis (NTA) of resuspended pellets after conditioned medium ultracentrifugation. Analyze realized for EVs of control UC-MSC (WT, top panel) and EVs of UC-MSC transduced by COL7A1 retrovirus (COL7A1, bottom panel). NTA profiles were similar and typical of an EV profile. B) *COL7A1* mRNA expression in EVs from control UC-MSC (WT, black bars) and UC-MSC transduced by COL7A1 retrovirus (COL7A1, dash bar) relative to 3 independent housekeeping genes *GAPDH*, *ACTB* and *PPIA*. C) Immunostaining of COL7A1 and DAPI staining of HCLE cell line as positive control, showing an endogenous COL7A1 expression and of BeFa cell line as negative control, without COL7A1 staining. BeFa cells were incubated 24h with EVs of UC-MSC control (BeFa + WT EV) or EVs of UC-MSC transduced by COL7A1 retrovirus (BeFa + COL7A1 EV) at MOI $5 \cdot 10^6$, scale bar 10µm. D) Immunostaining of COL7A1 and DAPI staining of HCLE cell line as positive control, showing the endogenous COL7A1 expression and in BeFa cell line as negative control without COL7A1 staining. BeFa cells are incubated 24h with EVs of UC-MSC

control (BeFa + WT EV) or EVs of UC-MSC transfected with COL7A1 plasmid and selected (BeFa + COL7A1 EV) at MOI $8 \cdot 10^5$, scale bar $10 \mu\text{m}$. In BeFa pathological REBD cell model, EVs derived from UC-MSC overexpressing COL7A1 were sufficient to increase COL7A1 staining.

Examples

Example 1: rPAX6 expression in umbilical cord mesenchymal stromal cells (UC-MSc)

1.1 UC-MSc culture

MSCs were isolated from the UC Wharton's jelly of a single healthy donor, using the explant
5 method [16]. Cells were grown in an incubator at 37°C with 5% of CO₂ in αMEM (Minimum
Essential Medium) containing, 10% of serum, antibiotic & antimycotic and 1ng/ml of bFGF
(Fibroblast Growth Factor).

1.2 Plasmids design and electroporation

10 PAX6 isoform A and PAX6 isoform B were independently introduced into a pFAR4 vector [9].
pFAR4 vector, a miniplasmid devoid of antibiotic resistance marker, was used as a gene
vector to deliver the *Sleeping Beauty (SB)* transposon system. pFAR4 vector successfully
demonstrated its safety and efficiency for gene delivery and is currently used in the clinic
(ACTRN12618001556235). SB system is composed of a two inverted terminal repeats
15 sequences (ITRs) surrounding the gene of interest (GOI) and its promoter (CAG). ITRs were
excised from the transposon plasmid and randomly inserted into the genome by the *SB*
transposase encoded by a second pFAR4 plasmid, allowing stable expression in cells.

After trypsinization, 1.10⁶ UC-MSc were resuspended in 500μL of αMEM without serum and
antibiotic. 20μg of pFAR4 plasmids containing PAX6 isoform A was added to the cells
20 suspension and incubated 5min on ice. The mixture was transferred in electroporation
cuvette (4mm wide). The cuvette was then inserted in a BTX ECM830 electroporation
system. 1 pulse of 50 ms at 200 volt was applied. Cells in the cuvette were then incubated
10 min in an incubator at 37°C, 5% of CO₂ before to be seeded in cell culture flask and cells
were grown as routinely. Using this procedure 15% of transfection rate was obtained (Figure
25 1C).

1.3 Lentiviral vector design and transduction

A nucleic acid coding *PAX6 isoform A* and a nucleic acid coding *PAX6 isoform B* were
independently introduced in a lentiviral vector under the control of EF1α promoter. The EF1α
30 promoter ensures ubiquitous and stable expression of the cassette. UC-MScs were
resuspended in αMEM with 10% of serum and 5 to 10μg/ml of Polybrene. Lentiviral particles

coding *PAX6 isoform A* were mixed with cells at MOI 25 to 50. After 48h the medium containing viral particles was washed and replaced by medium used for routine UC-MSC culture. Using this procedure more than 80% of transduced cells can be obtained.

5 *1.4 EVs production*

UC-MSCs stably expressing the GOI were grown as routinely until cells reach 80%-90% of confluency. Cells were then rinsed 3x with the starvation medium (αMEM without serum and dFGF). Cells were incubated for 2h at 37°C, 5% CO₂ in starvation medium, the medium was replaced on more time and cells were incubated for 48h. Additionally, EV production by cells
10 can be increased by increasing shear forces using EVERZOM "Turbulence technologies" [6].

The conditioned media produced was centrifuged a first time at 500 G for 5 minutes, the supernatant was centrifuged a second time at 1000 G for 10 minutes. The supernatant forming the conditioned medium was harvested and stored at -80° C. Evaluation of EV content and size distribution in conditioned medium was assessed by Nanoparticle Tracking
15 Analysis (NTA) and conditioned medium was stored at -80°C.

