

(Chan et al., 1996) [31]. The GIRK channel is predominantly closed at resting membrane potentials. After its activation by the $\beta\gamma$ subunit of a $G_{i/o}$ protein, potassium ions flow out of the cell, thus, hyperpolarizing the neuron (Figure 1B). It has therefore been possible to use vertebrate cone opsins SWO (for short wavelength opsin) and LWO (for long wavelength opsin) for repetitive $G_{i/o}$ activation of a specific wavelength *in vivo* in the anxiety circuitry, and the combination of cone opsins with GIRK has proven more efficient than microbial opsins at low light intensities (Masseck et al., 2014) [23].

Summary

In one aspect, the present disclosure refers to a vector comprising a nucleotide sequence encoding subunit 1 of G-protein-gated inwardly rectifying potassium channel (GIRK1) F137S. In another aspect, the present disclosure refers to a pharmaceutically acceptable carrier comprising the vector defined herein. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the vector as described herein, or the pharmaceutically acceptable carrier as described herein, with a pharmaceutically acceptable carrier, diluent or excipient. In a further aspect, the present disclosure refers to use of the vector according disclosed herein, the pharmaceutically acceptable carrier disclosed herein, or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating rod-cone dystrophy (RCD). In yet another aspect, the present disclosure refers to use of a cone precursor cell comprising a heterologous nucleic acid encoding subunit 1 of G-protein-gated inwardly rectifying potassium channel (GIRK1) F137S in the manufacture of a medicament for treating rod-cone dystrophy (RCD). In one example, the present disclosure refers to a method of preparing a cone precursor cell comprising a heterologous nucleic acid encoding GIRK1 F137S or a functional derivative thereof, said method comprising infecting a cone precursor cell with a viral vector as described herein, or a pharmaceutically acceptable carrier as described herein.

Description of the invention

The Inventors have thus investigated if it was possible to implement this cone opsin based system in a vision restoration setting and investigated patient retinas in preparation for clinical candidate selection. Therefore, in order to develop a light-sensitive cone reactivation strategy, the expression of the phototransduction cascade elements in cones during degeneration was first examined in two RCD mouse models. After exploring the phosphotransduction cascade in two RCD mouse models, a target molecule approach acting via $G_{i/o}$ proteins recruited by the activation of remaining cone opsin was proposed for the first

time. It has thereby created a new “short phototransduction cascade” that is independent from the expression of PDE and transducin. First, the state of the endogenous phototransduction cascade in degenerating cones through the progression of disease was investigated. In both mouse models, only opsin and arrestin were found to migrate to the cone cell bodies after outer segment degeneration. Thus it was hypothesized that cone reactivation based on cone opsin signaling may be feasible, which in turn will allow to recover high sensitivity vision. It was found that endogenous cone opsins were still expressed at the level of the cone cell body in rd10 mouse model (Figure 3) suggesting the possibility of linking their activity to GIRK channels, such as GIRK2 channel, even in absence of transducin and phosphodiesterase (Figure 1B). In this configuration, the opening of GIRK channel will allow the exit of potassium ions due to the resting membrane potential of dormant cones (Busskamp, 2010) [25]. K⁺ efflux via the GIRK channel will hyperpolarize the cones in response to light as it was seen in the two mouse models of RCD.

Next, GIRK2 channel activated by G proteins recruited by cone opsin was expressed in degenerating cones. Moreover, since the remaining opsin in the cone cell bodies is still functional and sufficient to induce a light response in the degenerated cones, the insertion of GIRK2 in all cones leads to light responses following the spectral properties of each of the opsins preserving color vision. The results pointed towards enhanced light-sensitivity in cones of RCD retinas during and after degeneration of cone outer-segments. The results thus demonstrated that vertebrate light sensitive proteins combined with GIRK channel, in particular

CLAIMS

1. A vector comprising a nucleotide sequence encoding subunit 1 of G-protein-gated inwardly rectifying potassium channel (GIRK1) F137S.

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2. A vector according to claim 1 wherein said vector is a virus, chosen from an adeno-associated virus (AAV), an adenovirus, a lentivirus, an SV40 viral vector.

3. A vector according to claim 1 or 2, wherein the vector is an AAV2 or AAV9 virus comprising a 7 to 11 amino acid long insertion peptide in the GH loop of the VP1 capsid protein, wherein the insertion peptide comprises amino acid sequence LGETTRP (SEQ ID NO: 7).

4. A vector according to any one of claims 1 to 3, wherein the nucleotide sequence encoding subunit 1 of G-protein-gated inwardly rectifying potassium channel (GIRK1) F137S is under the control of a cone-specific promoter.

5. A vector according to claim 4, wherein the cone-specific promoter is pR1.7 or a functional variant thereof, or minimal M-opsin promoter, in particular in a pMNTC expression cassette, or a GRK promoter or truncated version thereof.

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6. The vector according to any one of claims 1 to 4, wherein the nucleotide sequence encoding GIRK1 F137S comprises the sequence SEQ ID NO: 3.

