

infections and issues with stoma bags impact patients' quality of life, while the risk of cirrhosis and liver cancer still remains. Liver transplants are an effective treatment, but carry with them the risks involved with such a complicated procedure as well as a chance of re-emergence of the condition. Despite current available treatments, the quality of life and the life expectancy of patients with PFIC2 are still limited.

Gene therapy correcting the defective gene responsible for disease development is a promising treatment for a number of diseases. However, the technique remains still under study. RNA therapy to treat a liver condition such as progressive familial intrahepatic cholestasis 2 (PFIC2) using various potential therapeutic genes including ABCB11 was only suggested in WO2017/100551 or WO2017/001570. Thus, there is still a need to develop gene therapy methods which allow stable and long-term transgene expression.

Herein, it is described a new type of therapy for PFIC2 based on the delivery to the liver of a codon-optimized version of the *ABCB11* gene, by using an adeno-associated virus (AAV) as a vector.

SUMMARY

Surprisingly, the inventors found that contrary to the wild type human BSEP-coding gene (BSEP), the codon optimized sequence of BSEP encoding gene when administered *in vivo* showed an efficient expression specifically in the canalicular membranes of hepatocytes. In *Abcb11*^{-/-} knock-out mice which reproduce most of PFIC2 symptoms observed in patients, administration of AAV bearing a codon-optimized cDNA versions encoding BSEP achieve a therapeutic effect such as significant decrease of transaminases and restoration of the secretion of bile salts to bile.

A first aspect of the present disclosure thus refers to a nucleic acid construct comprising a transgene encoding BSEP, said transgene is the sequence SEQ ID NO: 1 or a sequence having at least 80 % of identity with SEQ ID NO: 1.

In specific examples, said nucleic acid construct further comprises a promoter which initiates transgene expression upon introduction into a host cell, preferably a liver specific promoter, more preferably an alpha-1-antitrypsin promoter or a bile salt-inducible promoter.

In specific examples, said nucleic acid construct further comprises a 5'ITR (inverted terminal repeat) and a 3'ITR sequences, preferably a 5'ITR and a 3'ITR sequences of adeno-associated virus (AAV), notably a 5'ITR and a 3'ITR sequences from the AAV2 serotype.

5 In more specific examples, said nucleic acid construct comprises or consists of the nucleic acid sequence SEQ ID NO: 3 or a nucleic acid sequence having at least 80% of identity with SEQ ID NO: 3.

In another aspect, in the present disclosure refers to an expression vector comprising a nucleic acid construct as disclosed herein, preferably a viral vector, more preferably an AAV vector.

10 Another aspect of the present disclosure refers to a viral particle comprising a nucleic acid construct or an expression vector as disclosed herein, and preferably comprising capsid proteins of adeno-associated virus such as capsid proteins selected from the group consisting of: AAV3 type 3a, AAV3 type 3B, NP40, NP59, NP84, LK03, AAV3-ST, Anc80 and AAV8 serotype.

15 Another aspect of the present disclosure refers to a host cell comprising the nucleic acid construct or the expression vector as disclosed herein, or a host cell transduced with a viral particle as disclosed herein.

Another aspect of the present disclosure refers to a pharmaceutical composition comprising the nucleic acid construct, expression vector, host cell, or viral particle as disclosed herein, in combination with one or more pharmaceutical acceptable excipient.

20 In yet another aspect, the present disclosure also refers to a nucleic acid construct, a vector, a viral particle, a host cell or a pharmaceutical composition as disclosed herein for use as a medicament, such as the prevention and/or the treatment of progressive familial intrahepatic cholestasis type 2 in a subject in need thereof.

25 In another aspect, the present disclosure refers to a use of a nucleic acid construct, a vector, a viral particle, a host cell or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for the prevention and/or treatment of Progressive Familial Intrahepatic Cholestasis Type 2 (PFIC2) in a subject in need thereof.

In another aspect, the present disclosure refers to a use of a nucleic acid construct, a vector, a viral particle, a host cell or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for the prevention and/or treatment of Progressive Familial Intrahepatic Cholestasis Type 2 (PFIC2) in a subject in need thereof, wherein said subject is a
5 neonate, an infant, a child or an adult, preferably a neonate, an infant or a child, more preferably a neonate or an infant.