Additionally, after harvesting the conditioned medium, a sample of cells was subjected to cytometry analysis to get the rate of cells expressing the GOI, and, a RNA sample was prepared and analyzed by RT-qPCR in order to get the expression of the GOI in the whole cell population (Figure 1B).
20

1.5 EVs isolation

EVs were isolated by tangential flow ultrafiltration (TFF) or ultra-centrifugation. First, conditioned medium was cleared (0.45 filtration) and subjected to TFF. We used a KFR2i device (Spectrum Laboratories) and a hollow fiber filtration system allowing ultrafiltration of
25 sample volumes from 50mL up to 10L. Control of continuous feed rate allows the filtration to operate at constant shear rate. Filtration was controlled by pressure sensors ensuring maximum reproducibility of the process.

Starting from a 1.1L solution of conditioned medium, it took about 4h to isolate EVs with an expected 90% yield. The EV were isolated in starvation medium, which was then exchange
30 with another liquid medium such as defined saline solution.

Ultracentrifugation was performed at 150 000 G for 2h at 4°C. The medium was discarded, and the resulting pellet was resuspended in a smaller volume of PBS. Extracellular vesicles were stored at -80°C or directly used for experiment.

5 *1.6 Analysis of EVs content*

Each batch of EVs is controlled for the content of the mRNA and protein of interest by RT-qPCR and ELISA respectively.

mRNA of EVs was extracted from control and modified UC-MSC conditioned media using the exoRNeasy kit (Qiagen®), according to the manufacturer's recommendations. The reverse
10 transcription was carried out using the SuperScript™ III Reverse Transcriptase (Invitrogen®) according to the manufacturer's instructions. Quantitative PCR was established with the SYBR GREEN detection method. qPCR was performed over 40 cycles of amplification. The expression was relative to 3 independent house keeping genes *PPIA*, *ACTB* and *GAPDH*. Pairs of primers used have been validated within the laboratory. *PAX6* forward
15 ATGAGGCTCAAATGCGACTT (SEQ ID NO: 7) and reverse CATTGGCCCTTCGATTAGA (SEQ ID NO: 8). *ACTB* forward GAAGATCAAGATCATTGCTCCT (SEQ ID NO: 11) and reverse TACTCCTGCTTGCTGATCCA (SEQ ID NO: 12). *PPIA* forward CGCGTCTCCTTTGAGCTGTT (SEQ ID NO: 13) and reverse ACTTGCCACCAGTGCCATTA (SEQ ID NO: 14). *GAPDH* forward
20 TATCGTGGAAGGACTCATGACCA (SEQ ID NO: 15) and reverse GGATGATGTTCTGGAGAGCCC (SEQ ID NO: 16).

The results are presented in Figure 1D. *PAX6* mRNA content in EVs *PAX6* relative to *ACTB*, *GAPDH* or *PPIA* was increased as compared to EV from non-modified UC-MSC.

25 *1.7 In vitro analysis of PAX6 transcriptional activity, in target cells, incubated with EVs produced by UC-MSC expressing rPAX6 according to the invention*

In order to assess *PAX6* transcriptional activity a reporter was built. A repetition of consensus sequences of *PAX6* binding sites were inserted front of a CMV minimal promoter followed by GFP and luciferase. The activation of the promoter requires *PAX6* binding. The GFP fluorescence can be quantified by flow cytometry, microscopy or plate reader and the
30 luminescence by plate reader, allowing quantitative analysis of *PAX6* transcriptional activity in target cells [10]. The reporter was stably transfected using the sleeping beauty transposon system, described in Example 1.2, in skin epithelial cell line, which expresses a low level of *PAX6* in comparison to corneal cell lines. Cells carrying the reporter are

incubated with EVs, at various MOI, from non-modified WT UC-MSC and with EVs from UC-MSC overexpressing PAX6, in 96-well plate. After incubation, cells were lysed and luciferase activity was revealed using the ONE-Glo® Luciferase Assay System (Promega®) according to the manufacturer's instructions.

- 5 The results are presented in Figure 1E. The transcriptional activity of PAX6 in skin epithelial cells treated with EVs from non-modified UC-MSC was similar to non-treated cells, while, cells treated with EVs from UC-MSC expressing PAX6 had an increased PAX6 transcriptional activity.

10 *1.8 In vitro functional rescue of PAX6 haploinsufficiency in corneal cells*

PAX6 is knocked down on a single allele in an immortalized corneal epithelial cell line by Crispr/cas9 [11]. EVs produced by UC-MSC expressing rPAX6 according to the invention are incubated with these cells and PAX6, KRT3, KRT12 and MMP9 expression is assessed by RT-qPCR and immunofluorescence [11] [12] [13] in order to determine the transcriptional
15 rescue in haploinsufficient cells. Additionally, using the Incucyte® Scratch Wound Assays, the functionality of the cells is assessed, based on the rescue of cell migration [11].

1.9 In vitro functional rescue of myofibroblast transformation (inhibition of fibrosis)

An immortalized corneal stromal cell line is treated with TGFβ inducing a myofibroblast
20 transformation [14] which is characterized by the expression of ACTA2. Additionally, stromal primary cells, isolated from human corneoscleral rims used for endothelium transplant, are also treated with TGFβ to induce a myofibroblast transformation. The functional inhibition of said myofibroblast transformation by EVs produced by UC-MSC expressing rPAX6 according to the invention is assessed by the decrease of ACTA2 expression by immunofluorescence
25 and RT-qPCR [15].