7. The vector according to any of the above claims, wherein the vector is a recombinant AAV9 vector comprising:

- a VP1 capsid protein in a 7 to 11 amino acid long insertion peptide is inserted in the GH loop of said VP1 capsid protein relative to wild-type AAV9 VP1 capsid protein, at a position localized between amino acids 588 and 589 of wild-type AAV9 VP1 capsid protein, wherein said peptide comprises amino acid sequence LGETTRP (SEQ ID NO: 5); and
- the nucleotide sequence encoding GIRK1 F137S under the control of a pR1.7 promoter.

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8. The vector according to any of the above claims, wherein said insertion peptide comprises or consists of amino acid sequence AALGETTRPA (SEQ ID NO: 10), LALGETTRPA (SEQ ID NO: 11), or GLGETTRPA (SEQ ID NO: 12).

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9. The vector according to any of the above claims, further comprising a nucleotide sequence encoding a mammalian cone opsin.

10. A pharmaceutically acceptable carrier comprising the vector defined in any one of claims 1 to 9.

11. The pharmaceutically acceptable carrier according to claim 10, which further comprises a vector comprising a nucleotide sequence encoding a mammalian cone opsin.

12. The pharmaceutically acceptable carrier according to claim 10 wherein the vector comprising a nucleotide sequence encoding a mammalian cone opsin:

a) is selected from the group consisting of an adeno-associated virus (AAV), an adenovirus, a lentivirus, and SV40 viral vector; or

b) is an AAV2 or AAV9 virus comprising a 7 to 11 amino acid long insertion peptide in the GH loop of the VP1 capsid protein, wherein the insertion peptide comprises amino acid sequence LGETTRP (SEQ ID NO: 7); or

c) is a recombinant AAV9 vector comprising:

- a VP1 capsid protein in a 7 to 11 amino acid long insertion peptide is inserted in the GH loop of said VP1 capsid protein relative to wild-type AAV9 VP1 capsid protein, at a position localized between amino acids 588 and 589 of wild-type AAV9 VP1 capsid protein, wherein said peptide comprises amino acid sequence LGETTRP (SEQ ID NO: 7); and

- the nucleotide sequence encoding the mammalian cone opsin under the control of a pR1.7 promoter.

13. The pharmaceutically acceptable carrier according to any one of claims 10 to 12 wherein the carrier is chosen from the group consisting of solid-lipid nanoparticles, chitosan nanoparticles, liposome, lipoplex and cationic polymer.

14. The vector according to any one of claims 1 to 9 or pharmaceutically acceptable carrier according to any one of claims 10 to 13, wherein the mammalian cone opsin is a short wavelength cone opsin (SWO).

15. A pharmaceutical composition comprising the vector according to any one of claims 1 to 9 and 14, or the pharmaceutically acceptable carrier according to any one of claims 10 to 14, with a pharmaceutically acceptable carrier, diluent or excipient.

16. The pharmaceutical composition according to claim 15, further comprising a vector comprising a nucleotide sequence encoding a mammalian cone opsin.

17. Use of the vector according to any one of claims 1 to 9 and 14, the pharmaceutically acceptable carrier according to any one of claims 10 to 14, or the pharmaceutical composition according to claim 15 or 16, in the manufacture of a medicament for treating rod-cone dystrophy (RCD).

18. The use according to claim 17, wherein the vector, pharmaceutically acceptable carrier or pharmaceutical composition is to be administered by subretinal injection at distance of the fovea.

19. The use according to any of claims 17 or 18 wherein the vector, pharmaceutically acceptable carrier or pharmaceutical composition is to be administered by subretinal injection a) in a region adjacent to the superior or inferior temporal branch of retinal artery; b) at a distance of 2–3 optic disk diameter away from the center of the fovea; and c) at a position localized in the geometric shape delineated by the branches of temporal retinal artery and temporal retinal vein.

20. The use according to claim 17, wherein the vector, pharmaceutically acceptable carrier or pharmaceutical composition is to be administered by intravitreal injection.

21. A nucleic acid comprising a sequence encoding subunit 1 of G-protein-gated inwardly rectifying potassium channel (GIRK1) F137S for use in treating rod-cone dystrophy (RCD).

22. Use of a cone precursor cell comprising a heterologous nucleic acid encoding subunit 1 of G-protein-gated inwardly rectifying potassium channel (GIRK1) F137S in the manufacture of a medicament for treating rod-cone dystrophy (RCD).

23. The use according to claim 22, wherein the cone precursor cell has been obtained from the RCD subject to be treated.

24. The use according to claim 22 or 23, wherein the cone precursor cell has been obtained by differentiation induced pluripotent stem cells obtained from somatic cells of the RCD subject to be treated.

25. The cone precursor cell for the use according to any one of claims 22 to 24, wherein the cone precursor cell is to be administered by subretinal space injection.

26. A method of preparing a cone precursor cell comprising a heterologous nucleic acid encoding GIRK1 F137S or a functional derivative thereof, said method comprising infecting a cone precursor cell with a viral vector according to any one of claims 1 to 9 and 14, or a
5 pharmaceutically acceptable carrier according to any one of claims 10 to 14.