In another aspect, the present disclosure refers to a method of producing viral particles as disclosed herein, comprising the steps of: a) culturing a host cell as disclosed herein in a culture medium, and b) harvesting the viral particles from the cell culture supernatant and/or
10 inside the cells.

In yet another aspect, the present disclosure also refers to a kit comprising the nucleic acid construct, the expression vector, the host cell, the viral particle, or the pharmaceutical composition as disclosed herein, in one or more containers, optionally further comprising instructions or packaging materials.

CLAIMS

1. A nucleic acid construct comprising a transgene encoding BSEP, said transgene is the sequence SEQ ID NO: 1 or a sequence having at least 80 % of identity with SEQ ID NO: 1.
2. The nucleic acid construct of claim 1 further comprising a liver-specific promoter, preferably an alpha-1-antitrypsin promoter or a bile salt-inducible promoter.
3. The nucleic acid construct of claim 1 or 2 further comprising a 5'ITR and a 3'ITR sequences, preferably a 5'ITR and a 3'ITR sequences of an adeno-associated virus, more preferably a 5'ITR and a 3'ITR sequences from the AAV2 serotype.
4. The nucleic acid construct according to any one of claims 1 to 3 comprising nucleic acid sequence SEQ ID NO: 3 or a nucleic acid sequence having at least 80% of identity with SEQ ID NO: 3.
5. An expression vector comprising a nucleic acid construct according to any of claims 1 to 4.
6. The expression vector of claim 5 wherein said vector is a viral vector, preferably an adeno-associated viral (AAV) vector.
7. A viral particle comprising a nucleic acid construct according to any of claims 1 to 4 or expression vector of claim 5 or 6.
8. An AAV particle comprising a nucleic acid construct according to any of claims 1 to 4 or expression vector of claim 5 or 6 and preferably comprising capsid proteins of adeno-associated virus such as capsid proteins selected from the group consisting of: AAV3 type 3A, AAV3 type 3B, NP40, NP59, NP84, LK03, AAV3-ST, Anc80 and AAV8 serotype.
9. A host cell comprising a nucleic acid construct according to any of claims 1 to 4 or an expression vector of claim 5 or 6 or transduced with a viral particle of claim 7 or 8.
10. A pharmaceutical composition comprising a nucleic acid construct according to any of claims 1 to 4, a vector of claim 5 or 6, a viral particle of claim 7 or 8 or a host cell of claim 9 and a pharmaceutically acceptable excipient.
11. A nucleic acid construct according to any of claims 1 to 4, a vector of claim 5 or 6, a viral particle of claim 7 or 8, a host cell of claim 9 or a pharmaceutical composition of claim 10 for its use as a medicament in a subject in need thereof.
12. Use of a nucleic acid construct according to any of claims 1 to 4, a vector of claim 5 or 6, a viral particle of claim 7 or 8, a host cell of claim 9 or a pharmaceutical composition of

claim 10 in the manufacture of a medicament for the prevention and/or treatment of Progressive Familial Intrahepatic Cholestasis Type 2 (PFIC2) in a subject in need thereof.

13. Use of a nucleic acid construct according to any of claims 1 to 4, a vector of claim 5 or 6, a viral particle of claim 7 or 8, a host cell of claim 9 or a pharmaceutical composition of claim 10, in the manufacture of a medicament for the prevention and/or treatment of PFIC 2 in a subject in need thereof wherein said subject is a neonate, an infant, a child or an adult, preferably a neonate, an infant or a child, more preferably a neonate or an infant.

14. A method of producing viral particles according to claim 7 or 8, comprising the steps of:

- a) culturing a host cell comprising a nucleic acid construct or an expression vector according to claim 9 in a culture medium, and
- b) harvesting the viral particles from the cell culture supernatant and/or inside the cells.

15. A kit comprising a nucleic acid construct according to any of claims 1 to 4, a vector of claim 5 or 6, a viral particle of claim 7 or 8, a host cell of claim 9 or a pharmaceutical composition of claim 10, in one or more containers, optionally further comprising instructions or packaging materials.