1.10 In vivo functional rescue of Pax6 haploinsufficiency in mice.

In a *Pax6* deficient mice model (*Pax6^{Sey-Neu/+}*) [21], a topical treatment in the form of eye drops containing bioengineered EVs produced by UC-MSC overexpressing PAX6 according to
30 the invention, is applied on the ocular surface. The rescue of PAX6 expression is assessed by immunostaining of the cornea of *Pax6* and other epithelial markers (e.g. KRT3 and KRT12).

Example 2: COL7A1 expression in umbilical cord mesenchymal stromal cells (UC-MSc)

2.1 UC-MSc culture

Cells were grown under the same condition described in Example 1.1.

5 *2.2 Plasmids design and electroporation*

COL7A1 coding sequence under the control of EF α promoter was inserted in a vector containing inverted terminal repeat sequences ITR, allowing random insertion in the genome by the *Sleeping Beauty (SB)* transposon system described in Example 1.2. The vector also contained a neomycin resistant gene. UC-MSc were transfected by electroporation as
10 described in Example 1.2. 48h post transfection UC-MSc were treated with 300 μ g/ml of G418 for at least one week.

2.3 Lentiviral vector design and transduction

A nucleic acid coding *COL7A1* was introduced in an auto-inactivated retrovirus vector, under
15 the control of EF1 α promoter [17][18]. After trypsination, UC-MScs were resuspended in α MEM with 10% of serum and 5 μ g/ml of Polybrene. Retroviral particles were mixed with cells at MOI 30. A second transduction was carried out under the same conditions at an interval of 24 hours. After 24h, the medium containing viral particles was washed and replaced by medium used for routine UC-MSc culture. UC-MSc used as control did undergo
20 the same procedure, without virus.

2.4 EVs production

EVs were produced following the procedure described in the Example 1.4.

25 *2.5 EVs isolation*

To extract extracellular vesicles from conditioned medium, the medium was ultracentrifuged at 150 000 G for 2h at 4°C. The medium was discarded, and the resulting pellet was resuspended in a smaller volume of PBS. Extracellular vesicles were stored at -80°C or directly used for experiment.

30

2.6 EVs quantification

Evaluation of EV size distribution and concentration in conditioned medium and in PBS after ultra-centrifugation was assessed by Nanoparticle Tracking Analysis (NTA). Samples were diluted between 10 and 200 times for quantification.

- 5 The results are presented in Figure 2A. EVs from unmodified UC-MSC and UC-MSC overexpressing COL7A1 have a similar profile in term of size distribution.

2.7 Analysis of EV mRNA content

Analysis of EV RNA content was done under the same condition described in Example 1.6
10 COL7A1 forward GGGAGAGAGTGACCTGCACG (SEQ ID NO: 9) and reverse
CACCAGCCCTTCGAGAAA (SEQ ID NO: 10).

The results are presented in Figure 2B. COL7A1 mRNA content is EVs PAX6 relative to ACTB, GAPDH or PPIA was increased as compared to EV from non-modified UC-MSC.

15 2.8 Type VII collagen supplementation assay

BeFa cell line was derived from immortalized RDEB patient keratinocytes homozygous for the amino acid 425 (A->G) mutation in exon 3 of COL7A1 [19], this cell line has no measurable COL7A1 protein expression. Cells were grown in DMEM and 30% Nutrient Mixture F-12 Ham, 10% fetal calf serum, 1% antibiotic-antimycotic, 0.2% triiodothyronine, 0.2% adenine, 0.1% hydrocortisone, 0.2% cholera toxin, 0.1% insulin and 0.1% EGF. HCLE cell line (Human
20 Corneal Limbal Epithelial) is an immortalized human corneal epithelial cell lines [20] cultured in Keratinocyte SFM medium (1X) (without serum) + 20 µg/ml of bovine pituitary gland extract + 0.5 ng/ml of recombinant human EGF, 1% antibiotic-antimycotic. This epithelial cell line endogenously expresses type VII collagen.

- 25 BeFa cells were incubated for 24h with EVs from UC-MSC control and UC-MSC overexpressing COL7A1, at MOI $8 \cdot 10^5$ to $5 \cdot 10^6$. Cells were fixed with 4% paraformaldehyde for 20 minutes, permeabilized with Triton X-100-PBS 0.1% for 5 minutes, then incubated in a solution of PBS + 0.05% of Triton, 0.05% of Tween and 5% of FBS for 30 minutes. The cells were incubated with a mouse anti-collagen VII IgG-1 monoclonal antibody (Clone LH7.2
30 provided by Sigma-Aldrich®) for 2 hours. After 3 rinses with PBS the cells were incubated for 1 hour with an anti-mouse IgG secondary antibody and DAPI (4',6-diamidino-2-phenylindole). Images were taken using confocal microscope (ZEISS®) and analyzed with the

ImageJ® software. The results are presented in Figure 2C and 2D. In a model of cells lacking COL7A1, the staining of COL7A1 was increased following a treatment with EVs from UC-MSC overexpressing COL7A1, while a treatment with EVs from unmodified UC-MSC, the COL7A1 staining was similar as compare to non-treated cells.

5

2.9 In vitro functional rescue of myofibroblast transformation (inhibition of fibrosis)

An immortalized corneal stromal cell line is treated with TGFβ inducing a myofibroblast transformation [14] which is characterized by the expression of ACTA2. Additionally, stromal primary cells, isolated from human corneoscleral rims used for endothelium transplant, are also treated with TGFβ to induce a myofibroblast transformation. The functional inhibition of said myofibroblast transformation by EVs produced by UC-MSC expressing rCOL7A1 according to the invention is assessed by the decrease of ACTA2 expression by immunofluorescence and RT-qPCR [15].

2.10 In vivo functional rescue of Col7a1 deficiency in mice.

A Knock-in (KI) mouse model of Col7a1 deficiency having a reduced or missing Col7a1 expression at the basement membrane (skin, oesophagus and cornea) is provided. On the ocular surface of this mouse model, a topical treatment in the form of eye drops containing bioengineered EVs produced by UC-MSC overexpressing Col7a1 according to the invention is applied. The rescue of Col7a1 localisation is assessed by immunostaining of the cornea. Additionally, corneal inflammation, ulceration, blistering and wound closure is investigated following wound or not.

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Sequence listing

SEQ ID	Reference	Sequence
1	Amino acid sequence of rPAX6 isoform A	MQNSHSGVNLGGVFNVRPLPDSTRQKIVELAHSGARPCDISRILQVSNGC VSKILGRYYETGSIRPRAIGGSKPRVATPEVSKIAQYKRECPSIFAWEIRDRL SEGVCTNDNIPSVSSINRVLRLNLASEKQQMGADGMYDKLRMLNGQTGSWGT RPGWYPGTSVPGQPTQDGCQQQEGGGENTNSISSNGEDSDEAQMRLQLKR KLQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKIDLPEARIQVWFSNRR AKWRREEKLRNQRRQASNTPSHIPISSSFSTSVYQPIQPPTTPVSSFTSGSML GRTDTALTNTYSALPPMPSFTMANNLPMQPPVPSQTSSYSCLMPTSPSVNGR SYDITYPPHMQTHMNSQPMGTSGTTSTGLISPGVSVPVQVPGSEPDMSQYW PRLQ
2	Nucleic acid coding rPAX6 isoform A	atgcagaacagtcacagcggagtgaatcagctcgggtgtctttgtcaacgggcgccactgccg gactccaccggcagaagattgtagagctagctcacagcggggcccggcgtgacattccccg aattctgcaggtgtccaacggatgtgtgagtaaaattctggcaggtattacgagactggctccatc agaccagggaatcgggtgtagtaaaccgagagtagcactccagaagttgtaagcaaaatag cccagtataagcgggagtgccctccatcttctgctgggaaatccgagacagattactgtccgagg gggctgtaccaacgataacataccaagcgtgtcatcaataaacagagttcttcgcaacctggtag cgaaaagcaacagatgggcgagacggcatgtatgataaactaaggatgtgaacgggcagacc ggaagctggggcaccgcccctggttggtatccggggacttcggtgccaggggcaacctacgcaaga tggctgccagcaacaggaaggagggggagagaataccaactccatcagttccaacggagaaga ttcagatgaggctcaaagcgcacttcagctgaagcgggaagctgcaagaaatagaacatcctttac ccaagagcaaattgaggcctggagaaagagtttgagagaaccattatccagatgtgtttgccg agaaagactagcagcaaaaatagatctacctgaagcaagaatacaggtatggtttctaactgaag ggcaaatggagaagagaagaaaaactgaggaatcagagaagacaggccagcaacacaccta gtcatattcctatcagcagtagtttcagcaccagtgctaccaaccaattccacaaccaccacaccg gtttctccttcacatctggctccatgttgggcccgaacagacacagccctcacaacacctacagcgc tctgccgctatgccagctcaccatggcaataacctgcctatgcaacccccagtcccagccag acctctcactactctgcatgctgccaccagcccttcggtgaatgggaggatgatgatacttacac cccccacatgtagacacacatgaacagtcagccaatgggcacctcgggaccacttcaacag gactcattccccctggtgtgtagttccagttcaagttcccgggaagtgaacctgatgtctcaatact ggccaagattacagtaa
3	Amino acid sequence of rPAX6 isoform B	MQNSHSGVNLGGVFNVRPLPDSTRQKIVELAHSGARPCDISRILQTHADA KVQVLDNQNVSNCGVSKILGRYYETGSIRPRAIGGSKPRVATPEVSKIAQYKR ECPSIFAWEIRDRLSEGVCTNDNIPSVSSINRVLRLNLASEKQQMGADGMYDK LRMLNGQTGSWGTGTRPGWYPGTSVPGQPTQDGCQQQEGGGENTNSISSNGE DSDEAQMRLQLKRKLQRNRTSFTQEQIEALEKEFERTHYPDVFARERLAAKID LPEARIQVWFSNRRRAKWRREEKLRNQRRQASNTPSHIPISSSFSTSVYQPIQP PTTTPVSSFTSGSMLGRTDTALTNTYSALPPMPSFTMANNLPMQPPVPSQTSSY SCMLPTSPSVNGRSYDITYPPHMQTHMNSQPMGTSGTTSTGLISPGVSVPVQ VPGSEPDMSQYWPRLO
4	Nucleic acid coding rPAX6 isoform B	atgcagaacagtcacagcggagtgaatcagctcgggtgtctttgtcaacgggcgccactgccg gactccaccggcagaagattgtagagctagctcacagcggggcccggcgtgacattccccg aattctgcagacctcagatgcaaaagtccaagtgtggacaatcaaacggtgtccaacggat gtgtgagtaaaattctggcaggtattacgagactggctccatc agaccagggaatcgggtgtagtaaaccgagagtagcactccagaagttgtaagcaaaatag cccagtataagcgggagtgccctccatcttctgctgggaaatccgagacagattactgtccgagg gggctgtaccaacgataacataccaagcgtgtcatcaataaacagagttcttcgcaacctggtag cgaaaagcaacagatgggcgagacggcatgtatgataaactaaggatgtgaacgggcagacc ggaagctggggcaccgcccctggttggtatccggggacttcggtgccaggggcaacctacgcaaga

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5	Amino acid sequence of rCOL7A1	<p>MTLRLLVAALCAGILAEAPRVRAQHRERVCTRLYAADIVFLLDGSSSIGRSNF REVRSFLEGLVLPFSGAASAQGVRFATVQYSDDPRTFGLDALGSGGDVIRAI RELSYKGNTRTGAAILHVADHVFLPQLARPGVPKVCILITDGKSQDLVDTAA QRLKGQGVKLFVAVGIKNADPEELKRVASQPTSDFFFFVNDFSILRLLLPLVSRR VCTTAGGVPVTRPPDDSTAPRDLVLEPSSQSLRVQWTAASGPVTGYKVQY TPLTGLGQPLPSEKQEVNVPAGETSVRLRGLRPLTEYQVTVIALYANSIGEAVS GTARTTALEGEPLTIQNTTAHSLLVAVRSVPGATGYRVTVRVLSSGGPTQQQE LPGQGSVLLRDLEPGTDYEVTVSTLFGRSVGPATSLMARTDASVEQTLRPVIL GPTSILLSWNLVPEARGYRLEWRRETGLEPPQKVVLPADVTRYQLDGLQPGTE YRLTLYTLLEGHEVATPATVVPTGPELVPSPVTDLQATELPGQVRVRSWSPVP GATQYRIIVRSTQGVERTLVLPQSQAFDLDDVQAGLSYTVRVSARVGPREGS ASVLTVRREPETPLAVPGLRVVSDATRVRVAWGPVPGASGFRISWSTGSGPE SSQTLPPDSTATDITGLQPGTTYQVAVSVLRGREGPAAVIVARTDPLGPVRT VHVTQASSSSVTITWTRVPGATGYRVSWHSAHGPEKSQLVSGEATVAELDGL EPDTEYTVHVRHVAGVDGPPASVVRTAPEPVGRVSRQLILNASSDVLRTW VGVTGATAYRLAWGRSEGPMRHQILPGNTDSAEIRGLEGGVSVRVTALV GDREGTPVSIVVTTTPEAPPALGTLHVVRQGEHSLRLRWEVPPRAQGFLLHW QPEGGQEQSRVLGPELSSYHLDGLEPATQYRVRLSVLGPAGEGPSAEVARTTE SPRVPSIELRVVDTSIDSVTLAWTPVSRASSYILSWRPLRPGQEVPGSPQTLF GISSSQRVTLGEPGVSYIFSLTPVLGVRGPEASVTQTPVCPRLADVVFLPHA TQDNAHRAEATRRLERLVLALGPLGPQAVQVGLLSYSHRPSPLFPLNGSHDL GIIILQIRIDMPYMDPSGNNLGTAVVTAHRYMLAPDAPGRRQHVPVGMVLLVD EPLRGDIFSPIREAQASGLNVVMLGMAGADPEQLRRLAPGMDSVQTFFAVDD GPSLDQAVSGLATALCQASFTTQPRPEPCPVYCPKGQKGEPEGEMGLRGQVGP PGDPGLPGRTGAPGPQPPGSATAKGERGFPGADGRPGSPGRAGNPGTPGAP GLKGSPLPGPRGDPGERGPRGPKGEPGAPGQVIGGEGPLPGRKGDGPGSG PPGPRGPLGDPGRGPPGLPGTAMKGDGDRGERGPPGPEGGIAPGEPGLP GLPGSPGPQGPVGPVGGKKEKGDSEDPGLPGQPGSPGEQGPVGGPAGIGP KGDRGFPGPLGEAGEKGERGPPGAGSRGLPGVAGRPAGKPEGPPGPTGRQ GEKGEPRPGDPAVVGPAVAGPKGEKGDVGPAGPRGATGVQGERGPPGLVLP GDPGPKGDPGDRGPIGLTGRAGPPGDSGPPGEKGDGPRGPPGPVGPGRGRD GEVGEKGDGEPGDPGLPGKAGERGLRGAPGVRGPVGEKGDQGDPEGDGRN GSPGSSGPKGDRGEPGPPGPPGRLVDTGPGAREKGEPEGDRGQEGPRGPKGD PGLPGAPGERGIEGFRGPPGPQGDVGRGPAKEKGDGRGPPGLDGRSGLDGKP GAAGPSGPNGAAGKAGDPGRDGLPGLRGEQGLPGPSGPPGLPGKPGEDGKPG LNGKNGEPGDPGEDGRKGEKGDGSGASGREGRDGPKGERGAPGILGPQGP LPGPVGPVGGQGFPGVPGGTGPKGDRGETGSKGEQGLPGERGLRGEPGSPVNV DRLLETAGIKASALREIVETWDESSGSFLVPERRRGPKGDSGEQGPVGGKGP GFPGERGLKGDGDPGQGPVGLALGERGPPGSPGLAGEPGKPGIPGLPGRA</p>

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6	Nucleic acid coding rCOL7A1	<p>atgacgctgcggtctctggtggccgctctgcgccggatcctggcagagggcgcccagtgcg agcccagcacagggagagagtgacctgcacgctcttacgctgacattgtgttctactggat ggctcctcatccattggccgagcaattccgagaggtccgagcttctcgaaggctggtgctgc ctttctctggagcagccagtgcacaggtgtgctgttccacagtgagtagcagcagtagccac ggacagagttcggcctgtagtgcacttggtctgggggtgtagtgcagcagcagcagcagcagc gctacaaggggggcaacactcgacaggggctgcaattctcatgtggtgacctgcttctgccc ccagctggcccagctggtgtcccaaggctgcatcctgatcacagacgggaagtcccaggacct ggtggacacagctgcccaggctgaaggggaggggggcaagctattgctgtggggatcaag aatgctgacctgaggagctgaagcgagttgctcagagccaccagtgacttcttctctgctcaa tgactcagcatctttaggacactactgcccctcgttcccggagagtgtagcagcagcagcagc gtgctgtgacctgacctcggatgactcgacctctgctccacagacctggtgctgtctgagccaa gcagccaatcctgagagtagcagtgagcagcagcagcagcagcagcagcagcagcagcagcag tacactcctgacgggctgggacagcactgcccagtgagcagcagcagcagcagcagcagcagc ctggtgagaccagtgtagcggctgtaggggtctccggcactgaccagtagccaagtactgtgattg ccctctacgccaacagcatcggggaggctgtgagcgggacagctcggaccactgcctagaagg gccggaactgacctccagaataccacagcccacagcctcctggtggcctggcggagtgtagcag gtgcaactggctaccgtgtgacatggcgggtcctcagtggtggccacacagcagcagcagcagc ggcctgggaggggtcagtggtgctgctgactggagcctggcagcagcagcagcagcagcagcagc agcaccctattggccgagtgtagggggcccactcctgatggctgcactgagcctctgttga gcagaccctgcccgggtcatcctgggcccacatccatcctccttctggaactggtgctgagg cccgtggctaccggttgaatggcggcgtgagactggctggagccaccgcagaagggtgtagcag ccctctgatgtgacctgacctgagtggtgagcagcgggactgagtagcagcagcagcagcagcagc aactctgctgaggggacagaggtggcaccctgcaaccgtggtcccactggaccagagctg cctgtgagccctgtaacagacctgcaagccaccgagctgcccgggagcggggtgtagtgctctg gagcccagtcctggtgcccaccagtagcagcagcagcagcagcagcagcagcagcagcagcagc ccctggtgcttctgggagtcagacagcattcgactggatgaggtcaggtcaggtggttagctact gtgagggtgctgctgagtggtcccgtgagggcagtgccagtgctcctactgtccgcccggag ccggaactcactgtgctccagggtgtaggggtgtagtgtagtgtagtgtagtgtagtgtagtgtag gcctggggaccctgctgtagcagcagcagcagcagcagcagcagcagcagcagcagcagcagcagc gtccagccagacactgccccagactctactgccacagacatcacagggtgtagcagcagcagcagc cctaccaggtggtgctgctggtactgtagggcagagaggggcccctgctgagcagcagcagcagc gaacggaccactgggcccagtgaggacggtccatgtgactcagggcagcagcagcagcagcagcagc attacctgaccaggggtcctgtagccacagcagcagcagcagcagcagcagcagcagcagcagcagc</p>

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7	PAX6 forward	ATGAGGCTCAAATGCGACTT
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9	COL7A1 forward	GGGAGAGAGTGACCTGCACG
10	COL7A1 reverse	CACCAGCCCTTCGAGAAA
11	ACTB forward	GAAGATCAAGATCATTGCTCCT
12	ACTB reverse	TACTCCTGCTTGCTGATCCA
13	PPIA forward	CGCGTCTCCTTTGAGCTGTT
14	PPIA reverse	ACTTGCCACCAGTGCCATTA
15	GAPDH forward	TATCGTGGAAGGACTCATGACCA
16	GAPDH reverse	GGATGATGTTCTGGAGAGCCC

CLAIMS

1. Extracellular vesicles (EVs) or composition thereof for use as a medicament, wherein said EVs are produced by a genetically modified cell comprising one or more recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6) and/or recombinant type VII Collagen (rCOL7A1), and wherein said EVs contain (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs.
2. Extracellular vesicles (EVs) or composition thereof for use in the treatment or the prevention of an ocular surface blinding disorder (OSBD), wherein said EVs are produced by a genetically modified cell comprising one or more recombinant nucleic acid sequence(s) expressing recombinant Paired Box 6 (rPAX6) and/or recombinant type VII Collagen (rCOL7A1); and wherein said EVs contain (i) rPAX6 proteins and/or rPAX6 mRNAs; and/or (ii) rCOL7A1 proteins and/or rCOL7A1 mRNAs.
3. EVs or composition thereof for use according to claim 2, wherein the OSBD is a condition associated with corneal ulceration, a condition associated with conjunctivalization of the cornea and/or a condition associated with fibrosis of the cornea.
4. EVs or composition thereof for use according to claim 3, wherein the condition is a limbal stem cell deficiency (LSCD).
5. EVs or composition thereof for use according to claim 4, wherein the LSCD is the consequence of:
 - a lesion of the cornea, such as burn of the cornea, physical lesions of the cornea;
 - a chronic inflammation of the cornea, such as severe dry eye, mucous membrane pemphigoid, toxic epidermal necrolysis; or
 - a congenital diseases of the cornea, such as congenital aniridia, aniridia-associated keratopathy (AAK) or recessive dystrophic epidermolysis bullosa (RDEB).

6. EVs or composition thereof for use according to claim 3, wherein the condition is neurotrophic keratitis, recurrent corneal erosion syndrome or mild dystrophic epidermolysis bullosa.
- 5 7. EVs or composition thereof for use according to any of the preceding claims, wherein the EVs prevent opacification of the cornea and/or improve corneal healing.
8. EVs or composition thereof for use according to any of the preceding claims, wherein the EVs are produced by an immortalized cell line.
- 10 9. EVs or composition thereof for use according to any of the preceding claims, wherein the cell is an induced pluripotent stem cell (iPS cell) or a mesenchymal stromal cell (MSC), such as a bone marrow-derived MSC (BDMSC) or an umbilical cord MSC (UCMSC).
- 15 10. EVs or composition thereof for use according to any of the preceding claims, wherein the cell is a UCMSC.
- 20 11. EVs or composition thereof for use according to any of the preceding claims, wherein the EVs or composition thereof is administered in the eye.

FIGURES

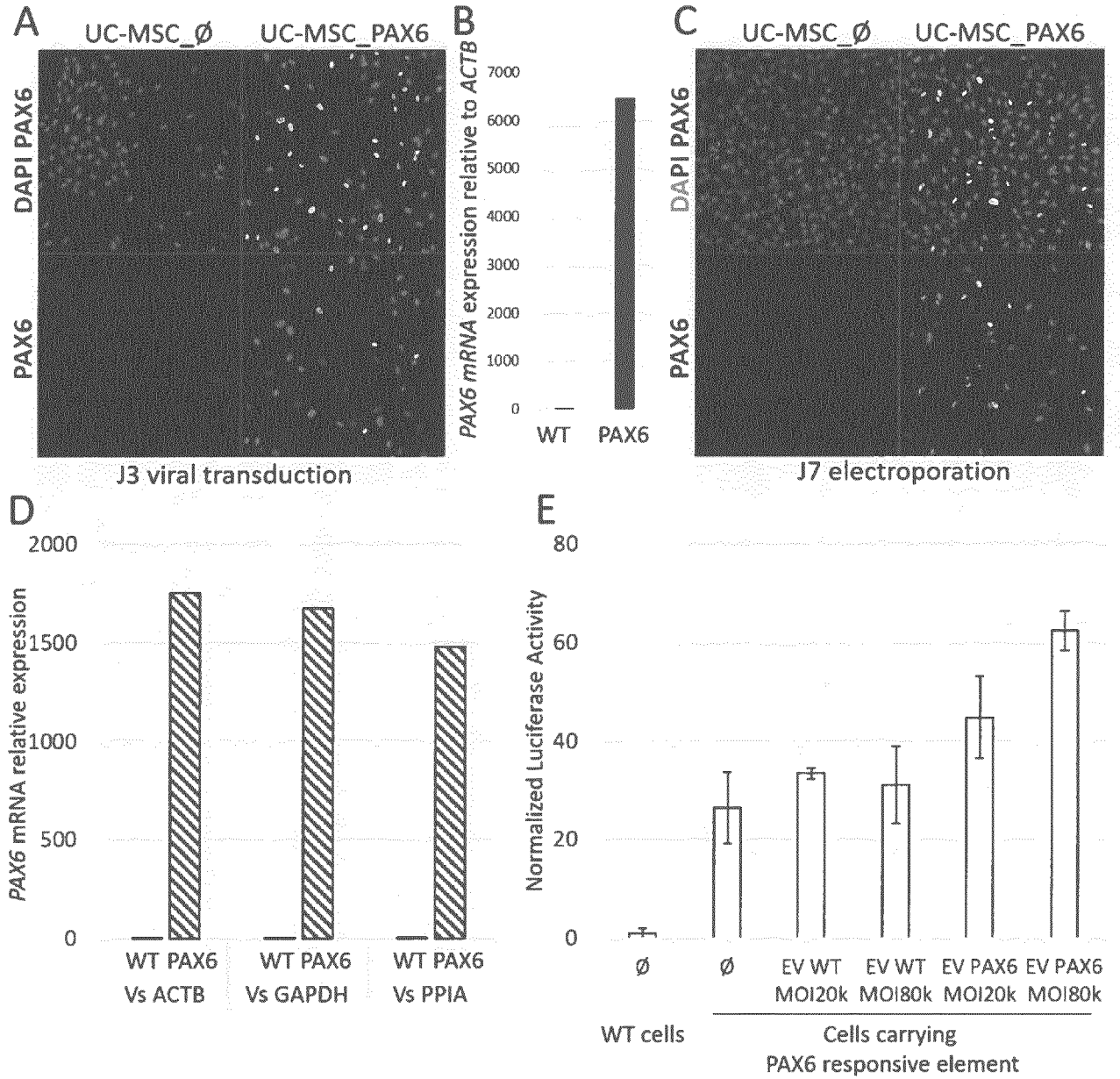


Fig. 1

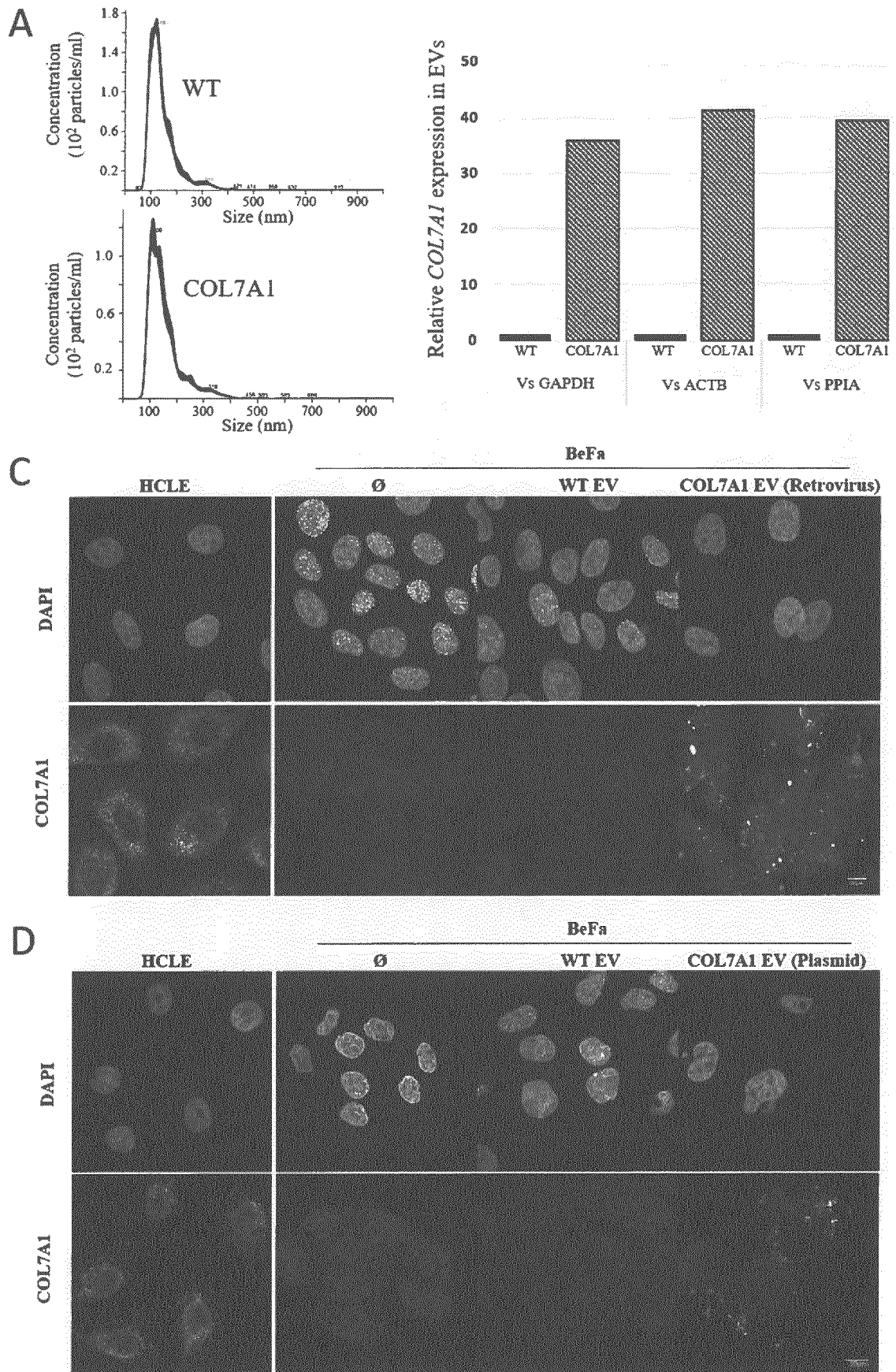


Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2022/080864

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. forming part of the international application as filed.
 - b. furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)).
 accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/080864

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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